University of Nevada, Reno

Impact of triploid Rainbow Trout and naturalized Rainbow Trout (*Oncorhynchus mykiss*) on recovery of Lahontan Cutthroat Trout (*Oncorhynchus clarkii henshawi*) in the Truckee River watershed

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Cell and Molecular Biology

by

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ABSTRACT

Historically the Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*, LCT) occurred throughout the Truckee River basin, supporting important commercial fisheries and was extirpated in the 1940s due to water diversions, predation, competition and hybridization with non-native trout. To provide angling opportunities, Rainbow trout (*Oncorhynchus mykiss,* RBT) has been planted, and there is a robust naturalized population throughout the Truckee River. Recovery efforts are underway to reintroduce the threatened LCT back into their native habitat in the Truckee; however, planting LCT sympatric with naturalized RBT can support hybridization between the species and hamper LCT recovery. Since 2004, in an effort to limit hybridization, 90% of the RBT stocked are non-reproductive triploid RBT. Over 3,400 trout samples were collected in the Truckee River and its tributaries from 2007-2010. These trout were identified as pure LCT, pure RBT, LCT/RBT hybrids or triploid RBT using bi-parentally inherited markers that differentiate between RBT and LCT and microsatellite markers that revealed triploidy in a proportion of the RBT. A mitochondrial marker was sequenced in hybrids to determine the maternal contribution to hybridization and to look at spawning success. The highest level of hybridization was found in 2008 from samples in the river tributaries. This correlates to the time period when fry stocked in 2005 and 2006 would reach sexual maturity. Backcrossing of hybrids with RBT was detected, and a low level of introgression indicates that hybridization has been occurring in the river for multiple generations. Mitochondrial sequences show that

LCT is successfully competing for spawning gravels; however continued stocking of LCT without the removal of the naturalized RBT will likely lead to a hybrid swarm. Triploidy was successfully identified in the hatchery supplied known triploids; despite high levels of stocking of trpRBT, less than 10% of the RBT sampled in the Truckee River were identified as triploid. The diploid RBT samples represent the naturalized RBT population in the river. The genetic population structure of the naturalized RBT was investigated using 11 microsatellite loci to look for potential RBT eradication units allowing for LCT reintroduction. Barriers along the Truckee River contribute to developing population structure, but these barriers are transient, and structure varies year to year. No clear eradication units or regions of the river to potentially isolate a translocated LCT population from RBT encroachment were identified. Six of the 11 microsatellites cross amplified and showed variation in LCT. Comparison of the LCT and HYB sampled in the Truckee River to the LCT strains stocked indicates that the Pilot Peak Strain of LCT has a higher survivorship in the Truckee River compared to the contemporary Pyramid Lake or Independence Lake strains. Reintroduction of LCT into the Truckee River is possible, but would require the eradication of the reproductive RBT and extensive monitoring to detect hybridization.

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Introduced species in freshwater ecosystems: utility of genetic tools to inform management promoting species recovery of threatened native trout

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INTRODUCTION

Conservation of biodiversity is important for overall ecosystem stability (Kaufman et al. 1998). Currently we are losing species at such a high rate that many scientists believe we are heading to a "sixth extinction" comparable in loss to each of the five mass extinctions shown in the fossil record (Chapin et al., 2000; Frankham, 2005; Pimm et al., 2014; McCallum et al., 2015). Many factors contribute to the loss of biodiversity including habitat fragmentation (Carlisle et al., 1998, Fischer et al., 2007), pollution (Hernadez et al., 2016, Clapcott et al., 2016), and introduction of exotic non-native species (Fragoso-Moura et al., 2016). Non-native species contribute to the decline of native species and cause ecosystem instability by altering food web dynamics (Baxter et al., 2004, Reissig et al., 2006, Martin et al., 2010). Introduced species can impact native species through habitat destruction as is known from the zebra mussel (*Dreissena polomorpha*) introductions into North American watersheds. The zebra mussel enters a foreign water body in ballast water of ships and spreads rapidly in the new environment after arrival. The mussel is an effective invading species because it grows quickly and becomes reproductive, reaching high densities and quickly expanding the geographic spread of the invasion (Vitousek et al., 1997). Zebra mussels can alter the habitat by covering the river and lake bottoms, and thereby alter the nutrient availability and algae populations of the ecosystem (Idrisi et al., 2001, Marsden et al 2001). Similarly, introduced fishes such as brook trout (*Salvelinus fontinalis*) compete for food resources and spawning habitat displacing native rainbow trout (*Onchorhynchus mykiss*, RBT) (Vitsousek et al., 1997) and cutthroat trout (*Onchorhynchus clarkii,* CT) (Peterson et al., 2004). On the other hand, when intentionally translocated, introduced RBT have been shown to directly impact native Atlantic salmon and brook trout populations through competition for habitat (Thibault and Dodson, 2013), and predation of small endemic fishes (Shelton et al., 2015). Humankind has introduced species both deliberately and unintentionally with many of these species becoming established and invasive in their new habitat causing a wide variety of ecological and economic problems (Vitousek, 1997).

Intentional translocations of fish species to create or enhance angling opportunities have been occurring for centuries, with little concern for the impact on native fauna (Gozlan et al., 2010). Since the late 1800s, fish culture-farming and stocking of artificially propagated fishes has become commonplace, and a variety of native and non-native fishes are planted throughout the world. Worldwide, freshwater fish are the most introduced taxa and lack of containment of introduced species has resulted in the decline of many native fish and other aquatic organisms (Hitt et al., 2003; Reissig et al., 2006; Sharma et al., 2011; Neville and Dunham, 2011). For example tilapia, *Oreochromis spp,* is often introduced into new waterways through human mediated translocations, using them for a food source or for bait. Tilapia is adapted to live in high densities and harsh living conditions, which increase their invasion success (Ovenden et al., 2015). The high density of tilapia negatively impacts community ecology of native fishes by degrading riverbeds and impacting spawning habitat, through aggression towards other species (Martin et al., 2010) and reductions in water quality (Doupe and Burrows, 2008). Regulations against transporting this fish are in place; however, tilapia continues to be introduced into new systems (Ovenden et al., 2015).

Introduction of Rainbow trout

One of the most widely introduced non-native fish species is RBT. This species has been introduced into waters in at least 99 countries, producing naturalized populations and heavily impacting native fishes (Stankovic et al., 2015). Intraspecific hybridization occurs with native *O. mykiss* subspecies, steelhead (Page et al., 2011) , and redband trout (Kozfkay et al., 2011) as well as interspecific hybridization with multiple native cutthroat trout subspecies (Campbell et al., 2002; Bennett, et al., 2009; Loxterman et al., 2013; Pritchard et al., 2015). Hybridization can have many negative consequences that can endanger the introgressed populations. Initially, hybrid vigor can occur, an apparent increase in fitness that increases the productivity and fitness of the first generation hybrid. This can be followed by outbreeding depression, or reduced fitness of backcrossed hybrids because of the loss of co-evolved gene complexes, and utlimately lead to the collapse of the hybridized populations, and complete replacement by the invader (Rhymer and Simberloff, 1996; Allendorf et al., 2001). Introgressive hybridization of RBT with native trout has played a role in the decline of all subspecies of native cutthroat trout. Three cutthroat trout subspecies have been listed as endangered or threatened under the United States Endangered Species Act (Lahontan cutthroat trout (LCT) *O. c. henshawi*; Paiute cutthroat trout (PCT), *O. c. seleniris;* and Greenback cutthroat trout (GCT) *O. c. stomias*) and two subspecies are considered to be extinct (Yellowfin cutthroat trout, *O. c macdonaldi* and Alvord cutthroat trout, *O. c. alvorensis*) (Behnke, 1992). Although the remaining subspecies of cutthroat trout (Coastal, Westslope, Yellowstone, Greenback, Rio Grande and Bonneville) are not listed as threatened, they are all at risk due to introduced species, and hybridization with RBT remains the largest threat for their long term persistence (Muhlfeld et al., 2009).

Cutthroat trout distribution and decline

Cutthroat trout have native distributions that span from the North Pacific Ocean to east of the continental divide with multiple physically isolated interior populations (Behnke, 1992, Figure 1). The cutthroat trout subspecies are thought to have diverged from a common ancestor approximately 2 million years ago due to geographic and reproductive barriers. These trout species evolved unique adaptations to particular stream and lake environments that have created both morphological and genetic evidence supporting differentiation (Leary et al., 1987; Behnke, 1992). The current range of eight of the extant cutthroat trout subspecies is shown on Figure 1. The yellowfin cutthroat trout (*O.c. macdonaldi*), once found in the headwaters of the Arkansas River and the Twin Lakes, in Colorado, was genetically and morphologically distinct from the GCT, found in the same region. Shortly after RBT and brown trout were introduced into the Twin Lakes, the yellowfin trout was displaced by the introduced trout and disappeared completely. GCT, once thought to also be extinct, was discovered in two small streams in the 1960s and 1970s, and currently the GCT populations are restricted to remote high-elevation streams protected by fish movement barriers (Young, 1995; Harig et al., 2000). GCT recovery efforts, including 44 attempted translocations, have been ongoing for more than 25 years and has reportedly resulted in 14 successful populations (Harig et al., 2000). However, later genetic evaluation comparing translocated populations to museum samples indicates that many of the early successful translocations may have mistakenly reintroduced similar trout from that region, the Colorado River cutthroat trout (*O. c. pleuriticus*) or the Rio Grande cutthroat trout (*O. c. virginalis*). Currently the GCT is thought to persist as a single population, transplanted outside it native range in the late 1800s, in Bear Creek in Colorado (Metcalf et al. 2012).

The Alvord cutthroat trout, another native cutthroat trout species, was thought to have lived in the Alvord basin of Nevada and Oregon and was only recognized in out-of-basin transplanted populations located in Trout Creek, Oregon and Virgin Creek, Nevada. These populations declined in the 1980s, and all known populations are hybridized with RBT or other cutthroat trout subspecies, therefore the Alvord cutthroat trout is now also considered extinct (Williams and Bond, 1983; Behnke, 1992).

Lahontan cutthroat trout was endemic to the Lahontan basin and long isolated from other *Oncorhynchus spp.* in pluvial Lake Lahontan. The desiccation of

Lake Lahontan 5000-8000 years ago separated the lake from its major tributaries and isolated this fish into six distinct river basins: the Carson, Walker, Truckee, Humboldt, Quinn and Willow-Whitehorse (Figure 2). Pyramid and Walker Lakes are remnants of the larger pluvial lake. A robust LCT fishery was once found throughout the Lahontan basin watersheds in California and Nevada; however, like most of the cutthroat trout subspecies, pollution, habitat degradation, and introduced non-native trout contributed to a major decline in distribution and abundance of LCT, and currently LCT is found in less than 10% of its historic fluvial habitat and 0.4% of its lacustrine habitat. Conservation programs are now attempting to protect extant populations, as well as to reintroduce the trout into its native habitat. The 1995 U. S. Fish and Wildlife Service Recovery Plan for Lahontan cutthroat trout (Coffin and Cowan, 1995), recognized three distinct population segments (DPSs) for LCT based on recent connectivity among the drainages and unique genetic composition (Figure 2). The Northwestern DPS consists of the Quinn River drainage (Brown), Coyote Lake and Summit Lake basins. The Eastern DPS consists of the Humboldt drainage (Red), and the Western DPS includes the Truckee (blue), the Carson (purple) and the Walker (green) river drainages. The Humboldt River basin supports the greatest number of LCT populations in the Lahontan basin in about 500 km of fluvial habitat, about 14% of the historic habitat (Coffin and Cowan, 1995). Seasonal flow reductions and water quality problems have created some isolated populations, threatening local extinction due to hybridization and loss of habitat. The Northwestern DPS, containing Summit Lake and the Quinn River drainage, contain small streams that harboring isolated LCT remnant populations (Coffin and Cowan, 1995), however, some of those populations may have gone extinct recently due to drought and livestock use of the riparian habitat (Coffin and Cowan, 1995). The largest self-sustaining lacustrine population is found in Summit Lake and its tributary streams. Introduced redside shiners (*Richardsonius egregious*), established in the 1970s, have contributed to a decline in this population; however Summit Lake is one of the few LCT populations not threatened by RBT introductions. The Western DPS is arguably the most at risk. The Walker River drainage has been negatively impacted in recent years by water diversions to support agriculture and have seen a dramatic increase in total dissolved solids and alkalinity has occurred in Walker Lake to such a degree that stocked LCT have very limited survival (Sedinger et al., 2012). Small endemic and introduced populations persist in the Walker River basin and the Carson River basins in less than 3% of historic habitat, and are declining rapidly (Coffin and Cowan, 1995). The Truckee River watershed (Figure 3), flows from oligotrophic Lake Tahoe to the terminal Pyramid Lake, and once had over 195 km of interconnected habitat for LCT. Natural meanders were removed to control the flow of the river, and Derby Dam was built in 1906. The watershed became fragmented, and the lacustrine forms of the LCT could no longer make their way into the Truckee River for spawning. The Pyramid Lake strain of LCT, the largest trout in North America, was thought to be extinct until 1978 when a translocated population was discovered in a small creek in Utah. This strain, called the Pilot Peak strain, is currently being propagated at the Lahontan

National Fish Hatchery in Gardnerville, Nevada (https://www.fws.gov/lahontannfhc). Currently, there is a hatchery supported fishery in Pyramid Lake, including the contemporary Pyramid Lake strain and, since 2005, the Pilot Peak strain. Independence Lake (Nevada and Sierra counties, California) contains the only extant lacustrine LCT population in the Truckee River watershed. Introduced brook trout threaten this population through reducing LCT spawning success and recruitment (Scoppettone et al., 2012). Introduced Kokanee (*O. nerka*) compete for resources and may impact this population. This population, however, is not impacted by introduced RBT. The Upper Truckee River, Lake Tahoe's largest tributary, flows 37 km from Red Lake Peak in Alpine County through the Truckee Marsh to Lake Tahoe, and historically was dominated by LCT. The biological assemblage of this tributary currently consists of suite of non-native species, including brook trout, brown trout and RBT. In 1989 and 1990, LCT were reintroduced into the head waters in Meiss Meadows. Prior to reintroduction, rotenone application, electro-fishing and gill-netting were done to remove non-native trout. Removal efforts were continued annually until 2009, when no non-natives were observed. Eight kilometers of stream habitat and 15 lake acres were reclaimed for LCT reintroduction and stocked with LCT from Macklin Creek. This transplanted population is currently one of the only high-elevation LCT populations found in the Sierra Nevada Mountain Range (Miller and Santora, 2013).

Tools for recovery

The re-introduction potential of LCT into its native habitat in the Truckee River is being considered in this study. Translocations, including those back to historic habitat are not without risk—the ecology of that habitat may have been altered to such a degree, that the re-introduction dynamics are more like those of an invasive species, disrupting the new "normal." Introduced species are one of the largest threats to cutthroat trout populations across their range due to the ease and popularity of planting various sport fishes to enhance recreational angling. However, in recent decades, the focus of translocations to enhance or maintain biodiversity has increased in frequency (Weeks, 2011; Seddon et al., 2014). With increasing species extinction rates and habitat losses due to climate change and anthropogenic alterations, translocating threatened or endangered species has become a powerful tool available to conserve biodiversity (Griffith, 1989; Seddon et al., 2014). Broad guidelines have been developed by the International Union for the Conservation of Nature Species Survival Commission (2013) (https://portals.iucn.org/library/sites/library/files/documents/2013-009.pdf) which offer a generalized framework for each reintroduction stage for plant and animal taxa. Briefly, the guidelines state: (1) A conservation translocation must take into consideration ecological, social, and economic interests, (2) the threat of extinction should be reduced, (3) potential benefits and negative impacts must be assessed, (4) long term negative impacts, which may be difficult to identify need to be considered, (5) translocations outside the indigenous range can have unintended consequences, (6) risk analysis should be done to minimize any negative impacts,

(7) risk must be balanced against the potential benefits, and (8) if a high level of uncertainty remains, the translocation should not proceed. It is imperative that prior to any large scale re-introduction effort, the potential impacts of the introduced species on the ecosystem be fully evaluated.

Study system

The Truckee River in eastern California and Western Nevada flows from oligotrophic Lake Tahoe in California to endorheic Pyramid Lake (Figure 3). Natural meanders and flood plains along the Truckee River were removed and the river was channelized to “control” the flow of water leading to destruction of much of the suitable spawning habitat and refugia for native species. Over the past century, eight dams and power diversions that act as complete fish passage barriers have been built (Figure 4, green diamonds). This has dramatically changed the distribution of many fishes native to the Truckee River, and completely eradicated LCT from the river and most of the watershed (Peacock et al. 2016). In the absence of LCT, to provide angling opportunities, state wildlife agencies have stocked rainbow trout (*Oncorhyncus mykiss*, RBT) throughout the Truckee River basin and as a result, RBT populations have become naturalized in the Truckee River. United States Fish and Wildlife Service (USFWS) has been mandated to recover native trout to their historic habitat if possible (Coffin and Cowan, 1995) and here, we are evaluating the reintroduction potential of the Truckee River system specifically. California and Nevada state game and fish agencies often have conflicting mandates in that they are required to attempt conservation and reintroduction of endangered species, while they are also required to provide angling opportunities and recreation in the system. Political conflicts among state and federal wildlife agencies are common in fishery biology, and can lead to delays in or abandonment of recovery projects if the recovery guidelines are not well established and maintain both public and political support.

Truckee River restoration projects include re-establishing meanders and riparian habitat to assist in recovery efforts and create spawning habitat; however the largest impediment to LCT recovery in the Truckee River is the presence of naturalized rainbow trout. RBT has been planted in the Truckee River since the 1800s for recreational angling; historically, LCT were able to resist hybridization and dominated the Truckee River until habitat destruction and reduced water flow in the Truckee River contributed to the decline of LCT. Since 1994, LCT have been planted in the river, primarily in the same stocking sites where RBT were planted. Hybridization was suspected, and stocking programs were changed to limit hybridization potential by primarily planting non-reproductive triploid RBT, and planting LCT in regions where RBT were not stocked. However, naturalized RBT in the river can contribute to hybridization with the planted LCT. Between 2004 and 2010, over 600,000 LCT were planted in an attempt to flood the system with LCT, hoping that the stocking of triploid RBT would interfere with the naturalized RBT reproduction and limit hybridization with LCT. Given the large naturalized RBT population in the river, hybridization was a probable outcome to this action, and therefore, the extent of hybridization and introgression was investigated in this study. To look at the impact of triploid RBT on the naturalized RBT population in the river, triploid RBT were identified and survivorship investigated. In addition, elucidation of naturalized RBT genetic population structure using nuclear microsatellite genetic markers was done to potentially identify isolated segments of the RBT population that can putatively be eradicated to provide habitat for reintroduction sites for LCT. Finally, by looking at the stocking history of LCT in the Truckee River and comparing the genetics of the stocked LCT strains, it can be determined if any of the strains survive better in the face of naturalized RBT and hybridization risk.

Genetic tools

In recent years, the use of molecular tools such as microsatellites and mitochondrial or nuclear sequence data has become more accessible and affordable (Blanchet, 2012). These molecular markers can be utilized to look at population structure of non-natives, identify hybridization or other threats to the reintroduction, and look at the genetic viability of the source population(s) available for reintroduction (Allendorf and Phelps, 1980; Estoup and Guillemaud, 2010; Blanchet, 2012).

Species specific nuclear markers

Bi-parentally inherited species-specific markers were developed that amplified a unique allele in RBT and cutthroat trout (Ostberg and Rodrigues, 2004). These eight markers cross-amplified in multiple cutthroat trout sub-species (westslope, coastal, Lahontan, and Yellowstone) and allowed for the simultaneous amplification of an allele in RBT and a different allele in cutthroat trout with a single PCR primer set. A pure cutthroat trout would display eight cutthroat alleles, a pure rainbow trout would display eight rainbow alleles, and a first generation hybrid would display both sets of markers. These markers were used to investigate the hybridization zone between native westslope cutthroat trout and RBT, identifying potential evolutionary mechanisms that may limit hybridization (Ostberg and Rodriguez, 2006; Ostberg and Chase, 2012). In a naturally sympatric population, asymmetric mating is observed, suggesting differential fitness or differences in mating behavior. This can lead to strategies for re-introduction that can give the advantage to the native species such as altering male/female ratios or the age of the LCT strain stocked.

Mitochondrial DNA

Mitochondria is passed down through generations via the maternal bloodline, therefore mitochondrial DNA (mtDNA) can be used to determine maternal contribution to the hybrid, establishing if the species of interest is able to compete for spawning sites (Ostberg and Rodriguez, 2006; van Herwerden et al., 2006). PCR amplicons can be differentiated through restriction enzyme digests or sequencing and compared to known RBT and cutthroat trout. Patterns usually differ when looking at hybridization between two naturally sympatric trout species compared to patterns seen when one of the hybridizing species is recently introduced. Ostberg et al. (2004) report that in two naturally sympatric populations of coastal cutthroat trout and anadromous rainbow trout (steelhead) the F_1 hybrids in the Ostberg et al. (2004) study had only RBT mtDNA but backcrossed individuals had a reduced proportion of the RBT mtDNA haplotype. It has been suggested that females of the rare species indiscriminately spawn with the abundant male species; whereas the common species is more likely to spawn with the hybrid. Investigations into the maternal contribution to hybridization can shed some light onto the ability of the introduced species to be competitive for spawning.. In the Truckee River watershed, LCT is the native species and therefore expected to to survive and contribute to reproduction because they evolved in this system and should be adapted to that environment.

Microsatellite markers

Microsatellite loci are highly variable, non-coding nuclear markers that have high variability and high mutation rates and have been invaluable tools for looking at population structure and effective population size. Normally, diploid individuals will display a maximum of two alleles at each microsatellite locus, and multiple loci can create a genetic fingerprint of the individual. The genotypes generated can be used in a plethora of statistical programs to look at characteristics of the population (structure, genetic variation) or the individual (identity or triploidy). Prior to initiating a re-introduction, it is necessary to evaluate the non-native species that are present in that system. If an exotic species is considered a threat, it is important to isolate the translocated population from the introduced species. Often, complete eradication from a watershed is not a viable alternative, so population structure can be used to identify "eradication units," or segments of the population that are isolated and can be removed as a unit (Finnoff et al., 2007; Adams et al., 2014). In a watershed, such as the Truckee River, barriers such as the dams shown in Figure 4, would be expected to isolate segments of the RBT population. In a fragmented population of Roanoke logperch (*Percina rex*), clustering models described discrete population segments, each one delineated by a major water diversion, indicating long term separation (Roberts et al., 2013). In the Truckee River, the RBT population has been stocked extensively, on all sides of the diversions with a hatchery strain of RBT. Stocking of triploids started in 2004. Therefore, it may take longer for population structure to develop and be detected than the time-line chosen for this study. In fact, population structure seen may be attributed to founder effect, and drift or selection acting on the population. I am using genotype clustering analysis in the program STRUCTURE (Pritchard et al., 2000) to look at the current genetic variation on the landscape as well as F-statistics to assess long-term connectivity between the collection sites (Wright, 1965).

Effective population size (Ne) is of conservation importance because it can allow managers to understand the impact that drift or inbreeding may be having if attempting to control reproduction in a watershed (Do et al., 2014). In the Truckee River watershed, triploid RBT are being planted to attempt reproductive containment that will interfere with the naturalized RBT reproduction. N_e was compared in the RBT sampled from 2007-2010.

Genetic evaluation of the strains used in reintroductions can be done in order to evaluate the genetic viability of the translocated populations (Frankham et al., 2014). Genetic tests evaluating strain survival and ability to adapt to a putative translocation site can be time consuming and costly, but genetic assessment can elucidate important characteristics contributing to successful reintroductions of endangered species (Griffith et al., 1989; Dodd and Segal, 1991; Cochran-Biederman et al, 2015). The genetic evaluation of the source stock for the reintroduction is important because many reintroduction projects utilize broodstock or small founder populations that have been reared in a hatchery potentially leading to to lower genetic diversity (Allendorf and Phelps, 1980) and a decline in adaptability (Weber and Fausch, 2003). Over time, captive stocks can become less viable and unable to thrive in a wild environment (Sedinger et al., 2012). Genetic diversity and local adaptation of the source stock are valuable predictors of survival, recruitment and reproduction necessary to establish a wild population from a captive stock (Cochran-Biederman et al., 2015). Here, microsatellite loci can be used to perform cluster analysis which allows for comparison of the samples of LCT found in the system to the LCT strains that are stocked in the system, determining the recovery value of the Independence Lake, the Pyramid Lake, and the Pilot Peak LCT, respectively. Sedinger et al. (2012) found that in the highly alkaline Walker Lake, transplanted LCT from the Pilot Peak strain had higher survival. Similar results can be expected here, as the Pilot Peak strain is more similar genetically to the strain that was originally found in the river.

Specific aims and hypothesis

This study aims to evaluate the re-introduction potential for LCT into the Truckee River. There are multiple factors present in this system that contribute to a failed re-introduction. The greatest impediment is the robust naturalized RBT population that can potentially hybridize with the introduced LCT. Prior to any recovery plan, the impact of planting triploid RBT needs to be evaluated, and the possibility of removal of the naturalized RBT population needs to be considered. In addition, because planted fishes often have trouble acclimating and die soon after stocking, the survival of the LCT needs to be evaluated, specifically the genetic characteristic of the strains surviving and contributing to reintroduction. To assess the feasibility of LCT reintroduction, I evaluated the following specific aims:

Specific AIM 1: Evaluate the hybridization dynamics of RBT and LCT in the Truckee River. Using bi-parentally inherited nuclear I identified the *Oncorhyncus* species in the Truckee River as either pure RBT, a pure LCT, a first generation hybrid, or a backcrossed hybrid. I looked at the appearance of hybrids in the river taking into consideration the stocking of triploid RBT, and LCT. Because the mitochondrial genome is passed down the maternal bloodline, the mitochondrial ND2 marker was used to look at the maternal contribution to the hybrid. LCT ND2 sequence indicate that the LCT is able to survive to reproductive age and contribute to spawning.

Hypothesis 1: The highest level of hybridization will be found in regions where both RBT and LCT were planted.

Hypothesis 2: High levels of hybridization will be found in regions where LCT are planted because of the presence of naturalized RBT that will readily breed with LCT.

Outcome:

LCT are overwintering successfully after stocking and becoming reproductively active in the Truckee River. Hybridization was found in all of the sites where the LCT have been planted in the river and Hybrid Index Scores (HIS) indicate back crossing of hybrids with pure RBT and high levels of introgression. Mitochondrial sequence data indicate that the LCT females are able to compete for spawning sites. Planting of triploid RBT has had little to no impact on the ability of the naturalized RBT to reproduce.

Specific AIM 2: Evaluate the ability of microsatellites to identify triploids and the ability of triploids to survive in the Truckee River. Eleven highly variable microsatellite markers were amplified on 75 known triploid RBT provided by the fish hatcheries, and over 3400 *Oncorhynchus* samples collected in the river.

Hypothesis 1: Microsatellite markers will display three alleles in at least one of the eleven loci in triploid fish.

Hypothesis 2: Because trpRBT are stocked in popular fishing spots for recreational angling, the triploid fish will only make up a small portion of the RBT sampled and triploids will have little impact on RBT or LCT reproduction and hybridization.

Outcome:

All of the hatchery triploids were identified as triploid using microsatellite markers. Although 90% of the stocked RBT are trpRBT, less than 10% of the RBT sampled in the Truckee River were identified as triploid, indicating that triploids do not remain in the system long-term.

Specific AIM 3: Evaluate the population structure of the Naturalized RBT in the Truckee River watershed. Using eleven microsatellite markers and Bayesian clustering analysis, the population structure of the salmonids identified as naturalized RBT was investigated.

Hypothesis 1: Barriers to gene flow such as Derby Dam and the Verdi Power Dam, will impact the population structure of the naturalized Rainbow trout.

Hypothesis 2: High water flow will eliminate the impact of barriers, allowing for connectivity among the segments of Truckee River thereby creating a panmictic RBT population.

Outcome:

The population structure of the RBT varied over the four years of the study. Regions that appeared as isolated population segments one year were connected with the rest of the population segment the next year. Although barriers such as Derby Dam and the Farad Power Diversion contributed to developing population structure over the four years of sampling, overall connectivity is indicated by low levels of differentiation and no eradication units were identified.

Specific AIM 4: Evaluate the genetic contribution of the different strains of LCT stocked in the river with the LCT sampled that have survived in the river and the hybrids identified in the system. Using microsatellite markers that cross amplified in LCT and RBT, the LCT and hybrids found in the Truckee River were compared to the Pilot Peak strain, Independence Lake strain, and the Pyramid Lake strain of LCT planted in the system.

Hypothesis 1: Because the Pilot Peak strain is a recent descendant of LCT from the Truckee River watershed, this strain contain more locally adapted traits, will have a higher survivorship and will contribute more to reproduction than the other strains stocked over the same time period.

Outcome:

Clustering analysis showed that most of the pure LCT found in the River were of Pilot Peak ancestry. This includes pure LCT found in all four years of the study. The hybrids in the system clustered primarily with the RBT, but showed genetic contribution from both the Pyramid Lake Strain and the Pilot Peak strain.

Overall, this research represents a significant increase in our understanding of naturalized RBT populations and the challenges of reintroducing LCT in the Truckee River watershed. Although the data presented here indicate that LCT are able to survive, become reproductive, and compete for spawning habitat with the RBT, the presence of RBT will lead to hybridization, and continued stocking of LCT without isolation from the naturalized RBT will likely lead to a hybrid swarm. Hybridization was found in the highest level in the Truckee River tributaries, Dog Valley and Hunter creeks. These creeks provide good spawning habitat, but gene flow between the main stem and the tributaries is evident and removal of the RBT from the tributaries would likely result in reinvasion from the main stem. Triploid RBT appear to have little impact on the naturalized RBT population, however, planting of triploids can provide angling opportunities without contributing to the existing RBT population, potentially allowing for RBT eradication. The lower region of the Truckee River, beyond Derby Dam, showed little RBT reproduction as the conditions are not optimal, however, recently, Pilot Peak LCT in Pyramid Lake have spawned in the lower Truckee River. Current management by the United States Fish and Wildlife Service include attempts to improve the habitat in the lower Truckee River, and this region could serve as a putative reintroduction site, if RBT can be fully eradicated. LCT recovery in the Truckee River watershed will be possible only with continued public support and cooperation between state and federal agencies.

Figure Legends

Chapter 1: Introduction

Figure 1. Range of Cutthroat trout subspecies in North America. Of the 14 recognized cutthroat trout subspecies the distribution of eight primary extant subspecies is shown. Lahontan Cutthroat trout, Greenback Cutthroat trout and Paiute Cutthroat trout (not shown) are listed as threatened on the U. S. Endangered Species act. Other LCT subspecies are at risk due to hybridization and competition with introduced species.

Figure 2. The Lahontan hydrographic basin with the river systems that support the Lahontan Cutthroat Trout designated. The Northern GMU contains the Quinn, Alvord and Owyhee river drainages, the Eastern GMU contains the Humboldt River drainage, and the Western contains the Carson, Truckee and Walker River drainage. (Map prepared by Robert E. Elston, UNR, Department of Biology)

Figure 3. The Truckee River watershed. Once a large interconnected watershed, the Truckee River watershed supported a fishery containing one of the largest trout species found in North America, the Lahontan cutthroat trout, *O. clarki henshawi*. (Map prepared by Robert E. Elston, UNR, Department of Biology)

Figure 4. Partial and complete barriers along the Truckee River. Complete in stream barriers (green diamonds) block fish passage in several stretches of the Truckee River, fragmenting the habitat. Partial barriers are also shown (red circles)

that may contribute to forming population structure in the fish populations.

Sampling transects are shown in the white circles.

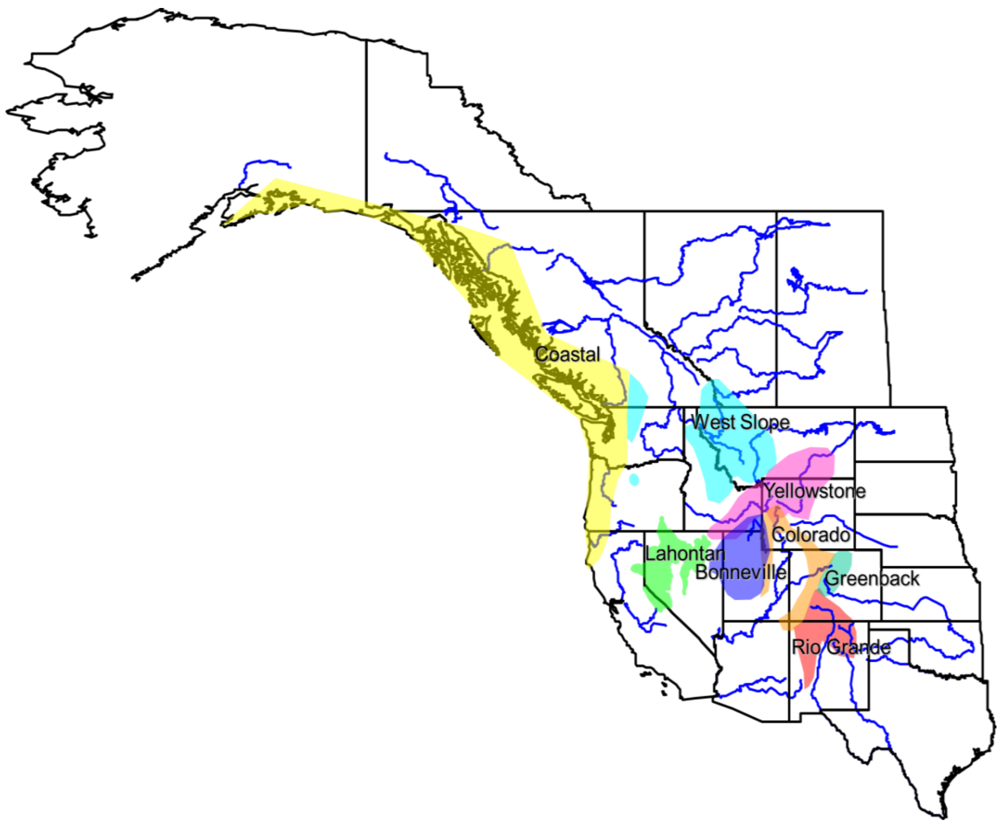
Coastal
West Slope
Yellowstone
Colorado
Lahontan
Bonneville
Greenback
Rio Grande

Figure 1.

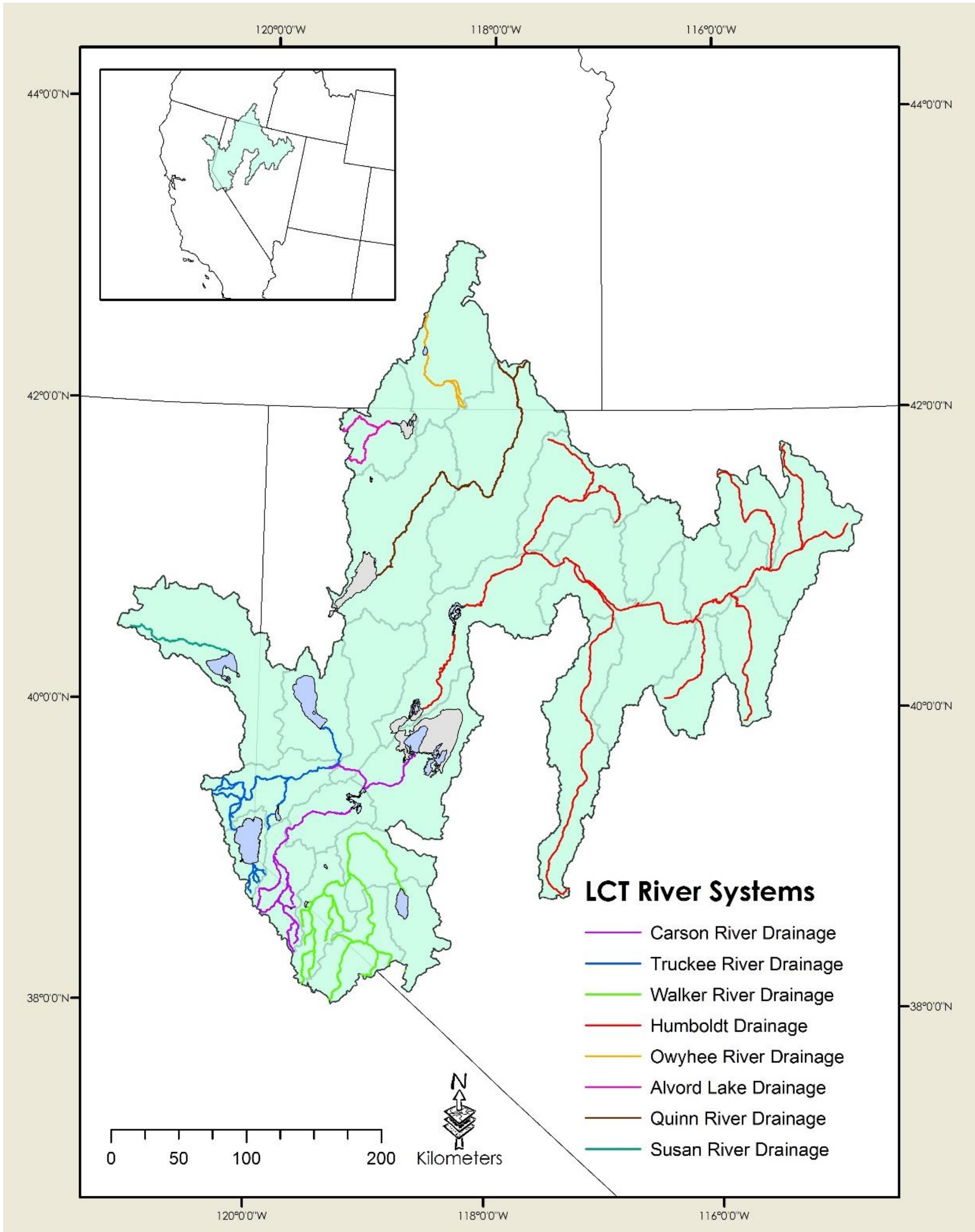
120°0'0"W
118°0'0"W
116°0'0"W
44°0'0"N
42°0'0"N
40°0'0"N
38°0'0"N
LCT River Systems
Carson River Drainage
Truckee River Drainage
Walker River Drainage
Humboldt Drainage
Owyhee River Drainage
Alvord Lake Drainage
Quinn River Drainage
Susan River Drainage
N
0
50
100
200
Kilometers

Figure 2.

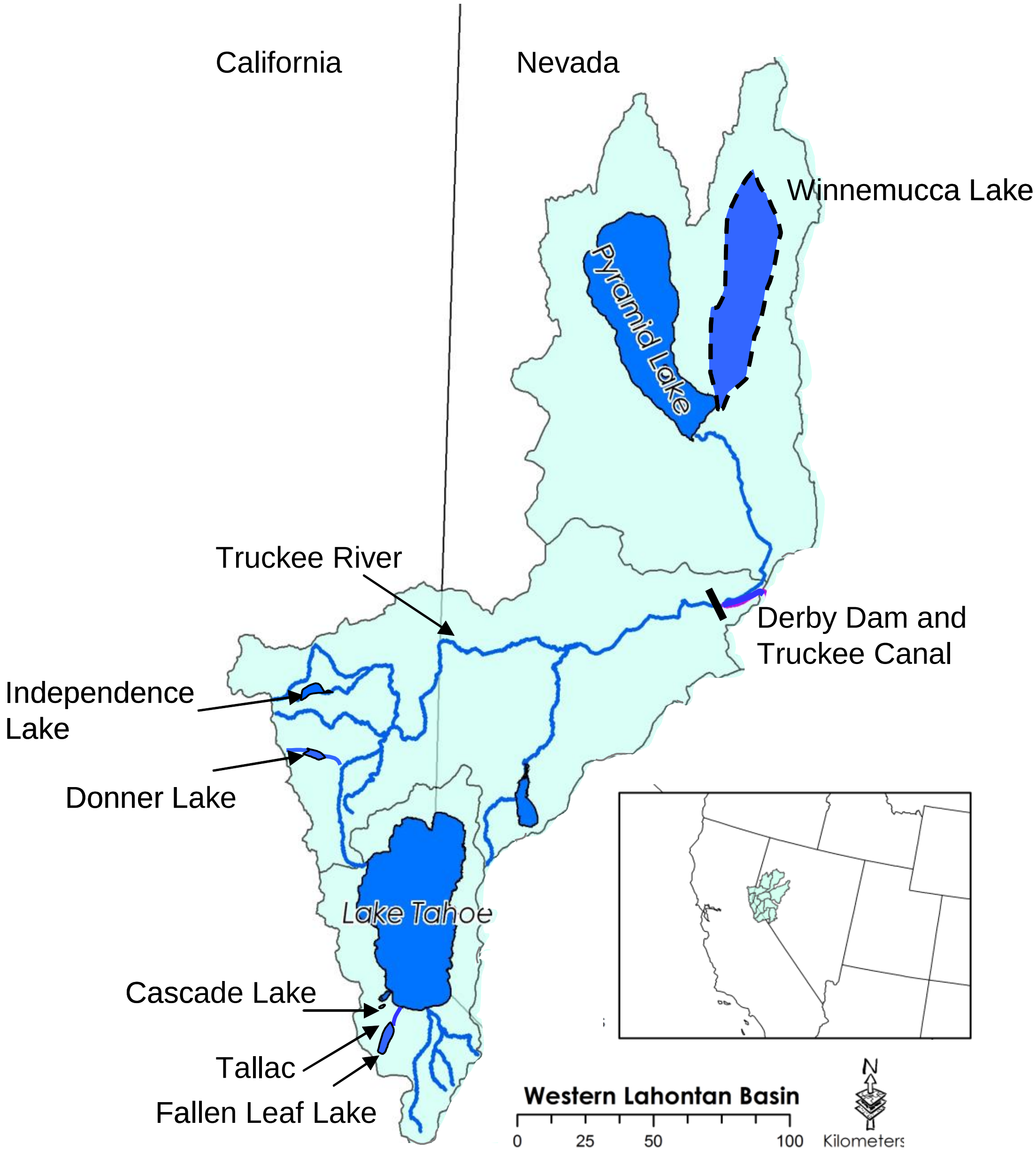
California
Nevada
Winnemucca Lake
Pyramid Lake
Truckee River
Derby Dam and
Truckee Canal
Independence
Lake
Donner Lake
Lake Tahoe
Cascade Lake
Tallac
Fallen Leaf Lake
Western Lahontan Basin
0
25
50
100
Kilometers
N

Figure 3.

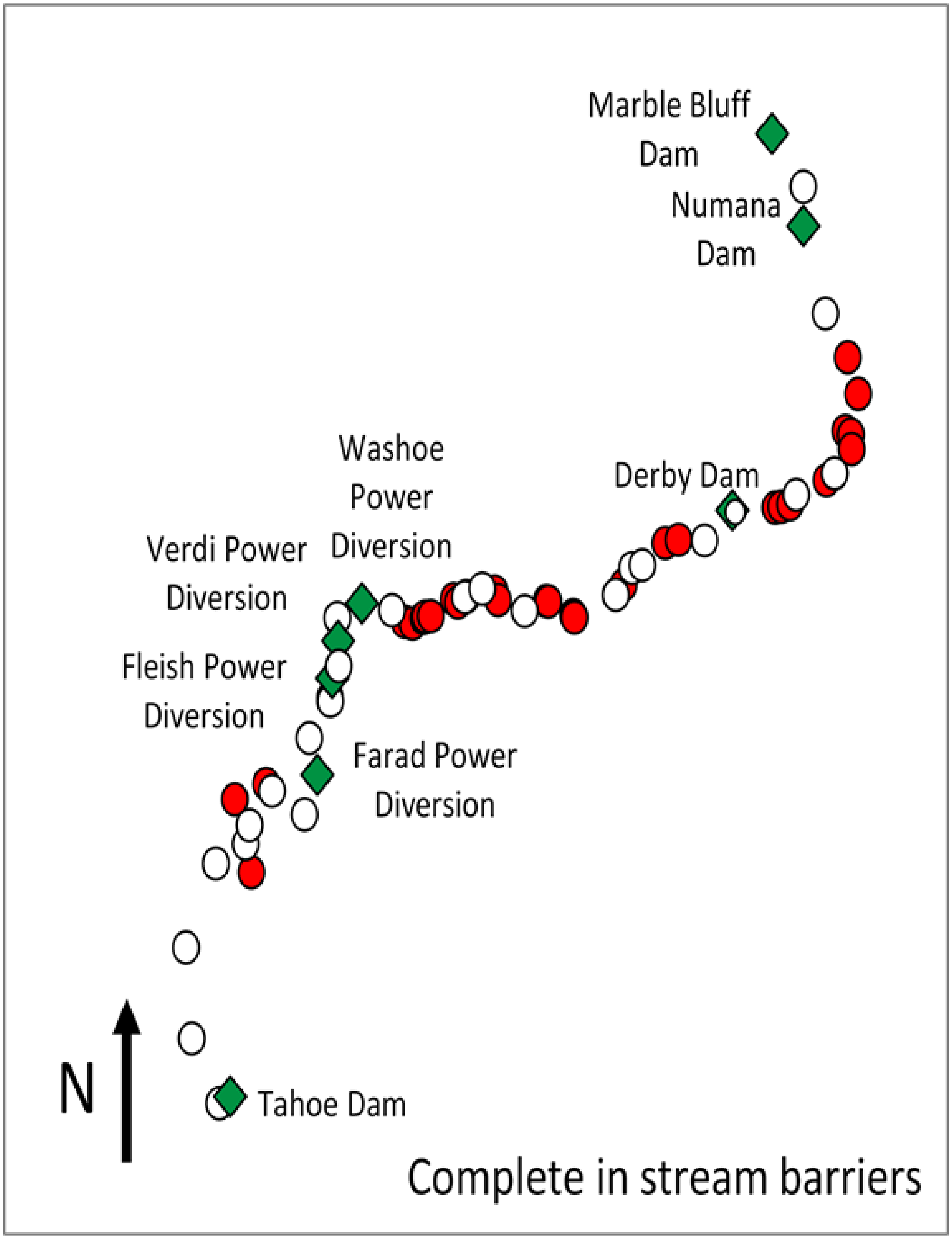
Marble Bluff
Dam
Numana
Dam
Washoe
Power
Diversion
Derby Dam
Verdi Power
Diversion
Fleish Power
Diversion
Farad Power
Diversion
N
Tahoe Dam
Complete in stream barriers

Figure 4.

LITERATURE CITED

Adams, A. L., Y. van Heezik, K. J. M. Dickinson, and B. C. Robertson. 2014. Identifying eradication units in an invasive mammalian pest species. *Biological Invasions,* **16**:1481-1496.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution,* **16**:613-622.

Allendorf, F. W., and S. R. Phelps. 1980. Loss of genetic-variation in a hatchery stock of cutthroat trout. *Transactions of the American Fisheries Society,* **109**:537-543.

Baxter, C. V., K. D. Fausch, M. Murakami, and P. L. Chapman. 2004. Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology,* **85**:2656-2663.

Behnke, R. J. 1992. Native trout of western North America. *American Fisheries Society Monograph,* **6**:i-xx, 1-275.

Bennett, S. N., and J. L. Kershner. 2009. Levels of introgression in westslope cutthroat trout populations nine years after changes to rainbow trout stocking programs in southeastern British Columbia. *North American Journal of Fisheries Management,* **29**:1271-1282.

Blanchet, S. 2012. The use of molecular tools in invasion biology: an emphasis on freshwater ecosystems. *Fisheries Management and Ecology,* **19**:120-132.

Campbell, M. R., J. Dillon, and M. S. Powell. 2002. Hybridization and introgression in a managed, native population of yellowstone cutthroat trout: Genetic detection and management implications. *Transactions of the American Fisheries Society,* **131**:364-375.

Carlisle, D. M., and C. P. Hawkins. 1998. Relationships between invertebrate assemblage structure, 2 trout species, and habitat structure in Utah mountain lakes. *Journal of the North American Benthological Society,* **17**:286-300.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. *Nature,* **405**:234-242.

Clapcott, J. E., E. O. Goodwin, and J. S. Harding. 2016. Identifying catchment-scale predictors of coal mining impacts on New Zealand stream communities. *Environmental Management,* **57**:711-721.

Cochran-Biederman, J. L., K. E. Wyman, W. E. French, and G. L. Loppnow. 2015.

Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology*, **29**:175-186.

Coffin, P.D., and W.F. Cowan. 1995. Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) Recovery Plan. U.S. Fish and Wildlife Service, Region I, Portland, Oregon.

Dodd, C. K., and R. A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles - are they conservation strategies that work. *Herpetologica*, **47**:336-350.

Doupe, R. G., and D. W. Burrows. 2008. Thirty years later, should we be more concerned for the ongoing invasion of Mozambique tilapia in Australia? *Pacific Conservation Biology*, **14**:235-238.

Estoup, A., and T. Guillemaud. 2010. Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology*, **19**:4113-4130.

Finnoff, D., A. Potapov, and M. Lewis. 2007. Eradication and the optimal management of a spreading invader. *Ecological Society of America Annual Meeting Abstracts*.

Fischer, J., and D. B. Lindenmayer. 2007. Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography*, **16**:265-280.

Fragoso-Moura, E. N., L. T. Oporto, P. M. Maia-Barbosa, and F. A. R. Barbosa. 2016. Loss of biodiversity in a conservation unit of the Brazilian Atlantic Forest: the effect of introducing non-native fish species. *Brazilian Journal of Biology*, **76**:18-27.

Frankham, R. 2005. Conservation biology - Ecosystem recovery enhanced by genotypic diversity. *Heredity*, **95**:183-183.

Frankham, R., C. J. A. Bradshaw, and B. W. Brook. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*, **170**:56-63.

Gozlan, R. E., J. R. Britton, I. Cowx, and G. H. Copp. 2010. Current knowledge on non-native freshwater fish introductions. *Journal of Fish Biology*, **76**:751-786.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool - status and strategy. *Science*, **245**:477-480.

Harig, A. L., K. D. Fausch, and M. K. Young. 2000. Factors influencing success of greenback cutthroat trout translocations. *North American Journal of Fisheries Management*, **20**:994-1004.

Hernandez, D. L., D. M. Vallano, E. S. Zavaleta, Z. Tzankova, J. R. Pasari, S. Weiss, P.

C. Selmants, and C. Morozumi. 2016. Nitrogen pollution is linked to US listed species declines. *Bioscience*, **66**:213-222.

Hitt, N. P., C. A. Frissell, C. C. Muhlfeld, and F. W. Allendorf. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences*, **60**:1440-1451.

Idrisi, N., E. L. Mills, L. G. Rudstam, and D. J. Stewart. 2001. Impact of zebra mussels (*Dreissena polymorpha*) on the pelagic lower trophic levels of Oneida Lake, New York. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**:1430-1441.

Kozfkay, C. C., M. R. Campbell, K. A. Meyer, and D. J. Schill. 2011. Influences of Habitat and Hybridization on the genetic structure of redband trout in the Upper Snake River Basin, Idaho. *Transactions of the American Fisheries Society*, **140**:282-295.

Leary, R. F., F. W. Allendorf, S. R. Phelps, and K. L. Knudsen. 1987. Genetic-divergence and identification of 7 cutthroat trout subspecies and rainbow-trout. *Transactions of the American Fisheries Society*, **116**:580-587.

Loxterman, J. L., E. R. Keeley, and Z. M. Njoroge. 2014. Evaluating the influence of stocking history and barriers to movement on the spatial extent of hybridization between westslope cutthroat trout and rainbow trout. *Canadian Journal of Fisheries and Aquatic Sciences*, **71**:1050-1058.

Marsden, J. E., and M. A. Chotkowski. 2001. Lake trout spawning on artificial reefs and the effect of zebra mussels: Fatal attraction? *Journal of Great Lakes Research*, **27**:33-43.

Martin, C. W., M. M. Valentine, and J. F. Valentine. 2010. Competitive Interactions between invasive Nile Tilapia and native fish: The potential for altered trophic exchange and modification of food webs. *Plos One*, **5**: 1-9.

McCallum, M. L. 2015. Vertebrate biodiversity losses point to a sixth mass extinction. *Biodiversity and Conservation*, **24**:2497-2519.

Metcalf, J. L., M. R. Siegle, and A. P. Martin. 2008. Hybridization dynamics between Colorado's native cutthroat trout and introduced rainbow trout. *Journal of Heredity*, **99**:149-156.

Miller, R., and M. Santora. 2013. Upper Truckee River Lahontan cutthroat trout restoration project, 2013 *Annual Report. USDA Forest Service, Lake Tahoe Basin Management Unit.* BO#: 2008-F-0434-R001. 1-26.

Muhlfeld, C. C., S. T. Kalinowski, T. E. McMahon, M. L. Taper, S. Painter, R. F. Leary, and F. W. Allendorf. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. *Biology Letters,* **5**:328-331.

Neville, H. M., and J. B. Dunham. 2011. Patterns of hybridization of nonnative cutthroat trout and hatchery rainbow trout with native Redband Trout in the Boise River, Idaho. *North American Journal of Fisheries Management*, **31**:1163-1176.

Ostberg, C. O., and D. M. Chase. 2012. Temporal genetic monitoring of hybridization between native Westslope Cutthroat Trout and introduced rainbow trout in the Stehekin River, Washington. Northwest. *Science,* **86**:198-211.

Ostberg, C. O., and R. J. Rodriguez. 2004. Bi-parentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. *Molecular Ecology Notes*, **4**:26-29.

Ostberg, C. O., and R. J. Rodriguez. 2006. Hybridization and cytonuclear associations among native westslope cutthroat trout, introduced rainbow trout, and their hybrids within the Stehekin River drainage, North Cascades National Park. *Transactions of the American Fisheries Society*, **135**:924-942.

Ovenden, J. R., G. M. Macbeth, L. Pope, P. Thuesen, R. Street, and D. Broderick. 2015. Translocation between freshwater catchments has facilitated the spread of tilapia in eastern Australia. *Biological Invasions,* **17**:637-650.

Page, K. S., M. T. Negus, M. C. Ward, and T. L. Close. 2011. Simulating Effects of Nonintrogressive hybridization with a stocked hatchery strain of rainbow trout on the sustainability and recovery of naturalized steelhead populations in Minnesota Waters of Lake Superior. *North American Journal of Fisheries Management*, **31**:1065-1076.

Peacock, M. M., M. Gustin, V. Kirchoff, M. L. Robinson, E. Hekkala, C. Pizzarro-Barraza and T. Loux. *In press 2016*. Native non-game fishes in the Truckee River: are barriers and patterns of population genetic structure related? *Science of the Total Environment, STOTEN-D-15-05108*

Peterson, D. P., K. D. Fausch, and G. C. White. 2004. Population ecology of an invasion: Effects of brook trout on native cutthroat trout. *Ecological Applications*, **14**:754-772.

Pimm, S. L., C. N. Jenkins, R. Abell, T. M. Brooks, J. L. Gittleman, L. N. Joppa, P. H. Raven, C. M. Roberts, and J. O. Sexton. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science,* **344**:987

Pritchard, J. K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics,* **155**:945–959.

Pritchard, V. L., J. C. Garza, and M. M. Peacock. 2015. SNPs reveal previously undocumented non-native introgression within threatened trout populations. *Conservation Genetics,* **16**:1001-1006.

Reissig, M., C. Trochine, C. Queimalinos, E. Balseiro, and B. Modenutti. 2006. Impact of fish introduction on planktonic food webs in lakes of the Patagonian Plateau. *Biological Conservation,* **132**:437-447.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics,* **27**:83-109.

Scoppettone, G. G., P. H. Rissler, S. P. Shea, and W. Somer. 2012. Effect of brook trout removal from a spawning stream on an adfluvial population of Lahontan Cutthroat Trout. *North American Journal of Fisheries Management,* **32**:586-596.

Seddon, P. J., C. J. Griffiths, P. S. Soorae, and D. P. Armstrong. 2014. Reversing defaunation: Restoring species in a changing world. *Science,* **345**:406-412.

Sedinger, J. S., E. J. Blomberg, A. W. VanDellen, and S. Byers. 2012. Environmental and population strain effects on survival of Lahontan Cutthroat Trout in Walker Lake, Nevada: A Bayesian Approach. *North American Journal of Fisheries Management,* **32**:515-522.

Sharma, R., and S. W. Raborn. 2011. Estimating critical abundance thresholds in exploited populations: a simulation approach based on species resilience to disturbance. *Computational Ecology and Software,* **1**:189-207.

Shelton, J. M., M. J. Samways, and J. A. Day. 2015. Predatory impact of non-native rainbow trout on endemic fish populations in headwater streams in the Cape Floristic Region of South Africa. *Biological Invasions,* **17**:365-379.

Stankovic, D., A. J. Crivelli, and A. Snoj. 2015. Rainbow Trout in Europe: Introduction, Naturalization, and Impacts. *Reviews in Fisheries Science & Aquaculture,* **23**:39-71.

Thibault, I., and J. Dodson. 2013. Impacts of exotic rainbow trout on habitat use by native juvenile salmonid species at an early invasive stage. *Transactions of the American Fisheries Society,* **142**:1141-1150.

van Herwerden, L., J. H. Choat, C. L. Dudgeon, G. Carlos, S. J. Newman, A. Frisch, and M. van Oppen. 2006. Contrasting patterns of genetic structure in two species of the coral trout Plectropomus (Serranidae) from east and west Australia: Introgressive hybridisation or ancestral polymorphisms. *Molecular Phylogenetics and Evolution,* **41**:420-435.

Vitousek, P. M., C. M. Dantonio, L. L. Loope, M. Rejmanek, and R. Westbrooks. 1997. Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology,* **21**:1-16.

Weber, E. D., and K. D. Fausch. 2003. Interactions between hatchery and wild salmonids in streams: differences in biology and evidence for competition. *Canadian Journal of Fisheries and Aquatic Sciences* **60**:1018-1036.

Weeks, A. R., C. M. Sgro, A. G. Young, R. Frankham, N. J. Mitchell, K. A. Miller, M. Byrne, D. J. Coates, M. D. B. Eldridge, P. Sunnucks, M. F. Breed, E. A. James, and A. A. Hoffmann. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications,* **4**:709-725.

Williams, J. E., and C. E. Bond. 1983. Status and life-history notes on the native fishes of the Alvord basin, Oregon and Nevada. *Great Basin Naturalist*, **43**:409-420.

Wright, Sewall. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution,* **19:**395-420.

Young, M. K. 1995. Colorado River cutthroat trout. U S Forest Service General Technical Report RM **256**:16-23.

CHAPTER 2:

Hybridization dynamics of Lahontan Cutthroat Trout (*Oncorhynchus clarkii henshawi*) and Rainbow Trout (*Oncorhynchus mykiss*) and implications on cutthroat trout reintroduction to their native habitat

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ABSTRACT

Historically the Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*, LCT) occurred throughout the Truckee River Basin, supporting important commercial fisheries and providing a local food source for the Paiute Indians. Because of water diversion, overfishing, pollution and introduction of non-native salmonids, LCT was extirpated from Lake Tahoe and the Truckee River by 1939, leaving only remnant natural populations in the Tahoe Basin and a hatchery-stocked LCT fishery in Pyramid Lake. To provide angling opportunities, Rainbow trout (*Oncorhynchus mykiss,* RBT) has been planted in the river, and there is a robust naturalized population throughout much of the Truckee River. LCT is listed under the United States Endangered Species Act as threatened, and recovery efforts by the United States Fish and Wildlife Service (USFWS) are underway to reintroduce LCT back into their native habitat in the Truckee River. However, planting LCT sympatric with naturalized RBT can support hybridization between the species and hamper LCT recovery. Since 2004, in an effort to limit hybridization, primarily non-reproductive triploid RBT have been stocked by the Nevada Department of Wildlife (NDOW). I evaluated over 3500 Truckee River salmonid samples collected between 2007 and 2010 using 6 bi-parentally inherited nuclear genetic markers to differentiate among RBT, LCT and their hybrids. The mitochondrial ND2 locus was sequenced in 96 of the hybrids detected in the samples to determine maternal contribution. The hybrid markers show an increase in hybridization and introgression over time due to the consistent stocking of LCT in regions where

naturalized RBT populations exist. Mitochondrial sequences show that LCT haplotype accounted for 14% of F_1 hybrids despite the fact that LCT only make up less than 5% of the salmonids found in the system. Most of the hybridization is localized to the two tributaries that were the focus of reintroduction efforts in 2005 and 2006, and LCT alleles decreased over the 4 year period. These data indicate that hatchery reared LCT can be reintroduced into their native habitat and have the potential to become sustaining; however, they will readily hybridize with the naturalized RBT population. Further efforts are needed to identify regions of the system that can be isolated from the RBT, and RBT need to be removed from that region to allow for the recovery of LCT.

INTRODUCTION

Trout species of the genus *Onchorynchus* have radiated throughout the western United States and resulted in a number of closely related species of rainbow trout (*O. mykiss*) and cutthroat trout (*O. clarkii*) that were thought to have diverged from a common ancestor approximately 2 million years ago (Behnke, 1992). Because of geographic and reproductive barriers, multiple subspecies of these trout have evolved, displaying both morphological and genetic evidence supporting differentiation (Leary et al., 1987; Behnke, 1992). Currently there are 14 recognized subspecies of cutthroat trout. Three cutthroat trout subspecies have been listed as endangered or threatened under the United States Endangered Species Act (Lahontan cutthroat trout *O. c. henshawi*, Paiute cutthroat trout *O. c. seleniris,* and Greenback cutthroat *O. c. stomias*), and two subspecies are considered to be extinct (Yellowfin cutthroat trout *O. c macdonaldi* and Alvord cutthroat trout *O. c. alvorensis*)(Behnke, 1992). These cutthroat trout sub-species evolved in allopatry and lack biological reproductive isolation mechanisms allowing for hybridization among sub-species (intraspecific hybridization) or between cutthroat and RBT (interspecific hybridization), if in the same watershed.

Hybridization is common in many fish species because they have external fertilization, and similarities in mating behavior of different fish species can allow for interbreeding (Allendorf et al., 2001). In undisturbed ecosystems, if two fish species evolved sympatrically, reproductive isolation can be maintained through spatial, temporal and behavioral isolation (Higgins et al., 2015). Westslope cutthroat trout and Yellowstone cutthroat trout are naturally sympatric, and there is limited hybridization, usually limited to first generation (F_1) with little backcrossing with parental genotypes (Leary et al., 1995). However, interspecific hybridization with introduced RBT is widespread throughout the westslope cutthroat range (Allendorf and Leary, 1988; Leary et al., 1995; Allendorf et al., 2001; Bennett and Kershner, 2009). Hybridization is one of the most devastating threats to cutthroat trout conservation and has contributed to the extinction of the Alvord cutthroat trout (Bartley and Gall, 1991) as well as a number of localized cutthroat trout populations (Kruse et al., 2000; Allendorf et al., 2001; Peacock and Kirchoff, 2004, Metcalf et al., 2008). RBT/cutthroat hybrids are fertile and viable, and can interbreed with parental types, resulting in introgression. Introgression can progress to the point where no individuals in a population contain the pure parental genotypes, and the population is considered to be a hybrid swarm (Leary et al., 1995). Geographic isolation can be disturbed through intentional introduction of a non-native fish for angling (as is common with RBT), through expansion of a non-nonnative range because of climate change, or through anthropogenic alteration of the landscape, removing natural barriers potentially resulting in invasive hybridization and leading to genetic extinction of a population or a species (Rhymer and Simberloff, 1996; Gilk et al., 2004; Muhlfeld et al., 2014).

Introgressive hybridization can spread rapidly in region where RBT are introduced into native trout habitats, suggesting hybrid vigor, where the hybrid exhibits superiority over its parental strains in growth rate or reproductive success

(Lippman and Zamir, 2006; Muhlfeld et al., 2009). However, reproductive success declines rapidly with increased introgression. Muhlfeld et al. (2009) looked at reproductive success in westslope cutthroat trout hybrids backcrossed with RBT. F_1 hybrids showed high fitness and readily contributed to introgression; however very few post-F_1 males displayed comparable high levels of reproduction. It is suggested that reduced fitness occurs in hybrids due to outbreeding depression, disrupting co-adapted gene complexes that would become more evident in backcrossed individuals, as they would no longer have one complete genome of the parental type (Gilk et al., 2004; Muhlfeld et al., 2009). Research attempting to determine the precise life history trait(s) in westslope cutthroat trout which contributed to the decline in reproductive fitness (looking at embryonic survival, egg diameter, egg energy, sperm motility, juvenile growth, juvenile survival and swimming endurance) were unable to identify any one characteristic that can directly explain the reduced fitness found with introgressive hybridization (Drinan et al., 2015). Work with other cutthroat trout species show that the effects of hybridization differ when different populations and species hybridize (Pritchard et al., 2011). From a conservation standpoint, anthropogenic alterations leading to hybridization threaten many different species, sub-species and individual populations (Allendorf et al., 2001; Peacock and Kirchoff, 2004). In addition, genetically distinct groups of populations within the range of a particular species have evolved unique traits that contribute to the full range of diversity within a species (Carlsson et al., 1999; Peacock and Kirchoff, 2004). Therefore, genetic evaluation of the at-risk populations are needed to look at levels of hybridization and genetic variation within populations on a case by case basis to determine if individual populations warrant conservation. In many cases, genetically pure populations can by separated from hybridized populations through the construction of barriers to prevent invasive hybridization (Peacock and Kirchoff, 2004), though the long-term efficacy of these barriers is questionable, and continued monitoring of the protected population is necessary (Avenetti et al., 2006).

The identification of hybrids in the field relies on morphological characteristics and assumes that hybrid individuals show a phenotypic intermediate to the parental species being identified. Morphological identification is notoriously imprecise, and does not allow for the determination of whether an individual is an F_1 individual or a back cross individual (Campton, 1987), nor does it inform on the direction of hybridization, whether the hybrid resulted from a spawning of a RBT female or a cutthroat female. In the mid-1980s, allozyme-based analysis was used to assess genetic divergence among hybrids (Leary et al., 1987; Sanz et al., 2009). Allozymes are variant forms of an enzyme that are encoded by different alleles for a locus. To assess allozymes, specimens are sacrificed and proteins are isolated from muscle, liver or eye tissue and separated through horizontal starch gel electrophoresis (Utter et al., 1974). To evaluate hybridization, samples from known genetically pure populations are compared to the individuals from the hybrid population in question. Hybrids can be detected by identifying individuals that express an intermediate between the parental allozyme fingerprints; however, allozymes are difficult to quantitate and require sacrificing the animal in question (Leary et al., 1997). With the discovery of polymerase chain reaction (PCR), genetic techniques looking at either nuclear or mitochondrial DNA can provide meaningful information without the lethal sampling required for allozyme analysis. In protected species, small fin clips can be removed, and the DNA can be isolated and analyzed using various molecular markers to reliably identify F_1 and introgressive hybrids. Non-lethal sampling followed by genetic analysis can provide valuable information to determine if a population can potentially be recovered through selective removal of invasive species and/or hybrids, without further damage to an at risk population through destructive sampling. Many species-specific genetic markers have identified that differentially amplified through PCR in either RBT or cutthroat trout, amplifying a single product in one species only (Ostberg and Rodriguez, 2002). While this was allowed for the genetic identification of the sample in question, a failed PCR could cause false identification of a "pure" individual. Bi-parentally inherited species-specific markers were developed that amplified a unique allele in RBT and cutthroat trout (Ostberg and Rodrigues, 2004). These eight markers allowed for the simultaneous amplification of an allele in RBT and a different allele in cutthroat trout with a single PCR primer set and cross-amplified in multiple cutthroat trout sub-species (westslope, coastal, Lahontan, and Yellowstone). A pure cutthroat trout would display eight cutthroat alleles, a pure rainbow trout would display eight rainbow alleles, and a first generation hybrid would display both sets of markers. Introgression could be detected when the individual displayed an unequal number of cutthroat and RBT alleles and would either favor RBT (#RBT>#cutthroat) or the cutthroat trout in question (#cutthroat >#RBT). The use of these markers greatly simplifies description of hybridized population and allows for more accurate evaluation of the hybrid index of an individual or a population. The hybrid index describes the sum of the species-specific nuclear DNA contained in an individual, but can also consider mitochondrial DNA (Ostberg and Rodriguez, 2006; Ostberg and Chase, 2012). Mitochondrial DNA is passed down the maternal bloodline; therefore, maternal contribution to the hybrid can be determined to establish if the species of interest is able to compete for spawning sites (Ostberg and Rodriguez, 2006; van Herwerden et al., 2006). PCR amplicons can be differentiated through restriction enzyme digests or sequencing compared to known RBT and cutthroat trout. Historically, the Lahontan cutthroat trout native to the hydrographic Lahontan basin of eastern California, southeastern Oregon and northern Nevada, was the apex predator in the Truckee River watershed. However, habitat degradation, water management, overfishing, and the introduction of non-native salmonid species in the 19th and 20th centuries led to the extirpation of LCT populations from throughout much of the historical LCT range (Peacock and Kirchoff, 2004) including the Truckee River watershed. In 1970, LCT was listed as endangered, and was reclassified as threatened in 1975 to allow for regulated angling and facilitate management, including decisions on where to attempt reintroduction. Since 1993, LCT has been planted into regions of the Truckee River, primarily for recreational fishing purposes, as have both diploid and triploid RBT

(NDOW and USFWS public stocking records); however, there is a robust naturalized RBT population throughout the Truckee River, and although there has been much anecdotal evidence for the appearance of cutbows (LCT/RBT hybrids), the hybridization dynamics of LCT and RBT in this system have not been evaluated. Recent stocking activities were found to increase the incidence of both F_1 hybridization and introgression between westslope cutthroat trout and RBT in the Salmon River watershed in central Idaho (Loxterman et al., 1914). The planting of LCT for recovery or for recreational fishing activities will likely lead to hybridization as there is no spatial segregation of LCT from naturalized RBT in the Truckee River.

A common conservation strategy for threatened and endangered species is translocation into regions previously uninhabited or regions where the native fish has been extirpated (Griffith et al. 1989; Harig and Fausch, 2002). This strategy was successful in rebuilding the Pyramid Lake Fishery, re-introducing the native LCT. This trout now occupies less than 10% of its historic range, and is listed as "Threatened" under the U. S. Endangered Species Act (Hickman and Behnke, 1979; Coffin and Cowen, 1995; Pritchard et al., 2015). The Pyramid Lake fishery, once known for being highly productive and for producing a strain of LCT that grew to over 9 kg (20 lbs), declined rapidly in the 1930s due to overfishing, pollution and most importantly, large water diversions at Derby Dam on the Truckee River (Sumner, 1940). The surface elevation of the lake dropped more than 50 feet in a 30 year period, and the salinity, alkalinity, and total dissolved solids in the lake increased (Coleman, 1988). The Pyramid Lake strain of LCT was thought to be extinct, and attempts were made to rebuild sport fishing in the lake by stocking other trout species, as well as LCT from the Summit Lake and Walker Lake strains (Coleman, 1988). Due to limited access to spawning habitat and sub-optimal conditions in the lower Truckee River, hatchery propagated trout were regularly planted into the lake. LCT showed the highest survival in the alkaline conditions of Pyramid Lake and is currently the only trout stocked into Pyramid Lake. The contemporary Pyramid Lake strain is made up of primarily Summit Lake ancestry (Coleman, 1988; Peacock and Kirchoff, 2007) and although the rebuilt fishery is now thriving, the fish never regained the size and productivity found in the historical Pyramid lake LCT.

The Pilot Peak strain of LCT, discovered in 1979 in the Bonneville hydrographic basin, where they had been transplanted in the early 1900s, and now raised at the Lahontan National Fish Hatchery (LNFH), are morphologically (Hickman and Behnke, 1979) and genetically (Peacock and Kirchoff, 2007) shown to be descendants of the historical LCT from the Truckee River watershed. In 2006, this strain was also stocked into Pyramid Lake and monitored. The Pilot Peak strain has achieved larger size than found with the contemporary Pyramid Lake strain, reaching over 9 kg (20 lbs)(Schweber, 2013), and, for the first time since 1938, these LCT spawned up the Truckee River in 2014 (DeLong, 1914; Tim Loux, USFWS personal communication). This is a triumph for the successful reintroduction of LCT into part of its historical habitat, and could create a self-sustaining population in Pyramid Lake. This revitalized the interest in trying to expand LCT reintroduction into the Truckee River. However, one of the biggest threats to this LCT recovery is the potential for hybridization with RBT. Expansion of the LCT into the lower Truckee River, coupled with restoration projects in the lower Truckee River that improve conditions and allow for extension of the LCT into regions currently occupied by naturalized RBT, could lead to a hybrid swarm, hampering recovery. Current stocking in the Truckee River includes non-reproductive triploid RBT. Triploids are stocked as "catchables" for a put-and-take fishery. They are intended to provide fishing opportunities without contributing to the problems with stocking non-natives such as introgressive hybridization or creation of a self-sustaining naturalized non-native population (Wong and Van Eenennaam; Budy et al., 2012). It is hypothesized that stocking triploids may provide reproductive containment of the RBT by interfering with spawning behaviors (Koenig et al., 2011; Benfey, 1999). However, currently there is a large self-sustaining RBT population found in the river and investigations that have compared the physiology and behavior of diploid and triploid fishes were done with hatchery raised fish (Kim et al., 1995; Nam et al., 2004; Koenig et al., 2011; Budy et al., 2012). The impacts that stocking triploid fishes have on the behavior of a stable diploid population have not been thoroughly investigated. In order to allow for the spawning of LCT into the Truckee River from Pyramid Lake, it will be necessary to evaluate the naturalized RBT population and reproduction in the lower regions of the river, as well as to fully understand the effects that the stocked triploid RBT have on both LCT and RBT populations.

Continued isolation of the LCT population and monitoring of barriers is needed to evaluate the long-term success of LCT spawning in the lower Truckee River.

Conservation efforts for LCT include evaluating the re-introduction potential of this species into its native habitat (Coffin and Cowen, 1995). Both NDOW and USFWS have stocked LCT into regions of the Truckee River in various size classes in hopes that they can sustain in a portion of the river. However, hybridization with RBT is a continuous threat to LCT recovery. Here I investigated the hybridization dynamics of the LCT stocked sympatrically with the naturalized RBT population in the Truckee River. I used bi-parentally inherited nuclear molecular markers to identify salmonids sampled as pure LCT, pure RBT or hybrids (HYB) and estimated the frequency of RBT/LCT hybridization and the survival of LCT and HYB in this system. I calculated hybrid index scores to evaluate level of introgression in the regions that show hybridization. Finally, I used mtDNA analysis to determine the maternal contribution to the hybridization crosses and evaluated interspecies hybridization in the context of stocking history of reproductive RBT and LCT into this system. The stocking of triploid RBT will likely show little impact on hybridization with LCT. If the stocked LCT survive to reproductive age, they will readily hybridize with the naturalized RBT present in the system. Mitochondrial DNA analysis will likely show that both LCT and RBT can compete for spawning habitat and hybrids will have maternal haplotypes from both species. Given the increase in stocking of LCT fry in 2005 and 2006, those fish will become reproductive and will contribute to first generation (F_1) hybrids in 2008 and 2009, with back crossing and introgression favoring the dominant salmonid in the system, the RBT.

METHODS

Study Site

The Truckee River flows ~195 kilometers from Lake Tahoe in California (Elevation 1900m), to Pyramid Lake in Nevada (elevation 1157m) (Figure 1). It is the second largest river in Nevada, and the only outflow from Lake Tahoe. Over the last century, the Truckee River riparian habitats have been significantly altered, and flows are highly regulated to allocate water resources and control flooding (Chen et al., 2015). The Truckee River is characterized by fluctuating water flow, recurring drought causing water deficiencies for its water rights owners. This coupled with water management decisions and dams and barriers that fragmented the landscape and limited spawning habitat has left two of the rivers native fish species threatened (Cobourn, 1999). There are 41 irrigation ditch diversions and dams along the 195 km Truckee River (Figure 2, Table 1), which have been shown to have limited impact of movement of native fishes in the watershed (Peacock et al. 2016). Over the past several decades, the watershed has been stocked with LCT from the Independence Lake strain, the contemporary Pyramid Lake strain (mixed stock ancestry), and the Pilot Peak strain, as well as both diploid and triploid RBT. The Hunter Creek tributary flows into the Truckee River between the VerdiC and RenoA transects (Figure 2). Approximately 1.6 km of stream are accessible before it hits a diversion ditch that acts as a fish barrier. Dog Valley Creek enters the Truckee River near the VerdiC transect. Dog Valley has been a site for stocking of RBT and LCT over the past decade, whereas Hunter Creek has no record of being stocked with RBT. Between 2005 and 2007 large numbers of LCT fry were stocked in both of these tributaries. Both tributaries have suitable habitat for spawning for both LCT and RBT, but also have self-sustaining brook trout and RBT populations reported above the stocking locations use by the California and Nevada fish and game agencies. Dog Valley Creek has more rearing habitat for young fish, whereas Hunter Creek has a larger number of sizable drop pools that can provide good habitat for adult fish. In the summer months, even with low water flow, the water temperature in these streams was 15-16°C (Maples et al. 2007), which is well below the upper thermal tolerance range of 26°C found for LCT (Robinson et al. 2008). Hunter Creek has a large barrier that can limit migration; however, the upper regions of Hunter Creek are not very accessible and stocking takes place below this barrier.

Sample collection

Salmonid samples were collected by the United States Fish and Wildlife Service (USFWS) and Nevada Division of Wildlife (NDOW) from 2007 through 2010. USFWS collected samples from 8 primary sampling sections (n=1649). Each sampling section had 3 to 4 transects depending on the variation in habitat types and barriers within each site (Figure 2, Table 1). Transects were 500 m long and included both shore line and mid-stream regions. Raft, barge and backpack electrofishing methodologies were utilized to ensure all age classes were adequately sampled. Fin clips were placed in wax paper, dried, and placed in individual coin envelopes labeled with species (when possible), fork length, date collected and location. NDOW collected samples from five sites on the Truckee River main stem while doing their annual surveys. RBT samples collected by NDOW were primarily found in the Verdi to Reno transects (n=1820). In addition, NDOW collected samples from Hunter and Dog Valley creeks in 2007 (n=220) and 2008 (n=230). NDOWs tributary transects were below the diversion ditch barrier in Hunter Creek and below the California State Line in Dog Valley Creek, where fish had access to the main stem of the Truckee River. In 2009, the tributaries were sampled by USFWS; for this sampling period, samples were collected from two transects above the barrier in Hunter Creek (n=44) and above the California State Line in Dog Valley Creek (n=44), in addition to transects overlapping the NDOW collection sites (n=44).

Stocking Analysis

Official stocking records of diploid RBT, triploid RBT (trpRBT) and LCT were obtained from USFWS and NDOW for the time periods from 1990-2010. In addition to total numbers of fishes stocked, averages were calculated for each salmonid species during different time periods to determine if stocking events impacted the hybridization dynamics in the system. I also identified the LCT and RBT strains involved in hybridization to evaluate any differences among strains in the propensity to hybridize. Average fork length (mm) data of the fish stocked was used to compare to the size of the fish stocked to the incidence of hybridization.

Size class structure of salmonid samples

To determine if the planted LCT were surviving to adulthood, size class data was used to estimate the life stage of each salmonid type identified (RBT, LCT, and HYB). Young-of-the-year (yoy) (between 25-65 mm) and age 1 fish (80-100 mm) are not reproductive and often do not survive to the next size class (Novak et al. 2005). I looked at the overall size class structure of all of the salmonids sampled in 10 mm increments (Figure 6) and then grouped the RBT, LCT and HYB individually into 4 categories with 100 mm increments to look at overall patterns of growth and reproduction over the four year sampling period (Figure 7).

DNA isolation and genetic identification of hybrids

DNA was isolated using DNeasy96 Blood and Tissue Kits (QIAGEN) according to the manufacturer's protocol. Double stranded DNA was quantified at the University of Nevada, Nevada Genomics Center using a fluorescent nucleic acid stain (PicoGreen®) and read on a Labsystems Fluoroskan Ascent fluorescence plate reader, which measures only the double stranded DNA present. Samples were amplified with six diagnostic co-dominant nuclear markers that differentially amplify in LCT and RBT (Ostberg and Rodriques, 2004). Primer sequences and the specific allele expected in LCT and RBT are listed in Table 2. Primers for nuclear markers were purchased from Applied Biostystems, ThermoFisher Scientific (Grand Island, NY) with one of four fluorescent dyes (NED, VIC, FAM, and PET). The six primers can be amplified in a single multiplex polymerase chain reaction (PCR) with a final primer concentration of 0.05 μM each tailed forward primer, 0.1 μM each reverse primer, and in a 12 μl reaction. Qiagen Multiplex PCR Kit was used which contains HotStarTaq DNA Polymerase, dNTPs, and PCR buffer in a 2X concentration. PCR included 6 μl Multiplex Mix and 20-50 ng of DNA. PCR cycle parameters included a 15 minute hot start at 95°C, followed by 41 cycles of 95°C for 30 seconds, a touch down annealing temperature of 65°C to 55°C for 90 seconds, and elongation of 72°C for 30 seconds. The touch down annealing temperature starts with 7 cycles at 65°C, 7 cycles at 61°C, 7 cycles at 58°C and 20 cycles at 55°C. PCRs were completed using 96-well format, and an MBS Satellite 0.2G Thermal Cycler (Thermo Electron Corporation). PCR products were diluted to an appropriate concentration determined by dilution tests from 1:100 to 1:300. One μl of diluted PCR product was added to 19 μl of HiDye Formamide/LIZ500 size standard (Applied Biosystems) prepared by adding 5 μl size standard to 1 ml of Hi-Dye formamide(Applied Biosystems). Fragment analysis was carried out on a Applied Biosystems 3730 Genetic Analyzer at the Nevada Genomics Center (http://www.ag.unr.edu/genomics), and all alleles generated were scored, binned, and given allelic and genotypic designation using the ABI GeneMapper software (version 3.7).

Mitochondrial DNA sequencing

A 450 bp amplicon that has been used to distinguish a variety of *Oncorhynchus* species was amplified from the mitochondrial gene region NADH dehydrogenase 2 (ND2) (Novak, 2005; Campbell et al. 2011). PCR reactions were completed using a final concentration of 0.2 μM of both forward and reverse primers (Table 3), using 20 ng of template (dried down) in a 15 μl reaction. PCR included 7.5 μl Multiplex taq (1X final concentration) (QIAGEN) and 20-50 ng of DNA. PCR cycle parameters included a 15 minute hot start at 95°C, followed by 35 cycles of 95°C for 30 seconds, an annealing temperature of 65° for 90 seconds, and elongation temperature of 72°C for 30 seconds using 96-well format on a MBS Satellite 0.2G Thermal Cycler (Thermo Electron Corporation). PCR products were purified using the Qiagen MinElute filter plate, quantified and sequenced using the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 and sequenced on an ABI Prism 3730 genetic analyzer at the Nevada Genomics Center (http://www.ag.unr.edu/genomics/) using the reverse primers used for amplification. Reverse sequences with Q>20 were aligned and edited to remove ambiguous base calls using GENEIOUS version 7.1.5(http://www.geneious.com, Kerse et al., 2012).

Genetic analysis

The proportion of RBT and LCT alleles was determined for both nuclear and mitochondrial markers. Pure strain fish were homozygous for all 6 nuclear markers.

First generation hybrids (F_1) were heterozygous for all 6 nuclear markers, whereas introgression was detected if there was an unequal distribution. I used hybrid index scores (HISs) to quantify the levels of hybridization within individuals (Rubidge et al., 2001; Kovach et al., 2011). HISs were calculated by summing the number of RBT alleles and dividing by the total number of successfully amplified alleles for each individuals. Individuals were designated as pure RBT (HIS=1), pure LCT (HIS=0), F_1 (HIS-0.5), backcrossed LCT dominant (HIS between 0 and 0.5) and backcrossed RBT dominant (HIS between 0.5 and 1). HISs were compared over all years for each collection site, and over all sites for each year to identify regions of the river that are hot spots for hybridization, as well as trends or spikes in hybridization by year that could indicate a large number of LCT becoming reproductive.

mtDNA analysis

ND2 was amplified and sequences were analyzed for 104 salmonid samples, including 8 known pure LCT and 8 pure RBT, as well as 64 F_1 hybrids and 24 backcrossed hybrids. The resulting sequences were aligned using GENEIOUS Alignment software and a 65% similarity cost matrix (http://www.geneious.com, (Kerse et al., 2012). Known RBT and LCT samples were aligned with the hybrid samples sequenced in order to assign the hybrid samples a haplotype. Percent identity and number of bases that were not identical were calculated within this alignment (Table5). To investigate the distribution of the variation of the RBT and

LCT haplotypes found on the landscape, the haplotype assignment found at each of the transects is shown for both RBT (Figure 10) and LCT (Figure 11).

RESULTS

Stocking Analysis

The Truckee River supports a large sport-fishing population, and both native and non-native trout have been stocked to supplement the self-sustaining non-native Brown trout (*Salmo trutta*) and RBT populations. Official records of stocking of RBT, triploid RBT and LCT were obtained from USFWS and NDOW for the time periods from 1990-2010. Prior to the 1995 recovery plan (Coffin & Cowan, 1995), LCT were planted only sporadically, and the primary fish stocked in the Truckee River was rainbow trout. Stocking records show that federal and state agencies adjusted stocking of salmonids for angling opportunities as priorities shifted to allow for conservation of LCT. Between 1990 and 1994 a total of 622,494 rainbow trout (124,498 +/- 38,610 per year) were stocked throughout the Truckee River. Starting in 1994, to initiate LCT recovery (Coffin & Cowan, 1995) both the contemporary Pyramid Lake strain and Independence Lake strain LCT were also stocked in the river in large numbers. Between 1994 and 2000, a total of 287,674 (57,524 +/-14, 346 per year) RBT and 391, 778 (78,355 +/- 35,516 per year) LCT were stocked to provide angling opportunities. LCT were stocked sympatric to RBT stocking. As awareness of the potential to create a hybrid swarm in the river increased LCT stocking became much more limited from 2000-2003 and was not sympatric with

RBT stocking. Starting in 2004, federal mandates for LCT recovery in native streams and required that primarily non-reproductive triploid RBT were stocked (Figure 3). During that time period, large numbers of LCT fry were stocked, both sympatric with RBT and in regions that were considered to be more isolated from RBT (Figure 4). Rainbow trout and LCT were both stocked in Dog Valley Creek, and in Verdi and Reno transects in the Truckee River (Figure 4B), whereas only LCT were stocked during this time period in the Tahoe, Truckee, McCarren and Wadsworth transects, as well as the Hunter Creek tributary (Figure 4A). Thirty to fifty-thousand RBT were stocked in "catchable size" (33,930 +/-13,296 per year RBT; average fork length= 244 mm+/- 16). Of that amount, only 8.7% were designated as dipoid or unspecified strain, while the remaining were non-reproductive triploid RBT. LCT were also stocked as "catchable" (40%), but large numbers of fry were stocked (35%) primarily in Dog Valley and Hunter creeks. This was done in hopes of flooding the system with LCT determining if they would be able to survive and reestablish in the tributaries. The average fork length of all LCT stocked over this time period was 135mm +/-101.

Hybrid detection with nuclear markers

All seven markers (six nuclear markers and one mtDNA) were 100% diagnostic for all pure LCT (n=8) and RBT (n=8) trout tested. Among the 8 sections of the river over the 4 year period, a total of 2,931 pure RBT, 124 pure LCT, 65 F_1 hybrids and 73 backcrossed hybrids were identified (Table 3). RBT were found in all 8 sites sampled, with very few found downstream of Derby Dam in the Nixon transects (n=10), though 1 pure LCT was found downstream of Derby Dam 2009(Figure 5). LCT and hybrids were found primarily in the Dog Valley and Verdi sites, and the largest proportion of LCT were found in Dog Valley Creek (n=30) where they were most common in 2007(n=51). There were 14 F_1 hybrids in 2007 found in Reno transects and Hunter Creek. Most of the 2007 hybrids found only in Hunter Creek were backcrossed, showing introgression. In 2008, 42 pure LCT were found, 31 were identified in Dog Valley Creek. Thirty-seven F_1 hybrids were found in 2008, with 22 found in Dog Valley Creek. In 2009, I found a decline in hybridization and pure LCT in the system with 8 F_1 hybrids, only one in Hunter creek and 12 pure LCT, distributed in various sites in the river. The only year during which Dog Valley Creek and Hunter Creek were sampled in regions where NDOW did not stock LCT and RBT was in 2009. Hunter Creek had one F_1 hybrid in the lower region and three backcrossed hybrids with HISs of 0.58 to 0.75, indicating a second generation hybrid. In Lower Dog Valley Creek, one pure LCT was found, and no hybridization. Upper Dog Valley had 5 backcrossed hybrids, showing LCT alleles at only one locus; this indicates a longer term introduction of LCT into this system, and that, perhaps, selection may have maintained that allele in the population. Seven F_1 hybrids were found in the upper Truckee River, in a Tahoe transect. This is surprising because there was very little LCT stocking in that region, and it is unlikely that they were able to spawn. I cannot rule out that unrecorded stocking of hatchery produced hybrids may have occurred. In addition, in 2009 there were no hybrids found in lower Dog Valley Creek in the sites that overlapped with NDOW sampling in 2008 and 2007. However, in upper Dog Valley Creek, only sampled in 2009, there were 5 samples that showed an LCT allele at only one locus. Such a high level of introgression indicates that backcrossing must have occurred for more than two generations; thus, LCT alleles may have been introduced prior to the LCT fry stocking recorded in 2005 and 2006. In 2010, Dog Valley Creek and Hunter Creek were not sampled. There were only 6 F_1 hybrids found in 2010 in the Reno(4), Verdi(1), and McCarran(1) transects, and pure LCT showed a similar distribution with a total of 21 pure LCT in the Reno(13), Verdi (5), McCarran (1) and Wadsworth (1) transects. Introgression was still evident in the Reno (12) and Verdi (5) transects. Hybrid index scores (HIS) show a peak of hybridization occurring in 2008, both F_1 hybrids and backcrosses of hybrids with RBT spawners (Figure 8). HIS scores were then compared pooling hybrids for each year at each site. Although most sites have more F_1 hybrids than backcrosses, Hunter Creek shows consistent proportions of each hybrid index score. This confirms that hybridization was occurring in Hunter Creek before fry stocked in 2005 and 2006 could reach reproductively (Figure 9).

Salmonid reproduction

Figure 6 shows the size distribution of all RBT sampled over the four years of the study. Generally, both RBT and LCT spawn in the spring, and samples are collected later in the summer and fall. Salmonid growth varies among species and habitat, as was seen in coastal cutthroat trout (Rosenfeld et al. 2000). This can complicate associations of age with size; however, using a size histogram I was able to see a drop in frequency of fish between 80 to 100 mm. The high frequency of fish from 40 to 100 mm in length can indicate fish that have not yet overwintered (Rosenfeld et al., 2000). Therefore, to be able to compare size distributions among RBT, LCT and HYB, I grouped them into five size classes, ≤99 mm, 100-199mm, 200-299mm, 300-399mm, and ≥400mm over the 4 year period (Figure 7). Samples that were smaller than100 mm were considered to be young of the year (YOY). In 2007 and 2008, there were large numbers of YOY found in hybrids and LCT. In 2009, there was a decrease seen in HYB and LCT YOY, as well as a significant decrease in RBT YOY. In 2010 there was an increase in small hybrids found, of which most were back-crossed with RBT (74%), as well as an increase in small RBT found; however, there were very few small LCT surveyed. Stocking records for 2005-2010 indicate that fry were stocked in large numbers leading up to the sampling years and in three of the four years of this study; therefore the presence of pure LCT in small size classes does not necessarily indicate LCT reproduction, but some of the variation seen here may be due to changes in stocking. In Dog Valley Creek, the site that shows the highest level of hybridization, 34K fry were stocked in 2005, 36K fry were stocked in 2006, 21K fry were stocked in 2007, 5K fry were stocked in 2008; and 18K fry were stocked in 2009; in 2010, no fry were stocked. Therefore, in all years of the study with the exception of 2010, fry had only to survive from spring to fall to be included in the sampling. The primary indicator or LCT/HYB reproduction in Dog Valley is found in the increase of hybrids found in 2010 in the small size class.

Hybrids are not stocked in the river; therefore, YOY found were not a remnant of stocking. Also, despite the fact that 18K fry were stocked in 2009, there were very few YOY found in the 2009 sampling period. This indicates that stocking of fry in the spring is not sufficient to lead to recovery of small fish during the sampling period.

Mitochondrial sequence analysis

Hybrid genotypes at nuclear loci show that the Truckee River population exhibits a bias toward RBT alleles. This can be expected as such a large portion of the salmonid population are naturalized RBT. The presence of hybrids in this system (aside from any undocumented stocking of cut-bows) indicates that a portion of the stocked LCT were able to survive to reproductive age. Having a LCT mitochondrial haplotype indicates that the female LCT were able to compete for spawning sites. Sequence data for the ND2 mitochondrial gene show that, of the 64 F_1 hybrids, 9 individuals had the LCT maternal haplotype, whereas 55 had the RBT maternal haplotype. All of the backcrossed individuals tested had the RBT mtDNA haplotype and were dominant for RBT alleles at the nuclear loci (Table 4). There were 9 different RBT haplotypes identified. Most of the individuals belonged to one dominant haplotype (RBT A, n=50). Three different LCT haplotypes were identified, but differed from each other by only 1-3 single nucleotide polymorphisms (SNPs). The haplotypes found for RBT were 98.0 -99. 5% identical to each other, and those found for LCT were 99.5-99.7% identical. The LCT and RBT haplotypes were 92.9-94.2% identical. The significant difference between LCT and RBT indicates that these two species are very distantly related, and the ND2 sequences provided are distinct enough to identify the maternal contribution in the hybrids as either RBT or LCT definitively. The haplotypes found were compared to known Pyramid Lake and Pilot Peak LCT that were the stocking source for the Truckee River. There was not a distinguishable difference among either LCT strain or the three haplotypes found in the hybrids, as the difference between Pilot Peak and Pyramid Lake hatchery strains tested was only one SNP; Haplotype LCTA matched the Pilot Peak LCT sequenced, haplotype C matched the Pyramid Lake LCT sequenced, and haplotype B did not match either stocked strains sequenced (Table 5). Because of the low variation in found the ND2 sequence among the LCT screened, and the small number of control LCT compared (four Pyramid Lake, and four Pilot Peak), and the small number of F_1 hybrids that had the LCT haplotypes, I was unable to make a definitive assignment identifying the strain of the LCT that contributed to the hybridization. Further research using more variable markers such as microsatellites could help to elucidate which strain(s) of LCT contribute to reproduction in the Truckee River.

DISCUSSION

Here I have shown that stocking of LCT into the Truckee River and its tributaries without removal of the naturalized RBT population will lead to hybridization, regardless of the stocking of RBT or trpRBT in that system. I detected hybrids in every section of the river where LCT were stocked in large numbers (>20K). Barriers found in the Truckee River have little impact on the hybridization dynamics in the Truckee River because LCT were planted in every sampling section of the Truckee River and were planted on top of naturalized RBT; therefore for hybridization to occur, the planted fish need only to survive to reproductive age and be able to find suitable spawning habitat. Hybridization occurred primarily in regions where pure LCT were found in high numbers in previous years. Backcrosses and introgression increased in frequency over the years of the study, and were dominant in Dog Valley, Hunter Creek and Reno transects - the sites where most of the fry LCT were planted. While some researchers have found that reintroductions are more successful if planting adult fish when predators or competitors exist (Cochran-Biederman et al., 2014), others maintain that planting large numbers of fry will allow for more adaptability and acclimation and will result in overall higher survival, recruitment and eventual reproduction leading to an established population (Bigelow et al., 2010; Todd and Lintermans, 2015). However, because both fry and adult LCT were stocked in all of the years of the study, there is no way to determine is stocking of fry or stocking of adults led to spawning of the LCT and resulted in hybridization in the following year. Loxterman et al. (2014) investigated the influence of stocking history on the hybridization between two naturally sympatric trout, westslope cutthroat trout and RBT in the Salmon River, Idaho. These trout are sympatric for much of their range; however, this area is also stocked with hatchery reared RBT to support recreational angling. They found increased hybridization was correlated to both the total number of RBT stocked and the distance from the stocking event (Loxterman et al. 2014). The Truckee River is unique when compared to most other hybridization studies in that, although the LCT is the native fish to this region, LCT has been extirpated from the river for many decades, and RBT are naturalized throughout LCT native habitat. The stocking of RBT or stocking of trpRBT does not contribute to, or interfere with, hybridization in this system. This presents challenges to LCT recovery in this system because reproductive isolation from RBT would be necessary and requires identification of regions where RBT can be extirpated and isolated from reintroduction (Adams et al., 2014; Purcell and Stockwell, 2015). Such eradication units may not exist in the river. In systems such as the Truckee River, where water flow can vary dramatically, a putative barrier can exist if water flow is low, and can by bypassed in high water years (Peacock et al., 2016). Utilizing barriers to prevent reintroduction of a non-native species is usually only effective for the short term, and diligent monitoring of the barrier is needed to increase the probability of maintaining reproductive isolation of a translocated population (Avenetti et al., 2005).

The hybridization dynamics in the Truckee River were further investigated by looking at a segment of the mitochondrial ND2 gene. An unexpected finding in this study was the excess of LCT mitochondrial haplotypes found in the F_1 hybrids. Although LCT represented less than 5% of the salmonids identified over the four years of this study, 14% of the mitochondrial haplotypes were identified as LCT. This excess was not seen in the backcrossed hybrids showing introgression, with 100% showing the RBT mitochondrial haplotype. While Ostberg et al. (2004) report the opposite result for two naturally sympatric populations of coastal cutthroat trout and anadromous rainbow trout (steelhead), theF_1 hybrids in the Ostberg et al. (2004) study had only RBT mtDNA but backcrossed individuals had a reduced proportion of the RBT mtDNA haplotype. In that system, coastal cutthroat were the numerically dominant species, whereas in the Truckee River, RBT make up over 85% of the sampled salmonids. It has been suggested that females of the rare species indiscriminately spawn with the abundant male species; whereas the common species is more likely to spawn with the hybrid. More likely, in the Truckee River, backcrossing occurs due to precocious hybrid males sneak-spawning with RBT redds. Overwintering leads to high mortality and variable conditions in the Truckee River it difficult to determine the probability of survival. This study looked at size class data of the RBT population sampled over a four year period (Figure 6), which includes stocked triploid RBT, as well naturalized RBT. With the markers used in this study it is not possible to distinguish triploid RBT from naturalized RBT. However, the stocked triploid RBT were all stocked as catchables, with an average fork length of 244 ±16 mm. Figure 6 shows a large number of samples found in the smaller size classes, with a drop off indicated between 80 and 100 mm; the stocked salmonids were larger than this at the time of stocking, so this indicates naturalized RBT reproduction. Further evaluation of these samples is necessary using genetic markers with higher variation, such as microsatellites, to determine if the stocked triploid RBT are over wintering and included in these data. Based on the fact that most of the LCT planted in the Dog Valley and Hunter Creek tributaries prior to 2005 and 2006 were fry, which would have been recruited to the >100 mm size classes by 2007 and 2008, the presence of LCT >300 mm and HYB samples <100 mm suggests that the planted LCT are not only able to overwinter, but are surviving to reproductive age and are able to participate in spawning in those tributaries. There is little difference in preferred spawning gravels for RBT and LCT, and spawning gravels may be limited. LCT are able to compete for spawning gravels in the spring, when RBT are also spawning, leading to hybridization in regions of the river where LCT are stocked.

For LCT recovery, the presence of hybridization in this system can be interpreted with some good news. The fact that there are hybrids present predominately in the proximity of Dog Valley and Hunter Creek illustrates that a portion of the large number of LCT planted in 2005 and 2006 were able to survive to reproductive age and were able to spawn. At the same time, it is evident that for LCT, recovery to be successful, RBT need to be removed from the system or a portion of the system and large numbers of LCT must be planted annually in different size classes until a self-sustaining population is developed. The large naturalized RBT population present in the Truckee is more than adequate to maintain RBT in the system. The planted triploid fish are planted primarily for angling opportunities and it is thought that there is not a lot of overwintering (Kim Tisdale, NDOW, personal communication). This presents additional questions that can be genetically determined using samples from the Truckee River. First, can you molecularly identify triploids from diploids in the system? Generally, triploids are identified prior to planting by evaluating eggs through flow cytometry to insure that the eggs have the extra polar body post fertilization (Benfey, 1999; Wong & Van Eenennaam, 2008). Triploid eggs are then usually reared in the hatchery to “catchable" size prior to stocking. If trying to evaluate population structure and reproduction of a naturalized RBT population, the introduction of stocked fish can make that process more complicated. There are many variables that will dictate whether or not reintroduction of LCT into the Truckee River can be successful. As evidenced by the successful spawning seen for both LCT males and females creating F_1 hybrids, there is suitable habitat in the Truckee River for reintroduction. The difficulty becomes identifying regions of the system that may allow for reproductive isolation. To do this, the naturalized RBT population structure needs to be evaluated to determine if there are barriers to movement for the RBT or if there are regions that can be isolated from the rest of the river that can act as a strong hold for LCT reintroduction. Currently, the best sites for this are the two tributaries, Dog Valley and Hunter Creek. However, connectivity between the tributaries and the main stem as well as political and legal pressures to maintain angling of the RBT in those tributaries continues to complicate reintroduction.

Figure Legends:

Figure 1. The Truckee River watershed in eastern California and western Nevada. The Truckee River watershed in eastern California and western Nevada. Potential barriers to fish movement (Red) and collection sites (Black) for salmonid species along the Truckee River.

Figure 2. Sampling Transects and barriers in the Truckee River. Potential barriers and water diversions identified as (X). Numbers corespond to sites listed in Table 1. Collection transects (3-4 per site) are indicated by filled circles in alternating red and blue, which depict the sampling sites. .

Figure 3. Stocking of Salmonids over a 20 year time period. Stocking of Salmonids over a 20 year time period. Including data for Lahontan cutthroat trout (LCT), and Rainbow trout (RBT). Non-reproductive triploid RBT (trpRBT) and unspecified potentially reproductive diploid RBT (dipRBT) were all stocked.

Figure 4. Stocking of salmonids from 2004-2010 in the Truckee River. A. LCT stocking occurred throughout the Truckee River. Dog Valley and Hunter Creek the two tributaries, were stocked with a large amount of fry in 2005-2006. Many segments did not have sympatric stocking of RBT. B. Primarily non-reproductive triploid RBT were stocked into regions of the river for angling. RBT were not stocked in all of the collection sited, nor in both tributaries.

Figure 5. Proportion of each trout species identified in each river section. Sections correspond to the major collection sites from Table 1. Hybrids were found in all sections where LCT were stocked in large numbers, regardless of sympatric RBT stocking.

Figure 6. Size distribution of RBT samples collected along the Truckee River from 2007-2010. Size class distribution of RBT sampled along the Truckee River from 2007-2010. Measured fork-lengths were put into 10 mm graduations. Drop indicates reduced survivorship from young of the year to year class 1. Fish to the left of the arrow (100 mm or less) were considered young of year. Fish to the right of the arrow were considered to be fish that survived at least one winter.

Figure 7. Size class distribution of salmonids sampled. Size class distribution salmonids sampled from 2007 to 2010. LCT, RBT and hybrids found in the system were placed into one of 4 size classes based on fork-length. The distribution of sizes for LCT, HYB and RBT is similar, showing primarily small fishes and fewer large adults. In 2009, and less dramatically in 2010, LCT and HYB smaller than 100 mm declined. LCT were stocked in multiple size classes, so it can be difficult to determine if pure LCT are or if stocking is influencing sampling efforts. Hybrids indicate reproduction between LCT and RBT as they were not stocked and are found in both small and large size classes, indicating survival. RBT are stocked at ~200mm. RBT smaller than ~200 mm indicate reproduction.

Figure 8. Hybrid Index Scores by year. Hybrid Index Scores for each year of the study. Scores are calculated by summing the total number of alleles scored as RBT divided by the total number of alleles scored. Hybrid index scores of 0.5 are first generation hybrids, containing one of each of the LCT and RBT alleles. Scores above 0.5 contain more RBT contribution, and those below 0.5 contain more LCT contribution. There was a peak in F_1 hybrids found in 2008; however, introgression was evident in 2007, showing multiple generations of hybrids backcrossing with RBT.

Figure 9. Hybrid Index Scores by transect. First generation hybrids were common in Tahoe, DogValley and Reno transect, indicating recent hybridization. Hunter Creek showed HIS's that were evenly distributed, showing backcrossing of hybrids with RBT for multiple years. Hunter Creek was only stocked with LCT fry in 2007, therefore, the LCT stocked in the main stem must have access to Hunter Creek and have used it as spawning habitat. Very few hybrids were found north of Reno in McCarren or Wadsworth, indicating limited spawning habitat.

Figure 10. Distribution of RBT ND2 haplotypes. There were 9 different RBT haplotypes found in the hybrids sampled. Of the 64 F_1 hybrids sequenced, 55 showed a RBT mitochondrial haplotype. Backcrossed hybrids sequenced all had HIS over 0.5, and all showed RBT mitochondrial haplotype. The major haplotype found was found in all of the sections where hybrids were found. Most other haplotypes were only found in one or few individuals.

Figure 11. Distribution of LCT ND2 haplotypes. Of the 64 F_1 hybrids sequenced, 9 showed an LCT haplotype. None of the backcrossed hybrids showed evidence of backcrossing with pure LCT.

Table 1. List of all transects (T) per section (S) elevation, latitude and longitude. (S8 = Tahoe, S7 = Truckee, S6 = Farad, S5 = Verdi, S4 = Reno, S3 = McCarran, S2 = Wadsworth, S1 = Nixon and two tributaries)

Transect	Section(S) Transect (T)	Elevation (ft)	Latitude	Longitude
1. Tahoe-A	**S8T2**	**6225**	**39.16207**	**-120.159**
2. Tahoe-B	**S8T3**	**6079**	**39.20848**	**-120.198**
3. Tahoe-C	**S8T1**	**5909**	**39.27361**	**-120.206**
4. Truckee-A	**S7T3**	**5751**	**39.33366**	**-120.164**
5. **Truckee-B**	**S7T2**	**5676**	**39.34754**	**-120.123**
6. Truckee-C	**S7T1**	**5598**	**39.36092**	**-120.117**
7. **Farad-A**	**S6T1**	**5472**	**39.38554**	**-120.086**
8. Farad-B	**S6T2**	**5400**	**39.36848**	**-120.041**
9. **Farad-C**	**S6T4**	**5169**	**39.42332**	**-120.035**
10. Farad-D	**S6T3**	**5076**	**39.4497**	**-120.005**
11. **Verdi-A**	**S5T1**	**4984**	**39.475**	**-119.994**
12. Verdi-B	**S5T3**	**4854**	**39.50926**	**-119.996**
13. **Verdi-C**	**S5T2**	**4637**	**39.50651**	**-119.902**
14. **Reno-A**	**S4T3**	**4500**	**39.52391**	**-119.819**
15. Reno-B	**S4T2**	**4448**	**39.53013**	**-119.795**
16. **Reno-C**	**S4T1**	**4394**	**39.51424**	**-119.736**
17. **McCarran Ranch-A**	**S3T3**	**4309**	**39.52563**	**-119.61**
18. **McCarran Ranch-B**	**S3T2**	**4284**	**39.54498**	**-119.587**
19. McCarran Ranch-C	**S3T1**	**4277**	**39.54703**	**-119.573**
20. McCarran Ranch-D	**S3T4**	**4202**	**39.56469**	**-119.487**
21. **Wadsworth-A**	**S2T1**	**4189**	**39.58506**	**-119.444**
22. **Wadsworth-B**	**S2T2**	**4132**	**39.59062**	**-119.368**
23. Wadsworth-C	**S2T3**	**4099**	**39.61273**	**-119.306**
24. **Nixon-A**	**S1T1**	**3988**	**39.72697**	**-119.319**
25. **Nixon-B**	**S1T2**	**3903**	**39.81771**	**-119.35**
26. Nixon-C	**S1T3**	**3850**	**39.85426**	**-119.394**
27. Hunter Creek	**Tributary**	**5042**	**39.49131**	**-119.899**
28. Dog Valley Creek	**Tributary**	**5715**	**39.56431**	**-120.026**

Table 2. Differentiating primers used for identification of rainbow trout and cutthroat trout in known pure rainbow trout and pure cutthtoat trout populations and populations, alleles and proportion per species, forward (F) and reverse (R) primer sequences (OCC and OM primers from Ostberg and Rodriguez, 2004; ND2 mitochondrial sequencing primers from Nowak 2005)

Locus-DYE	Allele (bp)	RBT	LCT	F_1 HYB	BC HYB		Primer sequence (5'-3")
OCC34-	A-224	1.0		0.5		F:	ACGAGATGCCATTGATGTGAGTGC
NED	B-230		1.0	0.5		R:	ATGGGTGGATGGATGAATGGAATG
OCC35-	A-210	1.0		0.5		F:	GGCGGACGGACGGATGG
VIC	B-242		1.0	0.5		R:	GTCCCGGCGGAACCACAG
OCC36-	A-279	1.0		0.5		F:	CTGAAAGCACCTCCTCCATTAG
VIC	B-309		1.0	0.5		R:	GTTCTCTTCTCCTGTTTCGCTTAT
OCC37	A-259	1.0		0.5		F:	CCCTGGGAGCATCAGTTAGA
FAM	B-272		1.0	0.5		R:	AAAAGGTTGTGACCCACTGC
OCC38	A-152	1.0		0.5		F:	GCATGCTGTTATCAGACACTGAG
VIC	B-174		1.0	0.5		R:	GCTGGTCCCAATGTGATTTT
OM55	A-196	1.0		0.5		F:	GTGCATGTTAGCTGGTGCAA
FAM	B-219		1.0	0.5		R:	TGAGAACATGTCATTTGGGACT
ND2	RBTid	1.0		0.875	1.0	F:	GGTACAGTCCTCACCTTTGC
NDvarR	LCTid		1.0	0.125		R:	GCTTTGAAGGCTCTTGGTCT

Table 3. Proportion of each fish type identified differentiating markers in each river section. F_1 HYB are designated as first generation hybrids, back crossed (BC) hybrids show introgression with RBT.

2007	Pure RBT	Pure LCT	F_1 HYBRIDS	Post F_1-LCT	Post F_1-RBT
Tahoe	9	0	0	0	0
Truckee	41	0	0	0	0
Farad	23	0	0	0	0
Verdi	91	3	1	0	0
Dog Valley*	119	30	0	0	0
Hunter Creek*	76	13	4	2	19
Reno	64	5	9	0	0
McCarran	20	0	0	0	0
Wadsworth	0	0	0	0	0
Nixon	4	0	0	0	0
2008	Pure RBT	Pure LCT	F_1 HYBRIDS	Post F_1-LCT	Post F_1-RBT
Tahoe	38	0	2	0	1
Truckee	106	0	0	0	0
Farad	58	0	0	0	0
Verdi	180	4	1	0	3
Dog Valley*	91	31	22	0	4
Hunter Creek*	76	3	4	0	7
Reno	225	4	8	0	6
McCarran	80	0	0	0	0
Wadsworth	65	0	0	0	1
Nixon	0	0	0	0	0

2009	Pure RBT	Pure LCT	F_1 HYBRIDS	Post F_1-LCT	Post F_1-RBT
Tahoe	47	0	7	0	0
Truckee	83	0	0	0	0
Farad	73	0	0	0	0
Verdi	209	6	0	0	2
Dog Valley*	93	1	0	0	5
Hunter Creek*	81	0	1	0	6
Reno	226	3	0	0	0
McCarran	79	2	0	0	0
Wadsworth	20	0	0	0	0
Nixon	10	1	0	0	0
2010	**Pure RBT**	**Pure LCT**	**F_1 HYBRIDS**	**Post F_1-LCT**	**Post F_1-RBT**
Tahoe	7	0	0	0	0
Truckee	55	1	0	0	0
Farad	83	0	0	0	0
Verdi	217	5	1	0	5
Reno	131	13	4	0	12
McCarran	126	1	1	0	0
Wadsworth	25	1	0	0	0
Nixon	0	0	0	0	0

Table 4. Percent identity and number of SNPs among the salmonid haplotypes found in the Truckee River. Percent identity is above the diagonal, and number of SNPs found in the 395 bp alignment is below the diagonal. LCT comparisons with RBT are shaded grey, LCT comparisons with LCT are in bold.

	RBTH	RBTA	RBTB	RBTC	RBTD	RBTE	RBTF	RBTG	LCTA	LCTB	LCTC
RBTH		99.0	98.7	98.2	98.7	99.3	98.2	99.5	92.9	92.7	93.2
RBTA	4		98.7	98.2	98.7	99.6	98.2	99.5	92.9	92.7	93.2
RBTB	5	5		98.0	98.5	99.1	98.0	98.7	93.9	93.7	94.2
RBTC	7	7	8		99.5	98.5	98.5	98.2	93.7	93.4	93.9
RBTD	5	5	6	2		99.1	99.0	98.7	93.7	93.4	93.9
RBTE	3	2	4	6	4		98.5	99.3	93.2	93.0	93.5
RBTF	7	7	8	6	4	6		98.2	93.7	93.9	93.4
RBTG	2	2	5	7	5	3	7		92.9	92.7	93.2
LCTA	28	28	24	25	25	27	25	28		**99.7**	**99.7**
LCTB	29	29	25	26	26	28	24	29	**1**		**99.5**
LCTC	27	27	23	24	24	26	26	27	**1**	**2**	

Table 5. Lahontan Cutthroat trout ND2 haplotypes with RBT haptlotype. Differences between from most common LCT haplotype are highlighted in yellow for RBT and in blue for LCT. Pilot Peak Hatchery strain(PPLT) and Pyramid Lake Strain (PYRL) were used as LCT controls.

```
1        10        20        30        40        50        60
LCTAGGTAATTATGGTAAAGTTGAGGCGTCAGGGGGCAGTGGCAGTTAGGGTGTTGGGATAAAT
LCTBGGTAATTATGGTAAAGTTGAGGCGTCAGGGGGCAGTGGCAGTTAGGGTGTTGGGATAAAT
LCTCGGTAATTATGGTAAAGTTGAGGCGTCAGGGGGCAGTGGCAGTTAGGGTGTTGGGATAAAT
RBTAGGTAATTATGGTAAAGTTGAGGCGTCATGGGGCAGTAGCAGTTAGGGTGTTGGGATAAAT
PPLTGGTAATTATGGTAAAGTTGAGGCGTCAGGGGGCAGTGGCAGTTAGGGTGTTGGGATAAAT
PYRLGGTAATTATGGTAAAGTTGAGGCGTCAGGGGGCAGTGGCAGTTAGGGTGTTGGGATAAAT

         70        80        90        100       110       120
AGTGAGGGTTAAGGCGTAGCAGAGTCGTAGATAAAAGTAAAGGCTCAGGAGGGCTGTTAT
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AGTGAGGGTTAAGGCGTAGCAGAGTCGTAGATAAAAGTAAAGGCTCAGGAGGGCTGTTAT
AGTGAGGGTTAAGGCGTAGCAGAGTCGTAGATAAAAGTAAAGGCTCAGGAGGGCTGTTAT
        130        140        150        160       170       180
AGCAGCTAGTGTGGCAGATAGTGGGAGTCCTTGCTTTGTTAGTTCTTGCAAAATAAGTCA
AGCAGCTAGTGTGGCAGATAGCGGGAGTCCTTGCTTTGTTAGTTCTTGCAAAATAAGTCA
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AGCAGCTAGTGTGGCAGATAGTGGGAGTCCTTGCTTTGTTAGTTCTTGCAAAATAAGTCA
AGCAGCTAGTGTGGCAGATAGTGGGAGTCCTTGCTTTGTTAGTTCTTGCAAAATAAGTCA

        190       200       210        220       230       240
CTTTGGTATAAAGCCTGAGAGAGGGGGGAGACCTCCAAGGGATAGCAGTACAAGAGCGGC
CTTTGGTATAAAGCCTGAGAGAGGGGGGAGACCTCCAAGGGATAGCAGTACAAGAGCGGC
TTTTGGTATAAAGCCTGAGAGAGGGGGGAGACCTCCAAGGGATAGCAGTACAAGAGCGGC
TTTTGGTATAAAGCCCGAGAGAGGGGGGAGACCTCCAAGGGACAATAATACAAGAGCGGT
CTTTGGTATAAAGCCTGAGAGAGGGGGGAGACCTCCAAGGGATAGCAGTACAAGAGCGGC
TTTTGGTATAAAGCCTGAGAGAGGGGGGAGACCTCCAAGGGATAGCAGTACAAGAGCGGC
        250       260       270        280      290        300
TAATGCGGCAAGGGTCGGGGATTTAGTTCATGAAGTCGCGAGAGTGTTGATGGTTAAAGC
TAATGCGGCAAGGGTCGGGGATTTAGTTCATGAAGTCGCGAGAGTGTTGATGGTTAAAGC
TAATGCGGCAAGGGTCGGGGATTTAGTTCATGAAGTCGCGAGAGTGTTGATGGTTAAAGC
TAATGCGGCAAGGGTCGGGGATTTAGTTCATGAAGTTGCGAGAGTATTGACGGTTAAAGA
TAATGCGGCAAGGGTCGGGGATTTAGTTCATGAAGTCGCGAGAGTGTTGATGGTTAAAGC
TAATGCGGCAAGGGTCGGGGATTTAGTTCATGAAGTCGCGAGAGTGTTGATGGTTAAAGC

       310       320       330        340       350        360
GTTGTTGGTTTTTAATGTGAGGAATGCTGAAGATGTTATGATAATATATAGGGACAGGCT
GTTGTTGGTTTTTAATGTGAGGAATGCTGAAGATGTTATGATAATATATAGGGACAGGCT
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GTTGTTGGTTTTTAATGTGAGGAATGCTGAAGATGTTATGATAATATATAGGGACAGGCT
GTTGTTGGTTTTTAATGTGAGGAATGCTGAAGATGTTATGATAATATATAGGGACAGGCT
        370       380       390   395
GAGGAGTGTGAGGGAGGGTGCGAATTGTAAAATTA
GAGGAGTGTGAGGGAGGGTGCGAATTGTAAAATTA
GAGGAGTGTGAGGGAGGGTGCGAATTGTAAAATTA
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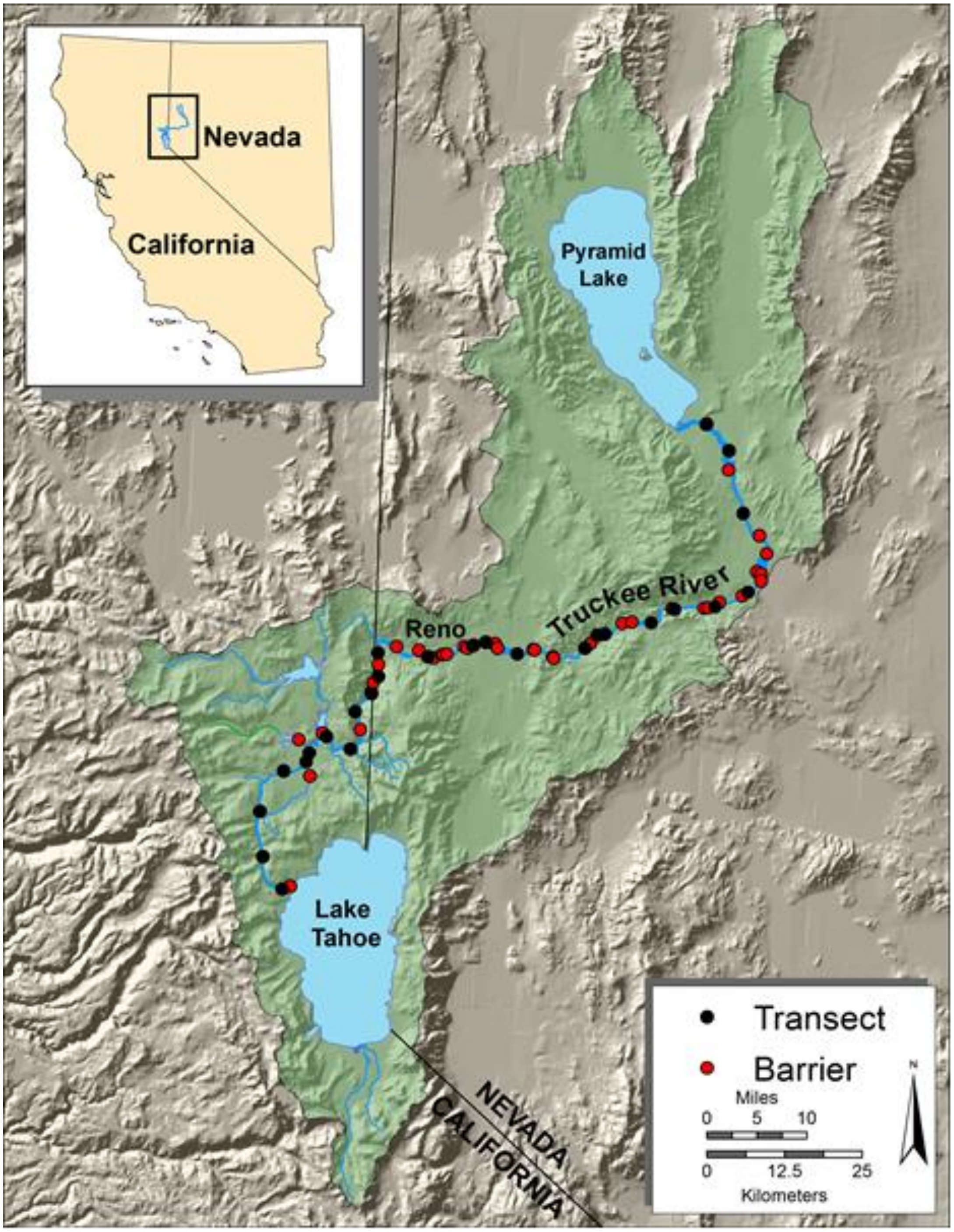
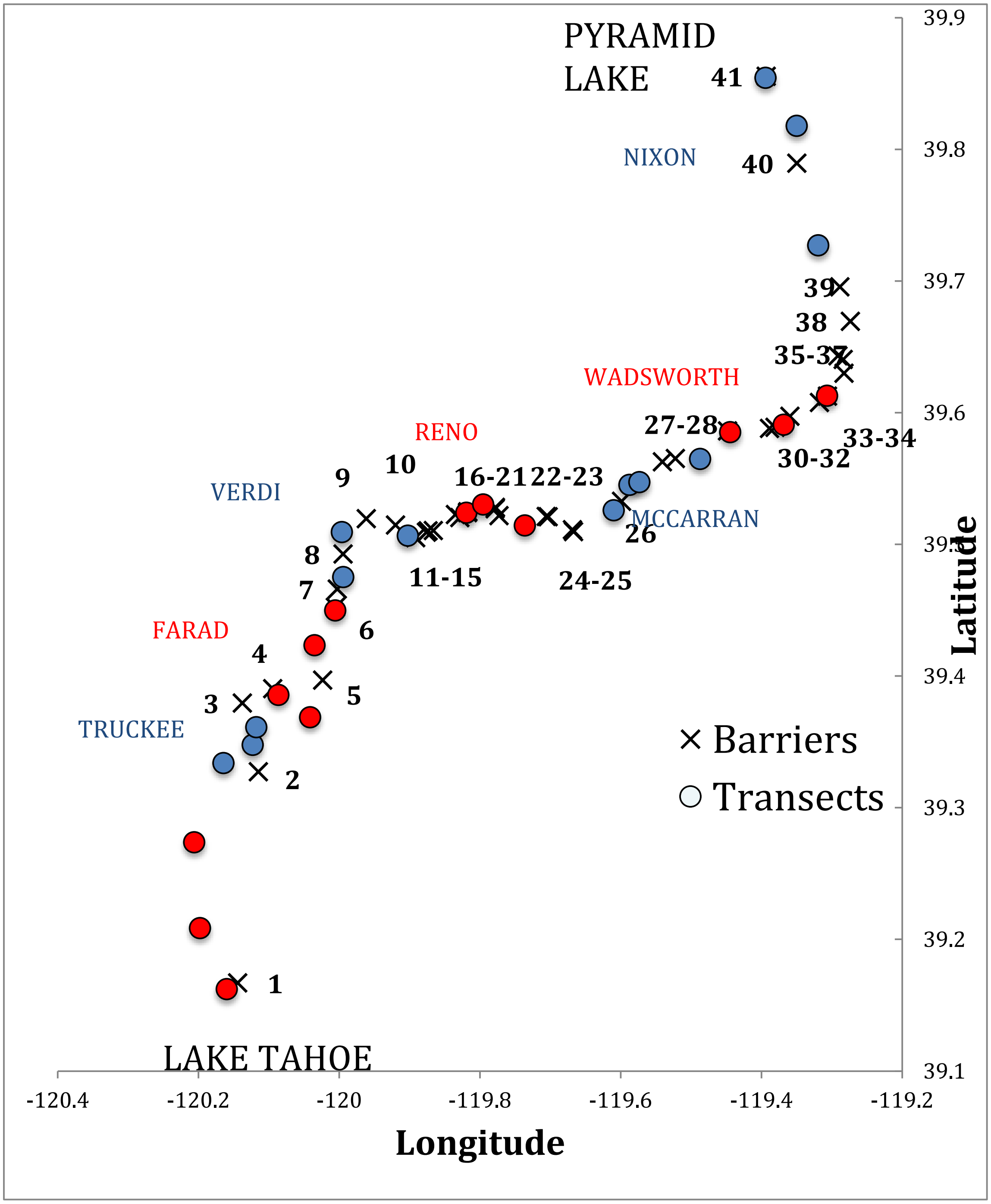


Nevada
California
Pyramid
Lake
Truckee River
Reno
Lake
Tahoe
NEVADA
CALIFORNIA
Transect
Barrier
Miles
0 5 10
0 12.5 25
Kilometers
N

Figure 1.



PYRAMID LAKE
41
NIXON
40
39
38
35-37
WADSWORTH
RENO
27-28
33-34
30-32
VERDI
9
10
16-21
22-23
MCCARRAN
26
8
11-15
24-25
7
FARAD
6
4
3
5
TRUCKEE
2
1
LAKE TAHOE
Barriers
Transects
39.9
39.8
39.7
39.6
39.5
39.4
39.3
39.2
39.1
Latitude
-120.4
-120.2
-120
-119.8
-119.6
-119.4
-119.2
Longitude

Figure 2.

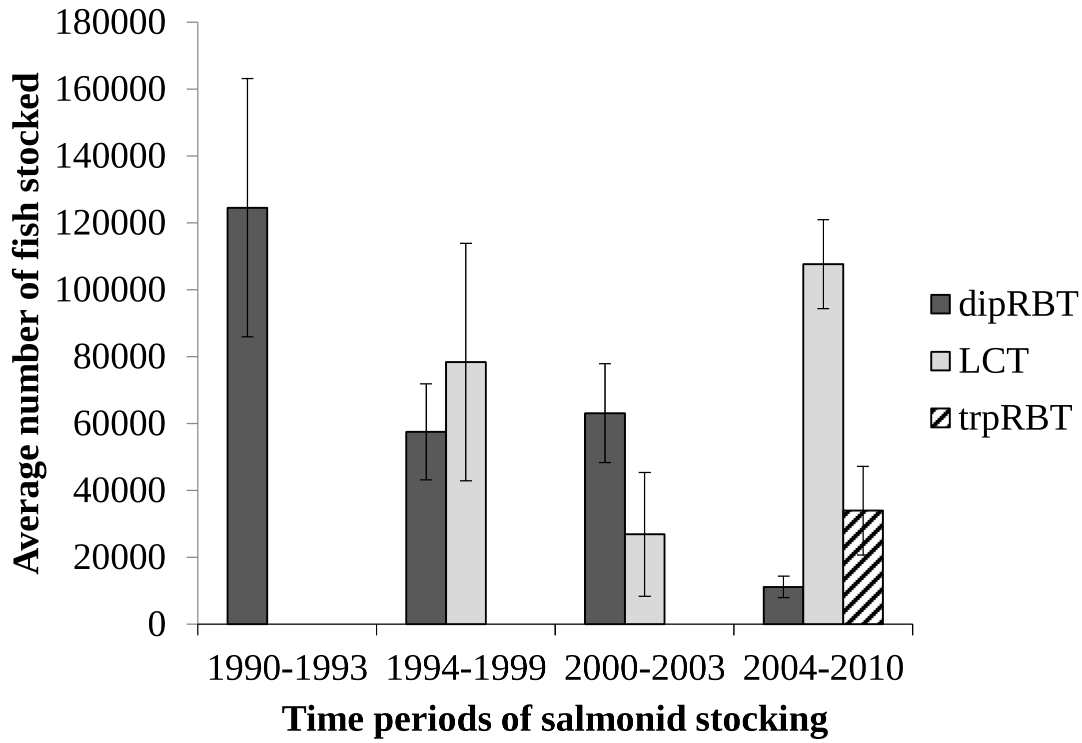
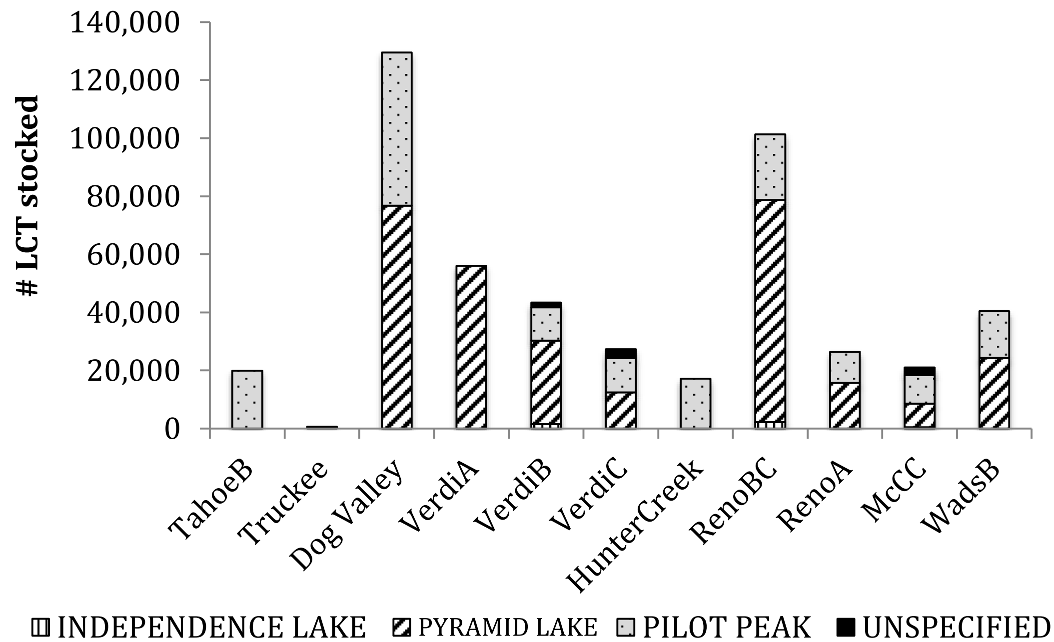

180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Average number of fish stocked
1990-1993
1994-1999
2000-2003
2004-2010
Time periods of salmonid stocking
dipRBT
LCT
trpRBT

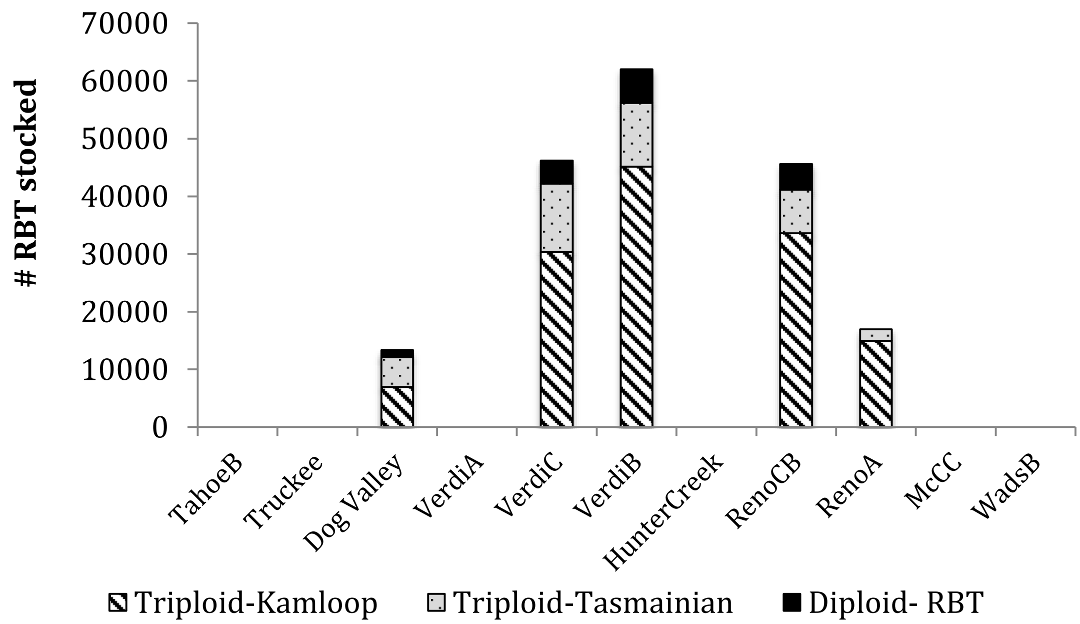
Figure 3.

A.



LCT stocked
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
TahoeB
Truckee
Dog Valley
VerdiA
VerdiB
VerdiC
HunterCreek
RenoBC
RenoA
McCC
WadsB
INDEPENDENCE LAKE
PYRAMID LAKE
PILOT PEAK
UNSPECIFIED

B



RBT stocked
70000
60000
50000
40000
30000
20000
10000
0
TahoeB
Truckee
Dog Valley
VerdiA
VerdiC
VerdiB
HunterCreek
RenoCB
RenoA
McCC
WadsB
Triploid-Kamloop
Triploid-Tasmainian
Diploid- RBT

Figure 4.

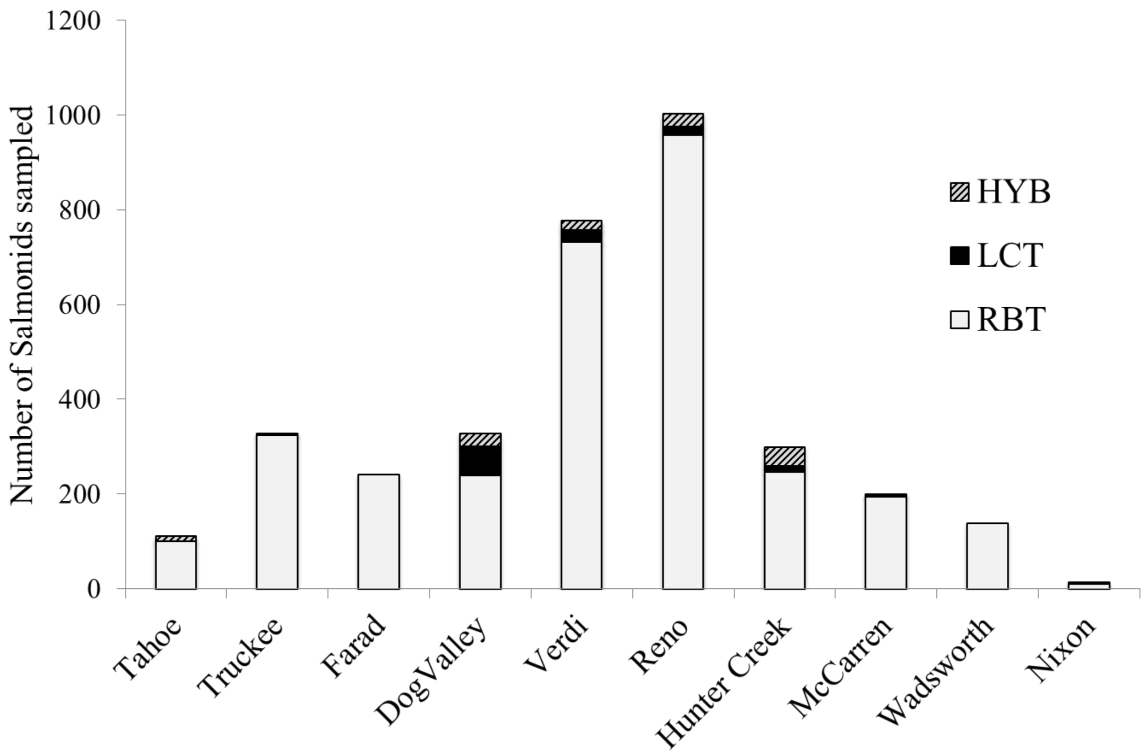

1200
1000
800
600
400
200
0
Number of Salmonids sampled
HYB
LCT
RBT
Tahoe
Truckee
Farad
DogValley
Verdi
Reno
Hunter Creek
McCarren
Wadsworth
Nixon

Figure 5.

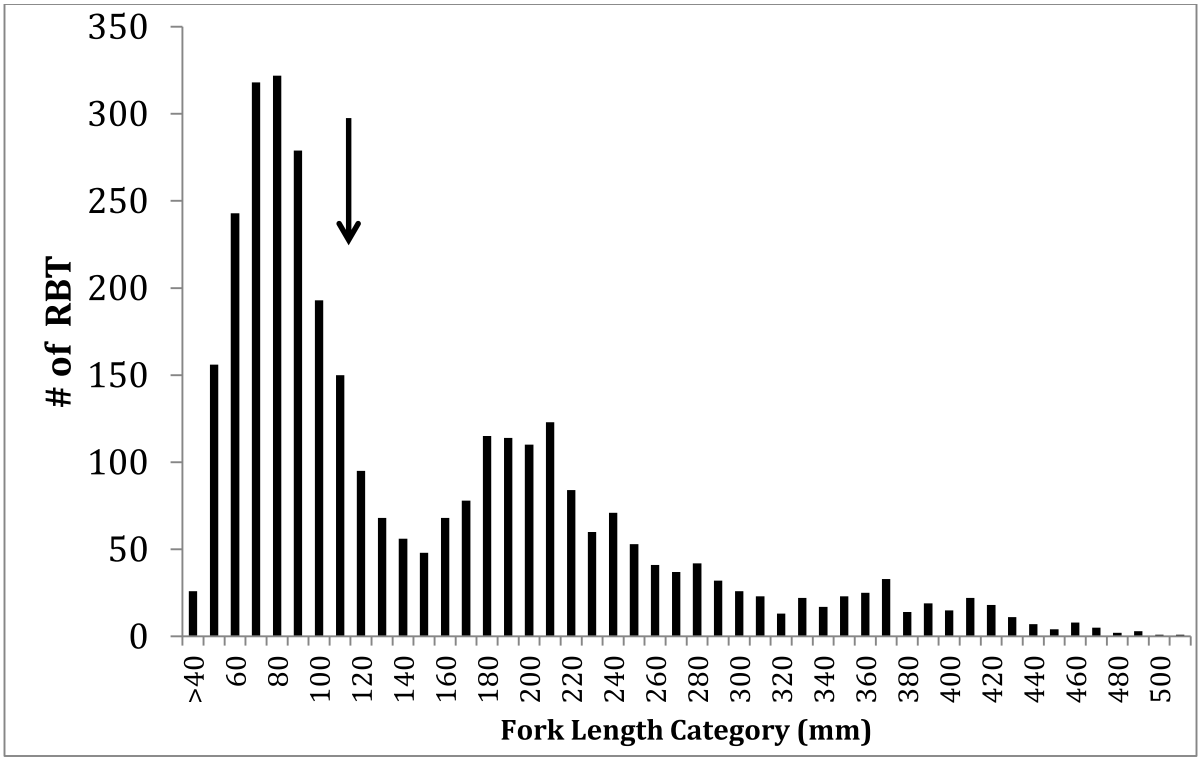
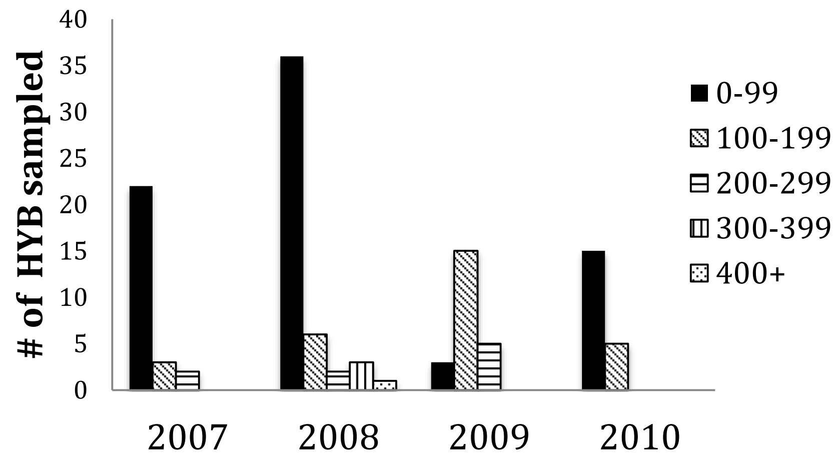
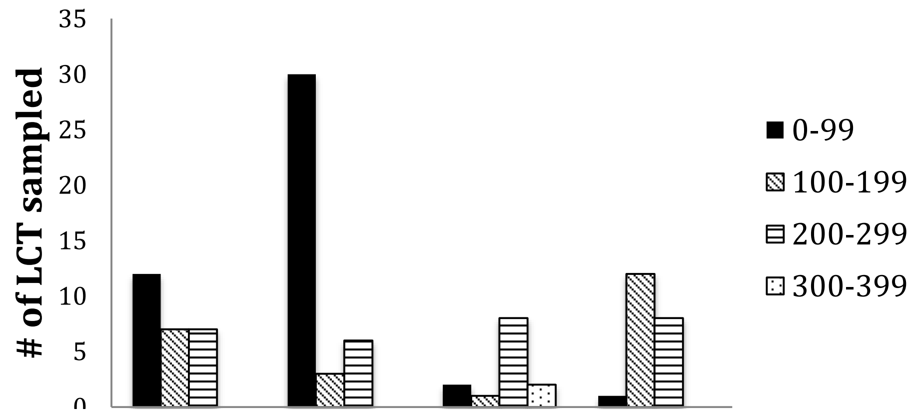

350
300
250
200
150
100
50
0
# of RBT
>40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
Fork Length Category (mm)

Figure 6.

A.


40
35
30
25
20
15
10
5
0
# of HYB sampled
0-99
100-199
200-299
300-399
400+
2007
2008
2009
2010

B


35
30
25
20
15
10
5
0
# of LCT sampled
0-99
100-199
200-299
300-399

C

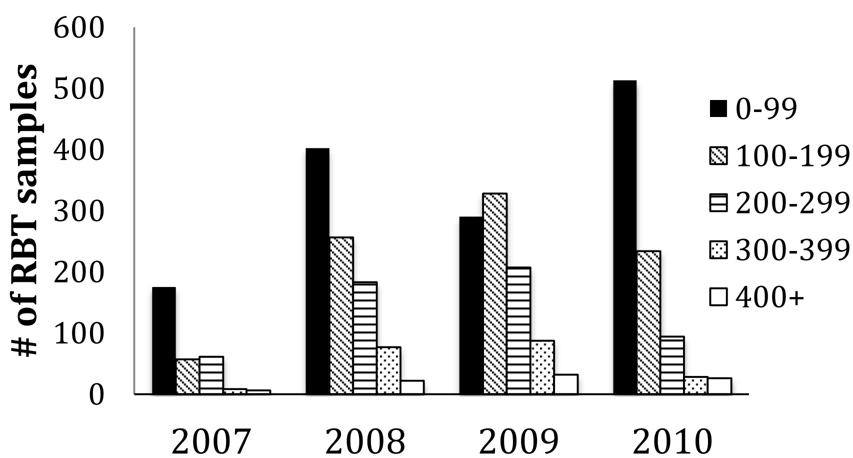

600
500
400
300
200
100
0
# of RBT samples
0-99
100-199
200-299
300-399
400+
2007
2008
2009
2010

Figure 7.

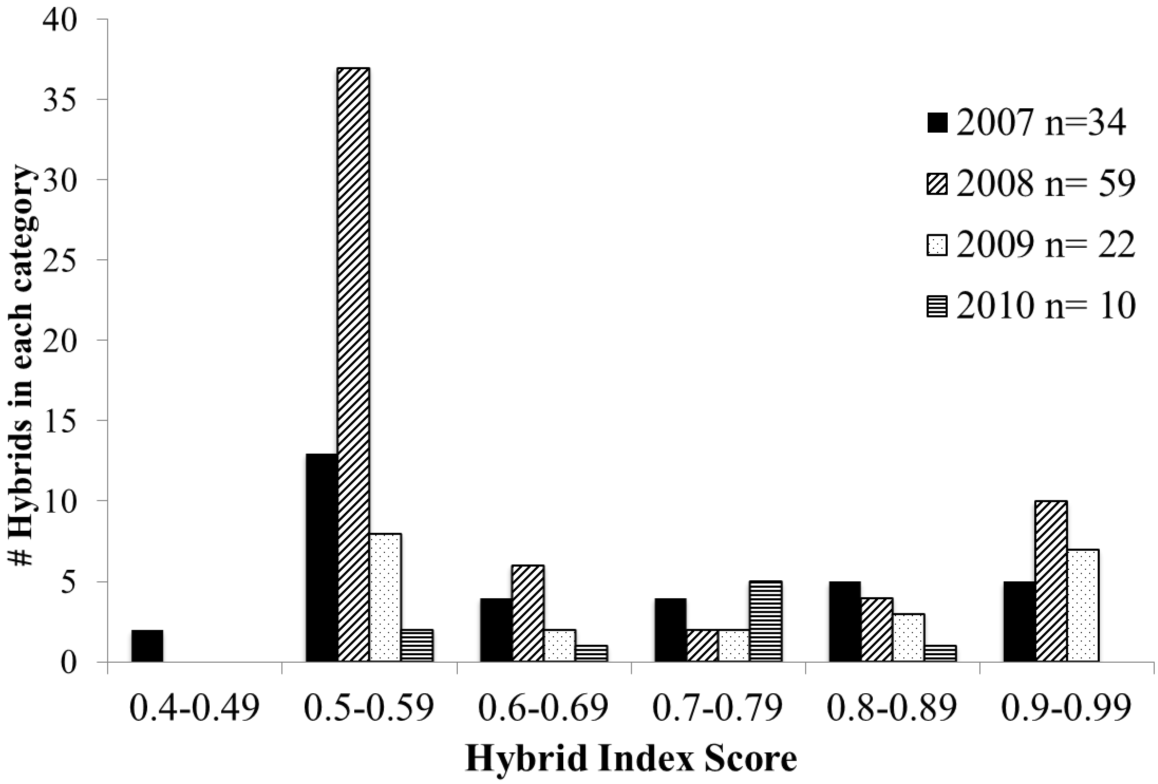
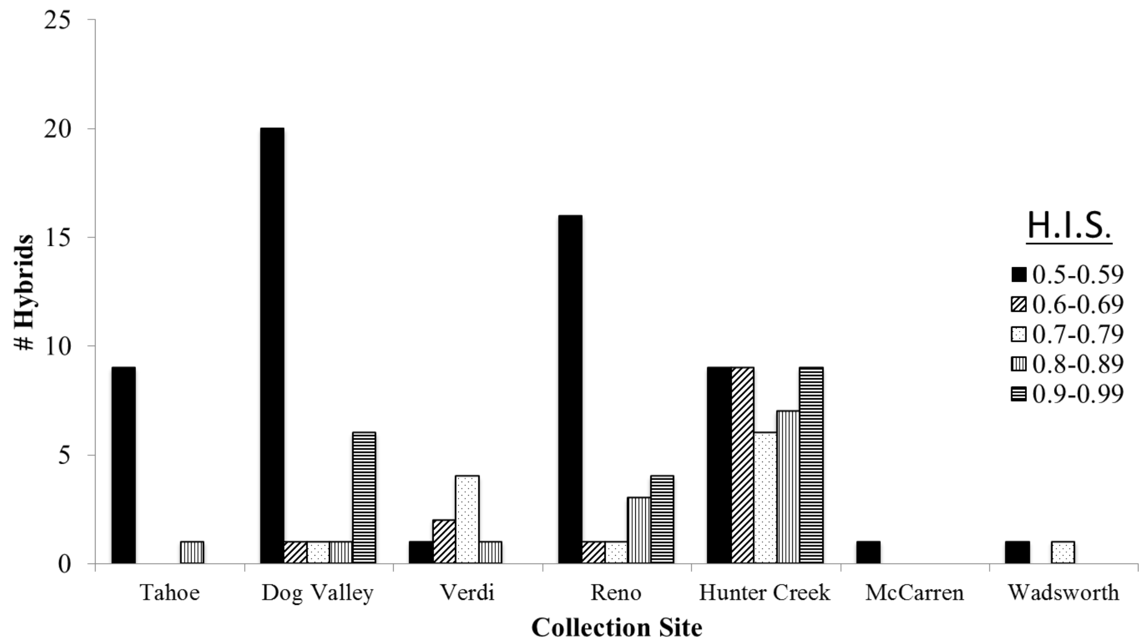

40
35
30
25
20
15
10
5
0
Hybrids in each category
2007 n=34
2008 n= 59
2009 n= 22
2010 n= 10
0.4-0.49
0.5-0.59
0.6-0.69
0.7-0.79
0.8-0.89
0.9-0.99
Hybrid Index Score

Figure 8.



25
20
15
10
5
0
Hybrids
H.I.S.
0.5-0.59
0.6-0.69
0.7-0.79
0.8-0.89
0.9-0.99
Tahoe
Dog Valley
Verdi
Reno
Hunter Creek
McCarren
Wadsworth
Collection Site

Figure 9.

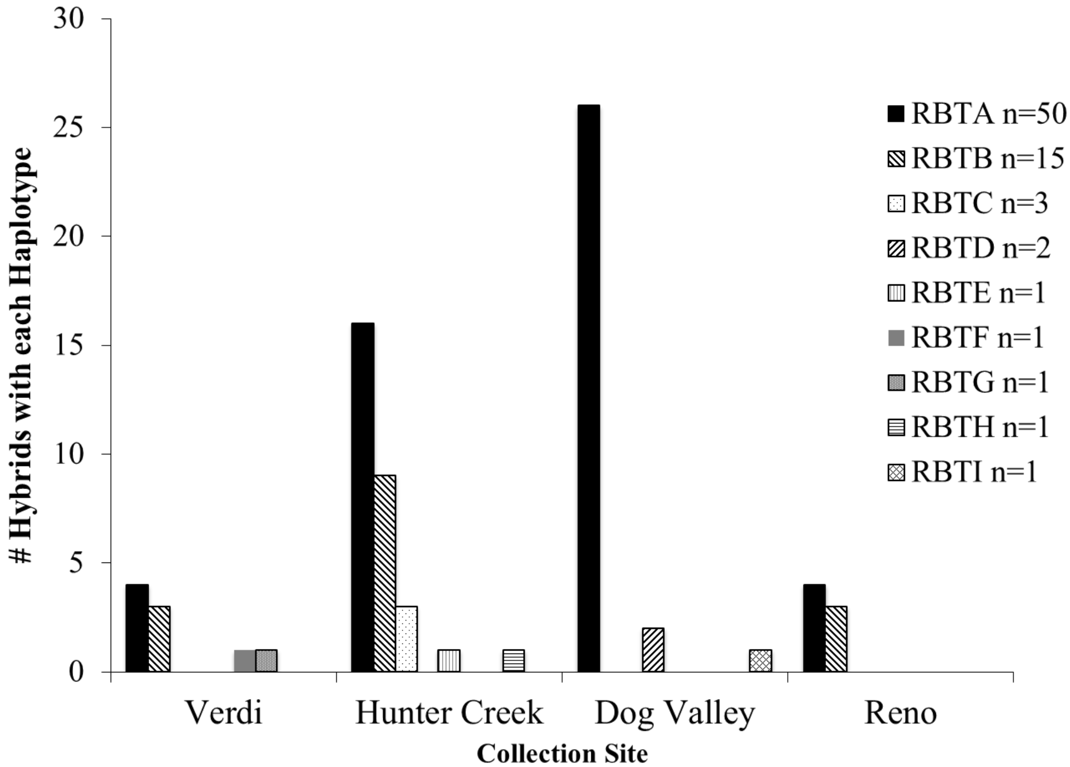
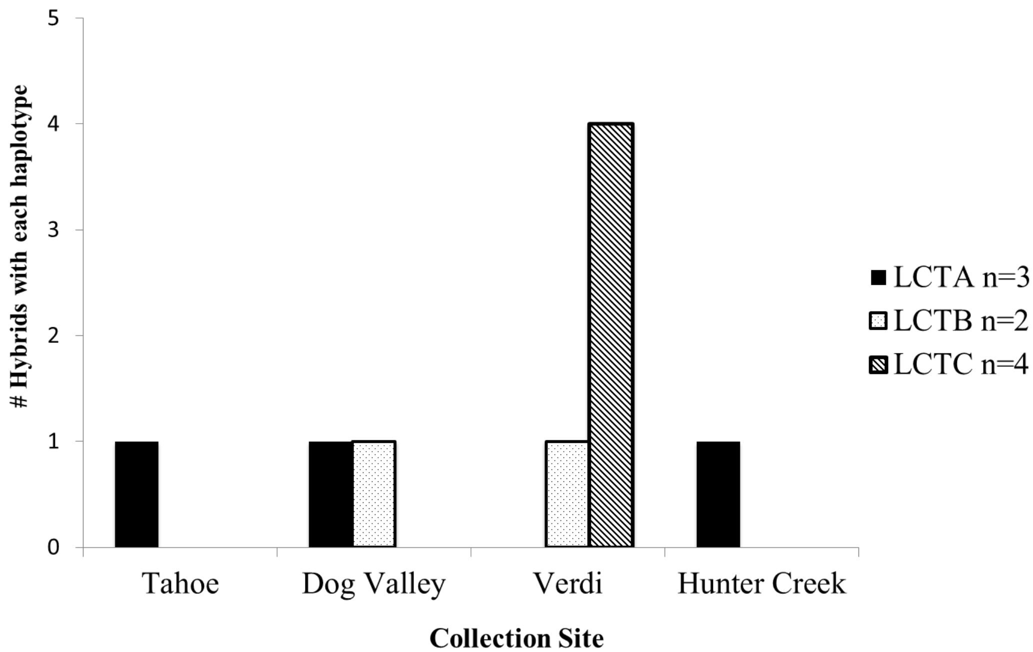

30
25
20
15
10
5
0
Hybrids with each Haplotype
RBTA n=50
RBTB n=15
RBTC n=3
RBTD n=2
RBTE n=1
RBTF n=1
RBTG n=1
RBTH n=1
RBTI n=1
Verdi
Hunter Creek
Dog Valley
Reno
Collection Site

Figure 10.



5
4
3
2
1
0
Hybrids with each haplotype
LCTA n=3
LCTB n=2
LCTC n=4
Tahoe
Dog Valley
Verdi
Hunter Creek
Collection Site

Figure 11.

LITERATURE CITED

Allendorf, F. W., and R. F. Leary. 1988. Conservation and Distribution of genetic variation in a polytypic species the cutthroat trout. *Conservation Biology* **2**:170-184.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution* **16**:613-622.

Avenetti, L. D., A. T. Robinson, and C. J. Cantrell. 2006. Short-term effectiveness of constructed barriers at protecting Apache trout. *North American Journal of Fisheries Management* **26**:213-216.

Bartley, D. M., and G. A. E. Gall. 1991. Genetic identification of the native cutthroat trout (*Oncorhynchus clarki*) and introgresive hybridization with introduced rainbow trout (*O.* mykiss) in streams associated with the Alvord Basin, Oregon and Nevada. *Copeia* **3:**854-859.

Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society Monograph **6**, Bethesda, Maryland. 1-275.

Benfey, T. J. 1999. The physiology and behavior of triploid fishes*. Reviews in Fisheries Science* **7**:39-67.

Bennett, S. N., and J. L. Kershner. 2009. Levels of introgression in Westslope Cutthroat Trout populations nine years after changes to rainbow trout stocking programs in southeastern British Columbia. *North American Journal of Fisheries Management* **29**:1271-1282.

Budy, P., G. P. Thiede, A. Dean, D. Olsen, and G. Rowley. 2012. A comparative and experimental evaluation of performance of stocked diploid and triploid brook trout. *North American Journal of Fisheries Management* **32**:1211-1224.

Campbell, M. R., C. C. Kozfkay, K. A. Meyer, M. S. Powell, and R. N. Williams. 2011. Historical influences of volcanism and glaciation in shaping mitochondrial DNA variation and distribution in Yellowstone Cutthroat Trout across its native range. *Transactions of the American Fisheries Society* **140**:91-107.

Campton, D. E. 1987. Natural hybridization and introgression in fishes- Methods of detection and genetic interpretations. Pages 161-192 in *Population Genetics and Fishery Management.* (N. Ryman and F. Utter eds) University of Washington Press: Seattle, Washington, USA

Carlsson-Graner, U., J. J. Burdon, and P. H. Thrall. 1999. Host resistance and pathogen virulence across a plant hybrid zone. *Oecologia* **121**:339-347.

Chen, X., L. Chen, J. Zhao, and Z. Yu. 2015. Modeling the hydrodynamic interactions between the main channel and the floodplain at McCarran Ranch in the lower Truckee River, Nevada*. Natural Hazards and Earth System Sciences* **15**:2161-2172.

Cobourn, J. 1999. Integrated watershed management on the Truckee River in Nevada. *Journal of the American Water Resources Association* **35**:623-632.

Coleman, M. E., and V. K. Johnson. 1988. Summary of trout management at Pyramid Lake, Nevada, with emphasis on Lahontan cutthroat trout, 1954-1987. *American Fisheries Society Symposium***4**:107-115.

Coffin, P.D., and W.F. Cowan. 1995. Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) Recovery Plan. U.S. Fish and Wildlife Service, Region I, Portland, Oregon.

DeLong, J. 2014. Cutthroats spawn at Pyramid Lake. *Reno Gazette-Journal.*

Drinan, D. P., M. A. H. Webb, K. A. Naish, S. T. Kalinowski, M. C. Boyer, A. C. Steed, B. B. Shepard, and C. C. Muhlfeld. 2015. Effects of hybridization between nonnative rainbow trout and native Westslope cutthroat trout on fitness-related traits. Transactions of the American Fisheries Society **144**:1275-1291.

Gilk, S. E., I. A. Wang, C. L. Hoover, W. W. Smoker, S. G. Taylor, A. K. Gray, and A. J. Gharrett. 2004. Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. *Environmental Biology of Fishes* **69**:287-297.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool - Status and strategy. *Science* **245**:477-480.

Harig, A. L., and K. D. Fausch. 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications* **12**:535-551.

Hickman, T. J., and R. J. Behnke. 1979. Probable discovery of the original Pyramid Lake cutthroat trout. *Progressive Fish-Culturist* **41**:135-137.

Higgins, C. L., A. Love-Snyder, W. Wiegreffe, and R. S. Pfau. 2015. Lack of hybridization between naturally sympatric populations of red and blacktail shiner (*Cyprinella lutrensis* and *C. venusta*) in Texas, but evidence of introgression among three lineages of the *C. lutrensis* species group. *Copeia* **103**:272-280.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., and

Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* ***28*****(12)**:1647-1649.

Kim, D. S., Y. K. Nam, and I. S. Park. 1995. Performance of diploid and triploid hybrid leach, *Misgurnus mizolepis* female x *M-anguillicaudatus* male. *Aquaculture* **137**:326-327.

Koenig, M. K., J. R. Kozfkay, K. A. Meyer, and D. J. Schill. 2011. Performance of diploid and triploid rainbow trout stocked in Idaho alpine lakes. *North American Journal of Fisheries Management* **31**:124-133.

Kruse, C. G., W. A. Hubert, and F. J. Rahel. 2000. Status of Yellowstone cutthroat trout in Wyoming waters. *North American Journal of Fisheries Management* **20**:693-705.

Leary, R. F., and F. W. Allendorf. 1997. Genetic confirmation of sympatric bull trout and Dolly Varden in western Washington*. Transactions of the American Fisheries Society* **126**:715-720.

Leary, R. F., F. W. Allendorf, S. R. Phelps, and K. L. Knudsen. 1987. Genetic-divergence and identification of 7 cutthroat trout subspecies and rainbow-trout. *Transactions of the American Fisheries Society* **116**:580-587.

Leary, R. F., F. W. Allendorf, and G. K. Sage. 1995. Hybridization and introgression between introduced and native fish. *American Fisheries Society Symposium* **15**:91-101.

Lippman, Z. B., and D. Zamir. 2007. Heterosis: revisiting the magic. *Trends in Genetics* **23**:60-66.

Loxterman, J. L., E. R. Keeley, and Z. M. Njoroge. 2014. Evaluating the influence of stocking history and barriers to movement on the spatial extent of hybridization between westslope cutthroat trout and rainbow trout. *Canadian Journal of Fisheries and Aquatic Sciences* **71**:1050-1058.

Maples, M. 2008. Field Trip Report: Truckee River tributaries electroshocking survey. Nevada Division of Wildlife, Reno, NV.

Metcalf, J. L., M. R. Siegle, and A. P. Martin. 2008. Hybridization dynamics between Colorado's native cutthroat trout and introduced rainbow trout. *Journal of Heredity* **99**:149-156.

Muhlfeld, C. C., S. T. Kalinowski, T. E. McMahon, M. L. Taper, S. Painter, R. F. Leary, and F. W. Allendorf. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. *Biology Letters* **5**:328-331.

Muhlfeld, C. C., R. P. Kovach, L. A. Jones, R. Al-Chokhachy, M. C. Boyer, R. F. Leary, W. H. Lowe, G. Luikart, and F. W. Allendorf. 2014. Invasive hybridization in a threatened species is accelerated by climate change. *Nature Climate Change* **4**:620-624.

Nam, Y. K., and D. S. Kim. 2004. Ploidy status of progeny from the crosses between tetraploid males and diploid females in mud loach (*Misgurnus mizolepis*). *Aquaculture* **236**:575-582.

Novak, M. A., J. L. Kershner, and K. E. Mock. 2005. Molecular genetic investigation of Yellowstone cutthroat trout and finespotted Snake River cutthroat trout. Utah State University, Report in partial fulfillment of a state of Wyoming grant agreement, Logan.

Ostberg, C., and R. J. Rodriguez. 2002. Novel molecular markers differentiate *Oncorhynchus mykiss* (rainbow trout and steelhead) and the *O. clarki* (cutthroat trout) subspecies. *Molecular Ecology Notes* **2**:197-202.

Ostberg, C. O., and D. M. Chase. 2012. Temporal Genetic Monitoring of Hybridization between Native Westslope Cutthroat Trout and Introduced Rainbow Trout in the Stehekin River, Washington. *Northwest Science* **86**:198-211.

Ostberg, C. O., and R. J. Rodriguez. 2004. Bi-parentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. *Molecular Ecology Notes* **4**:26-29.

Ostberg, C. O., and R. J. Rodriguez. 2006. Hybridization and cytonuclear associations among native westslope cutthroat trout, introduced rainbow trout, and their hybrids within the Stehekin River drainage, North Cascades National Park. *Transactions of the American Fisheries Society* **135**:924-942.

Peacock, M. M., and V. Kirchoff. 2004. Assessing the conservation value of hybridized cutthroat trout populations in the Quinn River drainage, Nevada. *Transactions of the American Fisheries Society* **133**:309-325.

Peacock, M.M. and V.S. Kirchoff. 2007. Analysis of genetic variation and population genetic structure in Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) extant populations. *Final report to US Fish and Wildlife Service*, Region 6, Reno, Nevada.

Peacock, M. M., M. Gustin, V. Kirchoff, M. L. Robinson, E. Hekkala, C. Pizzarro-Barraza and T. Loux. *In press* 2016. Native fishes in the Truckee River: are in-stream barriers and patterns of population genetic structure related? *Science of the Total Environment.*

Pritchard, V. L., L. Dimond, J. S. Harrison, C. C. S. Velazquez, J. T. Zieba, R. S. Burton, and S. Edmands. 2011. Interpopulation hybridization results in widespread viability selection across the genome in Tigriopus californicus. *BMC Genetics* **12**:1-13.

Pritchard, V. L., J. C. Garza, and M. M. Peacock. 2015. SNPs reveal previously undocumented non-native introgression within threatened trout populations. *Conservation Genetics* **16**:1001-1006.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* **27**:83-109.

Robinson, M. L., L. Gomez-Raya, W. M. Rauw, and M. M. Peacock. 2008. Fulton's body condition factor K correlates with survival time in a thermal challenge experiment in juvenile Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi). Journal of Thermal Biology* **33**:363-368.

Rosenfeld, J., M. Porter, and E. Parkinson. 2000. Habitat factors affecting the abundance and distribution of juvenile cutthroat trout (*Oncorhynchus clarki*) and coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* **57**:766-774.

Sanz, N., R. M. Araguas, R. Fernandez, M. Vera, and J. L. Garcia-Marin. 2009. Efficiency of markers and methods for detecting hybrids and introgression in stocked populations. *Conservation Genetics* **10**:225-236.

Schweber, N. 2013. 20 Pounds? Not too bad, for an extinct fish. *Pyramid Lake Journal, The New York Times*

Sumner, F. H. 1940. The decline of the Pyramid Lake Fishery. *Tranactions of the American Fisheries Society*, **69:**216-224.

van Herwerden, L., J. H. Choat, C. L. Dudgeon, G. Carlos, S. J. Newman, A. Frisch, and M. van Oppen. 2006. Contrasting patterns of genetic structure in two species of the coral trout *Plectropomus (*Serranidae) from east and west Australia: introgressive hybridization or ancestral polymorphisms? *Molecular Phylogenetics and Evolution* **41**:420-435.

Wong, A. C., and A. L. Van Eenennaam. 2008. Transgenic approaches for the reproductive containment of genetically engineered fish. *Aquaculture* **275**:1-12.

Young, W. P., C. O. Ostberg, P. Keim, and G. H. Thorgaard. 2001. Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O.clarkii clarki*). *Molecular Ecology* **10**:921-930.

Wong, A. C., and A. L. van Eenennaam. 2007. Transgenic containment of genetically engineered fish. *Transgenic Research*, **16**:862-862.

CHAPTER 3:

Use of microsatellite genotypes to differentiate between stocked triploid rainbow trout and naturalized diploid individuals in the Truckee River watershed

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ABSTRACT

The Truckee River contains a robust naturalized diploid rainbow trout (RBT) population, the result of multiple decades of planting hatchery raised rainbow trout. In recent years Nevada Department of Wildlife (NDOW) has begun planting triploid rainbow trout (trpRBT) instead of diploid in an effort to reduce the hybridization threat to reintroduced Lahontan cutthroat trout (LCT), the trout native to the Truckee River system. Salmonid samples were collected between 2007 and 2010 from the Truckee River and analyzed with co-dominant molecular markers to identify pure RBT, LCT and their hybrids; however, it was unknown if trpRBT could be easily distinguished from their diploid counterpart. To determine the feasibility of genetically distinguishing between naturalized diploids and planted trpRBT, genetic determination of triploidy was evaluated in 75 trpRBT individuals provided by NDOW hatchery personnel. Fragment analysis was carried out on these samples using 10 microsatellite markers. One-hundred percent of the hatchery triploid samples amplified three alleles for at least one locus, showing a high probability of being able to detect triploids if they were present in samples obtained from the river. Trout samples (n= 3,448) from 10 sampling locations along the Truckee River and its tributaries were identified to species (LCT, RBT, or HYB) and then evaluated with this suite of microsatellite markers. Of the salmonid samples identified as RBT, 91.9 % were identified as diploid, with only 8.1% identified as trpRBT. Our results indicate that there is a large naturalized RBT population in the river; although there is evidence that the some of the stocked trpRBT persist to adult size; however, they

make up a very small proportion of the rainbow trout in the river and have had potentially little impact to date on the breeding population of naturalized rainbow trout. The ability to distinguish stocked trpRBT from the naturalized RBT will allow for continued monitoring of naturalized RBT population structure in the river and may assist in recovery efforts for LCT by reducing hybridization risk.

INTRODUCTION

Global biodiversity is rapidly declining, and this contemporary drop in number and variation of species within ecosystems is considered by many to be the “sixth extinction,” comparable to the five major extinction events found in the fossil record (Chapin et al., 2000; Frankham, 2005; Pimm et al., 2014; McCallum, 2015). Fresh water ecosystems are especially vulnerable to a wide variety of threats, such as overfishing and over exploitation, habitat loss and fragmentation, as well as hybridization and competition with invasive species, leading to a large number of threatened and endangered species (Harig et al., 2000; Clausen and York, 2008; Krebs et al., 2010). One of the most significant causes of biodiversity loss is biological invasions and introductions of non-native species. Humankind has translocated species both deliberately and unintentionally, with many of these species becoming established and invasive in their new habitat (Vitousek, 1997).

Intentional translocations of fish species to create or enhance angling opportunities have been occurring for centuries, with little concern for the impact on native fauna (Gozlan et al., 2010). Depletion of natural aquatic resources due to overfishing, pollution and habitat destruction due to industrialization and logging practices led to fish culture-farming and raising artificially propagated fishes in the United States in the late 1800s (Halverson, 2010). Since that time, this practice has become commonplace, and a variety of native and non-native fishes have been planted throughout the world. Worldwide, freshwater fish are the most commonly introduced taxa, and lack of containment of introduced species has resulted in the decline of many native fish and other aquatic organisms (Hitt et al., 2003; Reissig et al., 2006; Sharma et al., 2011; Neville and Dunham, 2011). For example, tilapia, *Oreochromis spp,* are listed as 66th most invasive species on the global invasive species database (Ovenden et al., 2015). This species is often introduced into new waterways through human mediated translocations, as they are used for a food source or for bait. Tilapia is adapted to live in high densities and harsh living conditions, which increases their invasion success (Ovenden et al., 2015). The high density of tilapia negatively impacts community ecology of native fishes by degrading riverbeds and impacting spawning habitat through aggression towards other species (Martin et al., 2010), as well as causing reductions in water quality (Doupe and Burrows, 2008). Regulations against transporting this fish are in place; however, it continues to be introduced into new systems (Ovenden et al., 2015).

Another widely introduced non-native fish species is the rainbow trout, (*Oncorhynchus mykiss,* RBT). This species has been introduced into waters in at least 99 countries, producing naturalized populations and heavily impacting native fishes (Stankovic et al., 2015). Introduced RBT have been shown to damage populations of native Atlantic salmon and brook trout through competition for habitat (Thibault and Dodson, 2013), reduced survival in Atlantic salmon due to increased stress (Houde et al., 2015), and depleting small endemic fishes through predation (Shelton et al., 2015). Another major effect of RBT introduction is hybridization, which threatens steelhead (Page et al., 2011) , and Redband trout (Kozfkay et al., 2011) both native *O. mykiss* subspecies and multiple cutthroat trout subspecies (Campbell et al., 2002; Bennett, et al., 2009; Loxterman et al., 2013; Pritchard et al., 2015). However, because these fish are easy to propagate in captivity, easy to transport and desirable for angling, RBT have been planted in fishless watersheds or in watersheds where the native fish has been extirpated, in addition to streams occupied by native salmonid species. In the 1930s and 40s, the native Lahontan Cutthroat trout (*Oncorhynchus clarkii henshawi,* LCT) was extirpated from the Truckee River watershed, which flows from Lake Tahoe, California to Pyramid Lake, Nevada. The main causes for the decline of the fishery were overfishing and water diversions after the construction of Derby Dam in 1905 (Sumner, 1940). To rebuild the fishery, RBT was planted in the Truckee River and has become naturalized throughout the watershed. Water conditions and lack of spawning habitat precluded naturalization in Pyramid Lake (Coleman and Johnson, 1988). However, in 1975, LCT was classified as threatened under the United States Endangered Species Act. Recovery efforts by USFWS have included attempts to re-introduce this trout into its native habitat in the Truckee River watershed. The naturalized RBT populations are an impediment to these efforts. State and federal agencies face continued pressures to stock RBT for angling opportunities; however, it is imperative that some sort of containment be put in place to minimize the ecological impacts of this practice.

One method that can be used for genetic containment of an aquaculture species is to induce triploidy. Triploid fish have been planted in the Truckee River watershed for the past decade to enhance angling opportunities, while allowing for

LCT recovery and limiting hybridization. Although there is evidence for some behavioral and histological differences in triploid fishes, triploids are morphologically very similar to their diploid counterparts (Benfey, 1999; Budy, 2012.) This is argued by some to contribute to increased survival, better flesh quality and more rapid growth because the organism does not waste energy on sexual maturation (Thorgaard et al., 1981; Tiwary, 2004). On the other hand, triploids have also been found to have reduced fitness at higher temperatures (Fraser et al., 2012; Verhille et al., 2013), higher probability of deformities (Fraser et al., 2012) and slower growth of triploid fry (Quillet and Gaignon, 1990; Schafhauser-Smith and Benfey, 2001). There are many confounding variables that contribute to the contradictory finding on the fitness of triploids in comparison to diploids (Benfey, 1999); however, given the plausible sterility of triploids and the fact that triploids are often stocked as a "put and take" fishery and are not expected or needed to sustain in the watershed, triploid fish are the primary fish stocked in cases where reproductive containment is desired (Tiwary, 2004). The impact of planting triploid RBT in regions with an existing naturalized RBT population, such as that in the Truckee River, has not been evaluated.

In order to determine whether or not stocking of triploid RBT is likely to be an effective management tool for the reproductive containment of the RBT, it is necessary to have a protocol that can reliably identify triploid versus diploid individuals in the watershed. Previous studies looking at estimating the proportion of triploids and diploids in their natural environment focused on naturally occurring triploids that appear in various animal and plant species (Krieger and Keller, 1998). This process in nature appears to be the product of chromosomal changes due to hybridization between sympatric species with different numbers of chromosomes where reproductive isolation barriers have broken down because of changes in distribution (Kim et al., 1995; Nam et al,. 2004) or environmental changes that result in meiotic errors (Nanda et al., 1995). In these cases, triploids may coexist with diploids of the same species or may form an isolated triploid population, more commonly occurring in marginal habitats (Coates, 1995). Triploidy produces more genetic variation within the individual and is thought to increase survival in some cases (Coates, 1995; Piferrer et al., 2009). Previous studies have used microsatellite and allozyme markers to identify proportion of triploidy in a population based on heterozygosity and number of alleles (Ridout, 2000; Krieger and Keller, 1998).

Microsatellites are non-coding, highly variable, repetitive regions of DNA found throughout an organism's genome. In diploid organisms, if an individual is a heterozygote, they will display two distinct alleles for a microsatellite locus, and that is the maximum number of alleles that a single individual should display. This could be an effective tool to differentiate triploid RBT from diploid RBT because triploids could have three copies of a particular locus, and display three alleles. It is possible, however, that the triploidy may not be detected. The egg could retain an "identical" polar body and thus, would not result in three alleles. Multiple copies of the same allele could result in a higher expression of that allele in PCR, but "allelic dosage" is not always able to be determined accurately (Ridout, 2000). In addition, if heterozygosity is low for a particular locus, that locus would not be informative. The individual could appear homozygous, and have three copies of the same allele. Previous studies (Kreiger and Keller, 1998; Ridout, 2000) used maximum likelihood of an allele and allele frequency differences found in triploid and diploids from the same population to determine the proportion of triploids in the population. Populations containing triploids have been shown to have higher heterozygosity than diploid population from the same genetic background; however, these tools would not be very informative, and would not necessarily be applicable to populations that contain both triploids and diploids if the triploids were created from an unrelated hatchery stock and different allelic frequencies. These techniques cannot distinguish individual triploids from diploids in the population, which would allow for genetic investigations of only the diploid or the triploid populations or individuals.

The goals of this study were to evaluate the effectiveness of microsatellite loci at detecting triploidy in known hatchery triploids and in fish sampled from the Truckee River, where triploids are stocked sympatrically with naturalized diploid RBT. I specifically test the hypothesis that, due to crossing over and variation in microsatellite loci, there will be at least one nuclear marker out of multiple variable microsatellites that will display three alleles in the known triploids. This suite of microsatellite markers will then be used to screen RBT found in the Truckee river to address the following questions: (1) Are triploids sustaining and over wintering in the Truckee River? (2) What proportion of the RBT found in the Truckee River are triploid? Conversely, what proportion of RBT found in the river are naturalized diploid individuals, and (3) what are the implications of these results for LCT recovery?

METHODS

Study system and stocking history

The Truckee River flows from oligotrophic Lake Tahoe in California to endorheic Pyramid Lake in Nevada. For more than a century, state and federal agencies have been stocking non-native salmonid species in the Truckee River watershed to support recreational fishing. Stocking is carried out primarily by the United States Fish and Wildlife Service (USFWS) and the Nevada Division of Wildlife (NDOW). Currently, approximately 100,000 salmonids are stocked into the Truckee River annually , including native Lahontan cutthroat trout (LCT) and non-native rainbow trout , which in the past 10 years have been primarily non-reproductive triploid RBT(Figure 1A, public records NDOW and USFWS). Rainbow trout are stocked as “catchables,” with an average fork length of 246 mm (Figure 2A). LCT has been the focus of reintroduction efforts in the Truckee River. Therefore, in hopes of increasing the probability of reintroduction, it was stocked in high numbers and various sized classes (Figure 2B). LCT, native to the Lahontan hydrographic basin which includes the Truckee River, was extirpated from watershed, including Lake Tahoe and Pyramid Lake, in the 1930s and 1940s because of water diversions, pollution and competition and hybridization with non-native salmonid species

(Coffin and Cowen, 1995; Cordone and Frantz, 1968). The emphasis on trying to maintaining the historical fish assemblage in Pyramid Lake, along with the limited success of RBT in the lower Truckee River and Pyramid Lake, allowed for establishment and continued maintenance of a LCT fishery, created with LCT from other populations, and managed by the Pyramid Lake Paiute Tribe (http://www.pyramidlakefisheries.org/). Past efforts to re-introduce LCT into Lake Tahoe and the Truckee River have been largely unsuccessful (Cordone and Frantz, 1968). Due to the placement of the LCT on the United States Endangered Species list in 1973, re-introduction of LCT into the Truckee River watershed has become a priority (Coffin and Cowen, 1995). LCT was stocked in high numbers during the 2004-2010 from both the Pyramid Lake contemporary strain, as well as the Pilot Peak strain, which has been shown by morphological and genetic comparisons to be descended from the historical Pyramid Lake strain (Hickman and Behnke, 1979; Peacock and Kirchoff, 2007). In that same time period, NDOW stocked primarily triploid RBT in attempts to limit hybridization (Figure 3). However, the factors that contributed to the original decline of LCT in that watershed are still of concern. This watershed presents unique challenges for reintroduction of LCT due to river diversions, non-native salmonids and especially the hybridization threat posed by naturalized rainbow trout (Chapter 2).

Study species

RBT is a cold water fish species native to the west coast of North America. They are one of the most widely transplanted fish globally and have been stocked in the Truckee River predominantly over the past century (Cordone and Frantz, 1968). Rainbow trout have known stream resident, migratory, and anadromous forms and, historically, were found in rivers that drain into the Pacific Ocean from Mexico to the Aleutian Islands (Halverson, 2010). Although triploid, non-reproductive RBT are primarily stocked in the Truckee River, a large naturalized trout population is found throughout the river and its tributaries. RBT spawn in the early spring and are known to readily hybridize with LCT.

Triploid RBT can be made by heat-shock of the eggs (Thorgaard et al., 1981) or chemical or pressure treating the eggs to retain a polar body (Chourrout, 1984); thus the fertilized egg contains three copies of the genome. Triploid fishes have irregular meiotic division of chromosomes and have reduced gonad development, either failing to make gametes or resulting in aneuploid gametes, rendering them non-reproductive (Wong, 2007). Because these fishes are sterile, they are stocked in areas for the sole purpose of being fished out by recreational anglers and provide augmentation to the fishing in the area. Normal ovum are removed from the female, and the eggs(n) are triggered through heat shock for 5 minutes at 32°C to retain a polar body creating a diploid ovum (2n). The diploid eggs are created and fertilized, and triploidy is confirmed by flow-cytometry or hematology because the nuclei of triploid cells have 50% more DNA than the diploid counterpart (Benfey et al., 1986). The fish are reared and sold to various consumers and agencies when they reach the desired size class. Once planted in an existing ecosystem, there is no obvious morphological difference between the triploids and the naturalized “wild” RBT in the system.

Sample collection

Salmonid samples were collected by United States Fish and Wildlife Service (USFWS) and Nevada Division of Wildlife (NDOW) from 2007 through 2010. USFWS collected samples from 8 primary sampling sections. Each sampling section had 3 to 4 transects depending on the variation in habitat types and barriers within each site (Figure 3, Table 1). Table 1 lists each major sampling site, along with the latitude and longitude of each transect within that site, plotted in Figure 3 to present an overlay of the sampling sites and the relative amount of stocking of LCT and RBT. Transects were 500 m long and both shore line and mid-stream regions. Raft, barge and backpack electrofishing methodologies were utilized to ensure all age classes were adequately sampled. Fin clips were placed in wax paper, dried, and stored in individual coin envelopes labeled with species (when possible), fork length, date collected and location. NDOW collected samples from the two main tributaries (Hunter Creek and Dog Valley Creek) in 2007 and 2008, and collected from two transects below the diversion ditch barrier(Hunter Creek) and below the California State Line (Dog Valley Creek). In 2009, the tributaries were also sampled by USFWS; for this sampling period, samples were collected from two transects above the barrier and the California State Line, in addition to the sites overlapping the NDOW collection sites.

DNA isolation and PCR amplification

DNA was isolated using DNeasy96 Blood and Tissue Kits (QIAGEN) according to the manufacturer's protocol. Double stranded DNA was quantified at the Nevada Genomics Center using a fluorescent nucleic acid stain (PicoGreen®) and read on a Labsystems Fluoroskan Ascent fluorescence plate reader, which measures only the double stranded DNA. RBT microsatellites loci were selected from the literature (Rexroad et al., 2002; Palti et al., 2002; Rexroad and Palti, 2003) based on high number of alleles and ability to multiplex in PCR reactions. In addition, three LCT loci that were found to be highly variable and cross-amplified in RBT were also used (Peacock et al., 2004; Robinson et al., 2009). All primer sequences, heterozygosity, range and number of alleles, and primer conditions are listed in Table 2.

Locus primers were ordered with one of four unique M13 tails to allow for an economic method to label the PCR product with a corresponding fluorescent dye (Schuelke, 2000). The basic tailed primer protocol was amended to include 4 and 7 loci per reaction. A multiplex primer cocktail was prepared to give a final primer concentration of 0.05 μM each tailed forward primer, 0.2 μM each reverse primer, and 0.1 μM each labeled M13 primer in a 12 μl reaction. PCR reactions included 6 μl Multiplex taq (1X final concentration) (QIAGEN), and 50 ng of DNA was used whenever possible. PCR cycle parameters included a 15 minute hot start at 95°C, followed by 41 cycles of 95°C for 30 seconds, annealing temperature for 90 seconds, and 72°C for 30 seconds. Species specific primers were chosen to have an optimal annealing temperature from 58-63°C, allowing for a touch-down PCR reaction that has 7 cycles with an annealing temperature of 65°C, 7 cycles at 61°C, 7 cycles at 58°C, and 20 cycles at 55°C in which the first 21 cycles are amplifying a specific primer and the final 20 cycles are amplifying with the fluorescently labeled M13 tail. PCR reactions were completed using 96 well format on an MBS Satellite 0.2G Thermal Cycler (Thermo Electron Corporation). PCR products were diluted to an appropriate concentration determined by dilution tests from 1:50 to 1:200. One micro-liter of diluted PCR product was added to 19 μl of size standard (Applied Biosystems) prepared by adding 5 μl of LIZ500 size standard to 1 ml of HiDye Formamide and 0.5 ml of molecular grade water. Fragment analysis was carried out on an Applied Biosystems 3730 Genetic Analyzer and all alleles generated were scored, binned, and given allelic and genotypic designation using the ABI GeneMapper software (version 3.7)(Applied Biosystems, Grand Island, NY).

Genetic Analysis

The ability to distinguish triploids was tested using 75 known triploid fish provided by Troutlodge, Inc (www.troutlodge.com) (n=25) and NDOW Mason Valley hatcheries (n=50). "Triploidy" was calculated for each individual provided by the hatchery by summing the number of loci that had three alleles divided by the number of loci that successfully amplified. For the purpose of this study, I did not attempt to look for "hidden" triploids by trying to identify allelic dosage, as that would complicate analysis by requiring careful examination of each chromatogram and would result in more human error since peak intensity is not always consistent; given that 100% of the known triploids were detectable without looking at peak intensity, doing so was not necessary for this study.

The overall triploidy of each locus was determined by looking at the number of individuals that had three alleles for that locus divided by the total number of individuals amplified. This value was determined first for the hatchery provided known triploids based on samples and compared to the value for the same locus on the survey samples identified as triploid. Microsatellite Toolkit, an Excel add-in (Shaibi et al., 2008), was used to calculate observed (Ho) and expected (He) heterozygosity for each locus using the genotype frequencies of 94 rainbow trout surveyed in the Truckee River that were scored as diploid, having two or less alleles at all loci screened. Because previous studies reported that triploid individuals have higher heterozygosity (Koenig et al., 2011; Kriger and Keller, 1997) observed and expected heterozygosity of the triploids was also calculated for the hatchery and Truckee River triploid RBT. A random selection of any two of the three alleles was used for loci that displayed three alleles. Linear regression was done comparing reported heterozygosity and number of alleles reported in the literature per locus, with the ability of the locus to detect triploidy. This was used to search for a correlation that could permit a more efficient choice of loci used based on effectiveness of detecting triploids, allowing for use of fewer microsatellite loci to identify triploids in a natural setting.

Samples collected during the 2007-2010 surveys conducted on the Truckee River were first identified as RBT, LCT or hybrids based on bi-parentally inherited nuclear molecular markers (Ostberg, 2004; Chapter 2). For each year of the study, samples collected from the Dog Valley and Hunter creeks tributaries and the main stem Truckee River by NDOW biologists were combined with the samples collected from the 8 primary transects on the Truckee River collected by USFWS biologists. A total of 2,478 RBT DNA samples were analyzed using 11 microsatellite loci (Table 2). If an individual sample had three alleles at one of the 11 microsatellite markers, it was considered to be a triploid. Samples from the Truckee that did not show three alleles at any of the 11 loci were considered diploid. Fork length data was analyzed for all triploid individuals identified in the survey to determine if the triploid RBT were surviving and could potentially compete with and interfere with the naturalized RBT reproduction. Given that the average fork length of stocked triploid Rainbow Trout was 246 mm, triploids with 300 mm fork length or less were considered newly stocked and those that were over 300 mm were considered to have survived at least one winter.

RESULTS

Multiple alleles were evident when using microsatellite loci to identify triploids. Figure 4 shows a representative chromatogram on a single individual for the three panels. Within each panel, 3 or 4 molecular markers were run in a multiplex reaction and can be clearly distinguished by size or florescent dye. For the individual shown, OCH15, OCH17, OMM1036, OM1322, and OMM1329 clearly show three distinct alleles. Two of the remaining four loci (OMM1315 and OMM1220) show anomalies in intensity of the PCR product that are consistent with having extra copies of the allele marked with an "*" and the remaining loci appeared as diploid. From the 75 known triploid samples provided, four samples did not amplify successfully because of low DNA yield and were not included in this dataset. Twenty-three out of 25 genetic samples provided from Troutlodge and 48 out of 50 samples provided from Mason Valley hatchery successfully amplified at 10 of the 11 microsatellite markers used in this study. 100% of the known triploid individuals showed three alleles in at least one of the 10 loci used, and were unambiguously triploid (Figure 5). The average triploidy over all loci was 0.42 for Trout Lodge, 0.40 for Mason Valley and 0.27 for triploids found in the Truckee River (Table 3). Observed and expected heterozygosity was calculated for all samples and is included in Table 3. Expected heterozygosity was slightly lower than observed heterozygosity and consistent in the Troutlodge triploids and the Mason Valley triploid hatchery strains (P <0.05). There was no difference between expected and observed heterozygosity found in the Truckee River triploids. Heterozygosisty was higher in the triploid populations than what has been reported in the literature, with the average over all ten loci reported as 0.56 in the literature and overall for this study was 0.77-0.88 (Palti et al. 2002; Rexroad and Palti, 2003; Peacock et al., 2004; Robinson et al., 2009). There was no correlation between published heterozygosity or the number of alleles with the ability of the locus to pick up triploidy (Figure 6).

A total of 2,478 samples were amplified with 11 microsatellite markers. This included samples identified as hybrids and LCT; none of the individuals identified as hybrid or LCT showed three alleles at any of the loci used in this study. Bi-parentally inherited genetic markers identified 2,279 samples as pure RBT (Chapter 2), 186 of those RBT were designated as triploid (8.1%). The overall proportion of diploid and triploid RBT found in the Truckee was fairly consistent over the 4 years of the study (Figure 7A). Triploid RBT were found primarily from the Truckee through the Reno transects, consistent with where the triploids were reported as stocked, with the exception of Truckee transects (Figure 7B). The Truckee transects are in a region of the river that is sometimes stocked with triploid trout by private angling companies for special angling events, and not reported in the public stocking record. It is possible that the large triploids found in the Truckee region are remnants of that event that did not get fished out in 2009 and 2010 (Tim Loux, USFWS, personal communication).

The size class and distribution of the triploids was also consistent over the 4 years sampled (Figure 9), with the exception of 2009, for the reason discussed above. Generally speaking, the largest proportion of triploids found were in the stocked size class, though for each sampling year, there was a proportion of triploids that had overwintered and were sampled the following year. In 2009, the largest size class was over 300 mm, and these fish were found in large numbers in Truckee River (Figure 9). Unexpectedly, each year there were a few triploid fish found that were < 100 mm, smaller than the reported stocked triploids (200-299mm).

With the exception noted above, the triploid fish are found primarily where they were stocked, though they were found upstream of Verdi in Farad in large numbers, showing that they may move several miles. Interestingly, there were also small numbers of triploids identified in the section of the river closest to Lake Tahoe, and there are no records of triploids being stocked in that region.

DISCUSSION

Using three panels of microsatellite markers, I was able to unambiguously confirm that the hatchery RBT provided were indeed triploid fish. Surprisingly, there was no correlation between number of alleles or heterozygosity of a locus and its ability to detect triploidy (Figure 6). This could be because I selected only markers that had over 7 alleles and moderately high heterozygosisty (over 0.5) in the literature, and the variability found in the populations used for the published data was clearly different than for the samples from the hatcheries. Three of the known triploids showed only one locus with three alleles, but triploidy was indicated on the average of 4.1 of the 10 loci used.

Although I was able to identify all of the known triploids easily, there was some ambiguity when applying these loci to the identification of triploid fish in the river. First, there were a lot more individuals that were identified as triploid at only one locus (average number of triploid loci was 2.7). I went back to the traces on those individuals and double checked genotyping and found that many of the chromatograms showed irregular intensities on the diploid peaks (see "*" in Figure 4). Most likely there was less variability in the triploids found in the Truckee River, so heterozygosity and triploidy across all loci was lower.

Another unexpected finding in the analysis of triploidy was the prevalence of triploids that were smaller than 100 mm in each year of the sampling. These triploids came from different transects and had between 1 and 5 triploid alleles. This is unexpected because triploids are stocked in catchable size (average size 246 mm) and there should be no young-of-the-year triploid fish produced. This could be an indication of unsuitable spawning habitat or increased water temperatures in spawning sites and in situ production of triploid individuals. Triploidy can be reliably induced by holding the eggs at 32°C for 5 minutes, so perhaps spontaneous triploidy happens in a small number of young of year due to increased water temperatures. An increase in triploidy can be an indicator of marginal habitat (Coates, 1995; Piferrer et al., 2009).

One of the goals of this research was to determine if planted triploid fish were surviving and overwintering in the Truckee River. Although stocked salmonids consist primarily of triploid RBT and LCT (Figure 1B), less than 10% of the RBT identified in this study were triploid. This shows a low survival of triploid individuals, many of them are rapidly fished out of the river or unable to adapt to a wild environment, resulting in high mortality of stocked fishes. However, there is evidence that a small percentage of the triploid RBT do persist in the river for more than one year. In all four years of the study, there were many triploids larger than 300 mm. This indicates that either the triploids were stocked in a larger size class (likely in 2009) or that about 50% of the triploids that were not immediately removed from the river were able to find shelter and food in order to overwinter and grow. Figure 8 shows that in 2007, very few triploids were in the larger size class. This proportion increased in 2008 and 2009. Environmental conditions, temperature, water flow and ability to find refugia can contribute to survival.

The most important implication in this research for LCT recovery is that triploid individuals appear to have had little impact on naturalized RBT in the system to date. Surprisingly, they make up a very small percentage of the overall catch in the river. This may be a reflection the large population size for naturalized RBT. Although stocking of triploids is preferable to stocking of reproductive diploid RBT, resources may be better allocated for RBT removal to allow for reintroduction of the native LCT into this system. Reintroduction success for LCT is hampered by the presence of RBT in the Truckee River. LCT-RBT hybrids were found in every section of the river where LCT were planted and introgression is evident, indicating that hybridization has been occurring in this system for several years (Chapter 2). LCT re-introduction into its native habitat cannot occur without the eradication of the reproductive RBT in this system. It is possible that with reduced flows in the river due to drought, reproduction in RBT could be minimized, and planting of triploids could, over time, allow for angling, while weakening the naturalized RBT population. However, reinvasion of RBT would be likely in the river unless it was completely eradicated or barriers are constructed to isolate the re-introduced LCT population.

This research shows that 10 microsatellite markers are sufficient for differentiating stocked triploids from the naturalized RBT. This allows for the simultaneous amplification of all RBT in the system. Any individuals that display three alleles can be unambiguously identified as triploid. The allelic data on the diploid individuals can be retained and used to answer questions about the population structure and reproduction in the naturalized RBT. Population structure of the RBT can be used to look at dispersal patterns and reinvasion potential, as well as to identify vulnerable portions of the population that may be eradicated. Being able to identify the stocked triploid fish allows for needed tools to assist in LCT recovery.

Figure Legends

Figure 1 Salmonid Stocking in the Truckee River. (A)Salmonid stocking varied as the priorities for LCT recovery shifted. Early stocking (1993 and before) was mainly RBT. Recovery efforts for LCT started in 1994, but hybridization and low survival of LCT became a problem. (B) Salmonids stocked between 2004-2010 show larger numbers of LCT and triploid RBT stocked. Few diploid RBT were stocked.

Figure 2 Comparison of size variation of stocked salmonids. (A) Rainbow trout were stocked in sizes between 200 and 250mm, primarily as catchables for a “put and take” fishery. (B) LCT were stocked either as fry or as larger fish in hopes that they could sustain in the river.

Figure 3. Stocking sites and proportion of species stocked on the Truckee River for the time period 2004-2010. Stocking latitude and longitude were plotted (yellow squares) and grouped together based on nearest disinguishable sampling transect. The 8 major sampling sites are written in bold. Transects are indicated by blue diamonds. Size and color of circles represent the relative proportion of Lahontan Cutthroat trout (LCT), triploid rainbow trout (trpRBT) and diploid rainbow trout (dipRBT) stocked, shown in sampling locations.

Figure 4. Sample chromatogram of single triploid individual run on three Rainbow Trout microsatellite panels. Three alleles are evident in OMM1322, OMM1329, OMM1036 and OCH15. The two peaks indicated by an (*) indicate that the alleles may be double in intensity of normal when compared to a diploid chromatogram.

Figure 5. Detection of triploidy on hatchery supplied trpRBT. A. 48 individual triploids were identified from Mason Valley Hatchery (red). B. 23 triploids were

confirmed from Trout Lodge Hatchery (blue). The number of triploid loci found in each fish is on the x-axis.

Figure 6. Association between heterozygosity and number of alleles on a locus ability to detect triploidy. (A) Linear regression of published heterozygosity and triploid detection shows no correlation. (B) Linear regression of published number of alleles and triploidy dectection shows no correlation.

Figure 7. Triploids identified in Truckee River RBT. (A) Solid bar represents the proportion of triploids found in the Truckee River sampling. Hashed bar represents the portion of diploids found. (B) Triploids organized by river segment and year of the study. The number of fish found in each segment is represented on the x axis. River segments from south to north are represented on the y-axis. Most triploids were found between Farad and Reno, near to stocking sites, indicating that they

Figure 8. Fork length of triploids found in the Truckee River. (A)Fork length of each individual was plotted. The boxed area represents the reported size class of the triploids in this study. (B) Triploids were grouped into size classes for each year of the study.

Table 1. List of all transects (T) per section (S) elevation, latitude and longitude. (S8 = Tahoe, S7 = Truckee, S6 = Farad, S5 = Verdi, S4 = Reno, S3 = McCarran, S2 = Wadsworth, S1 = Nixon and two tributaries)

Transect	Section(S) Transect (T)	Elevation (ft)	Latitude	Longitude
1. Tahoe-A	S8T2	6225	39.16207	-120.159
2. Tahoe-B	S8T3	6079	39.20848	-120.198
3. Tahoe-C	S8T1	5909	39.27361	-120.206
4. Truckee-A	S7T3	5751	39.33366	-120.164
5. Truckee-B	S7T2	5676	39.34754	-120.123
6. Truckee-C	S7T1	5598	39.36092	-120.117
7. Farad-A	S6T1	5472	39.38554	-120.086
8. Farad-B	S6T2	5400	39.36848	-120.041
9. Farad-C	S6T4	5169	39.42332	-120.035
10. Farad-D	S6T3	5076	39.4497	-120.005
11. Verdi-A	S5T1	4984	39.475	-119.994
12. Verdi-B	S5T3	4854	39.50926	-119.996
13. Verdi-C	S5T2	4637	39.50651	-119.902
14. Reno-A	S4T3	4500	39.52391	-119.819
15. Reno-B	S4T2	4448	39.53013	-119.795
16. Reno-C	S4T1	4394	39.51424	-119.736
17. McCarran Ranch-A	S3T3	4309	39.52563	-119.61
18. McCarran Ranch-B	S3T2	4284	39.54498	-119.587
19. McCarran Ranch-C	S3T1	4277	39.54703	-119.573
20. McCarran Ranch-D	S3T4	4202	39.56469	-119.487
21. Wadsworth-A	S2T1	4189	39.58506	-119.444
22. Wadsworth-B	S2T2	4132	39.59062	-119.368
23. Wadsworth-C	S2T3	4099	39.61273	-119.306
24. Nixon-A	S1T1	3988	39.72697	-119.319
25. Nixon-B	S1T2	3903	39.81771	-119.35
26. Nixon-C	S1T3	3850	39.85426	-119.394
27. Hunter Creek	Tributary	5042	39.49131	-119.899
28. Dog Valley Creek	Tributary	5715	39.56431	-120.026

Table 2. Primers selected for identification of Triploid Rainbow trout. Product size and number of alleles (N_A), annealing temperature T_A(°C), observed heterozygosity H_O are reported as found in cited references.

Locus	Dye	Panel	Primer sequence (5'-3')	Product size (N_A)	T_A(°C)	H_O	GenBank	Reference
OCH15	Vic/Pet	1	F: ACCCGAGTTTCAGAGCATGTC RTTTGGGATCATCGTTATTATGGA	286-366 (21)	60	0.656	AY374432	Peacock et al. 2004
OCH17	Pet	1	F: CTGCCGTCTCTGAACTTGTCCAC R: GCTCAGGGGCAGAACGACAAATA	201-281 (16)	60	0.407	AY374434	Peacock et al. 2004
OCH20	Fam	1	F: CCCACGACAGAGCCCTACTAGAT R: CCTGGCCGTGCTTATGATAACTC	227-319 (16)	58	0.604	DQ979815	Robinson et al. 2009
OMM1315	Vic	3	F: TACAGGGCTTGGCTCTATCTC R: GCCAAATACTTTCGCAAGG	111-131 (6)	58	0.53	G73554	Palti et al. 2002
OMM1322	Fam	2	F: GCGCTCCTTTCATCTCTGATACAG R: GGTGAATACTTTCGCAAGCC	162-230 (12)	64	0.39	G73560	Palti et al. 2002
OMM1323	Fam	3	F: CTTTTTGCCAGCTCTGCTATGACA R: CATTACAGCACAACTACGAAACCC	106-206 (19)	58	0.6	G73561	Palti et al. 2002
OMM1036	Vic	2	F: TGTAGCAGGTGAGAATACCCA R: CACCATCTCCATCCTAGGC	210-278 (15)	60	0.563	AF346686	Rexroad et al. 2002
OMM1220	Pet	2	F: CTCTGGGACAGACTTATCAC R: CTATTGGACGATGCACAC	135-221 (16)	58	0.701	AF470002	Rexroad and Palti 2003
OMM1302	Ned	3	F: AGCCAGCCAATTAATACCCTG R: TTCTGTGTGGCCTAAACCTT	192-272 (15)	58	0.67	G73542	Palti et al. 2002
OMM1329	Fam	3	F: GGGAAGTGTTCACCATTACACAAG R: CATCCAGGAACGCACCTTTA	142-174 (10)	58	0.5	G73564	Palti et al. 2002
OMM1325*	Pet	3	F: TCTCTGCCAATGTGACATGCCT R: TAACTATCACTGCCACTCCTCGTG	253-297 (7)	58	0.5	G73562	Palti et al. 2002

*OMM1325 failed in PCR on Trout Lodge Triploids so was not included in Truckee River data. Used to ID triploids and for structure analysis of diploids in the Truckee River

Table 3: Comparison of Genetic variation found in RBT hatchery provided triploids, triploids and diploids found in the Truckee River.

Trout Lodge Triploids n=23

Locus	TRP	He_{ob}	He_{ex}	Range	N_A
OCH15	0.74	0.96	0.88	298-378	9
OCH17	0.83	1.00	0.86	201-289	8
OCH20	0.27	0.95	0.81	227-315	8
OMM1315	0.48	1.00	0.83	122-134	7
OMM1322	0.24	0.86	0.77	195-251	6
OMM1323	0.05	0.65	0.68	206-218	4
OMM1036	0.57	0.91	0.83	234-294	8
OMM1220	0.43	0.87	0.81	151-191	6
OMM1302	0.21	0.74	0.87	229-301	9
OMM1329	0.39	0.87	0.80	177-205	6
AVERAGE	0.42	0.88	0.81	---	7.10

Mason Valley Triploids n=47

Locus	TRP	He_{ob}	He_{ex}	Range	N_A
OCH15	0.64	0.98	0.86	306-354	11
OCH17	0.62	0.98	0.82	201-289	9
OCH20	0.43	0.94	0.82	227-315	8
OMM1315	0.34	0.96	0.75	134-158	5
OMM1322	0.43	0.87	0.83	195-279	8
OMM1323	0.17	0.76	0.68	206-222	5
OMM1036	0.15	0.83	0.80	234-203	9
OMM1220	0.44	1.00	0.86	151-203	9
OMM1302	0.16	0.61	0.82	217-301	9
OMM1329	0.64	0.91	0.80	177-205	7
AVERAGE	0.40	0.88	0.80	---	8.00

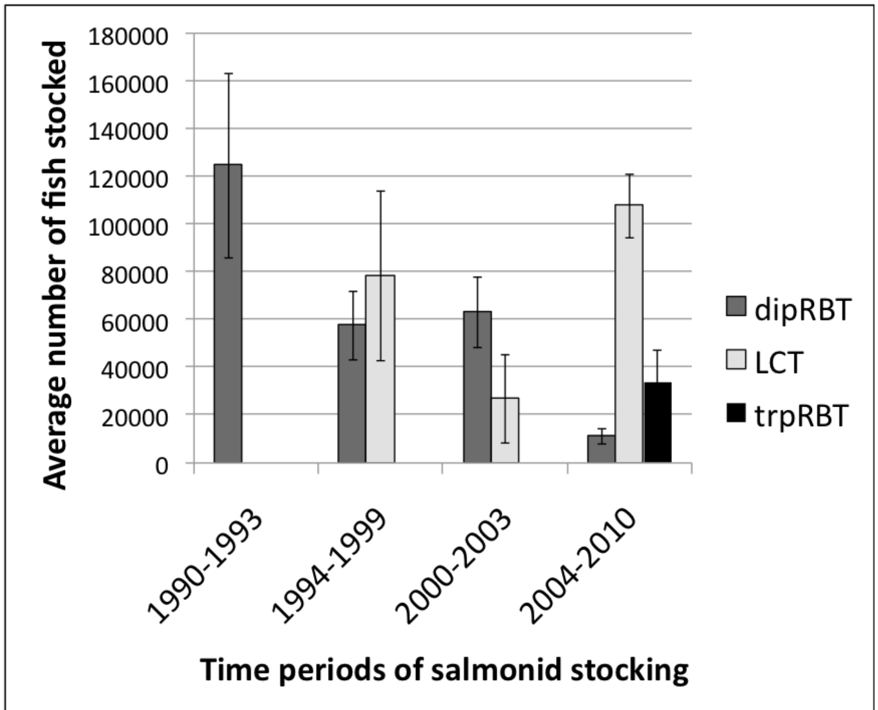
Truckee River Triploids n=123

Locus	TRP	He_{ob}	He_{ex}	Range	N_A
OCH15	0.53	0.92	0.92	298-378	18
OCH17	0.44	0.89	0.85	199-289	14
OCH20	0.15	0.74	0.87	227-319	19
OMM1315	0.31	0.79	0.78	122-158	8
OMM1322	0.27	0.83	0.88	195-271	13
OMM1323	0.06	0.62	0.67	206-226	5
OMM1036	0.17	0.86	0.82	234-310	14
OMM1220	0.24	0.85	0.85	151-215	10
OMM1302	0.11	0.65	0.85	217-301	17
OMM1329	0.44	0.87	0.78	177-209	8
AVERAGE	0.27	0.80	0.83	---	12.60

Truckee River Diploids n=94

Locus	TRP	He_{ob}	He_{ex}	Range	N_A
OCH15	0.00	0.76	0.76	286-366	21
OCH17	0.00	0.83	0.83	201-281	16
OCH20	0.00	0.86	0.86	243-311	19
OMM1315	0.00	0.78	0.78	122-158	7
OMM1322	0.00	0.86	0.86	207-271	17
OMM1323	0.00	0.60	0.60	206-222	5
OMM1036	0.00	0.85	0.85	234-322	17
OMM1220	0.00	0.56	0.56	127-215	14
OMM1302	0.00	0.79	0.79	225-299	15
OMM1329	0.00	0.85	0.85	169-209	11
AVERAGE	0.00	0.77	0.77	---	14.20

A.


Average number of fish stocked
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
1990-1993
1994-1999
2000-2003
2004-2010
Time periods of salmonid stocking
dipRBT
LCT
trpRBT

B.

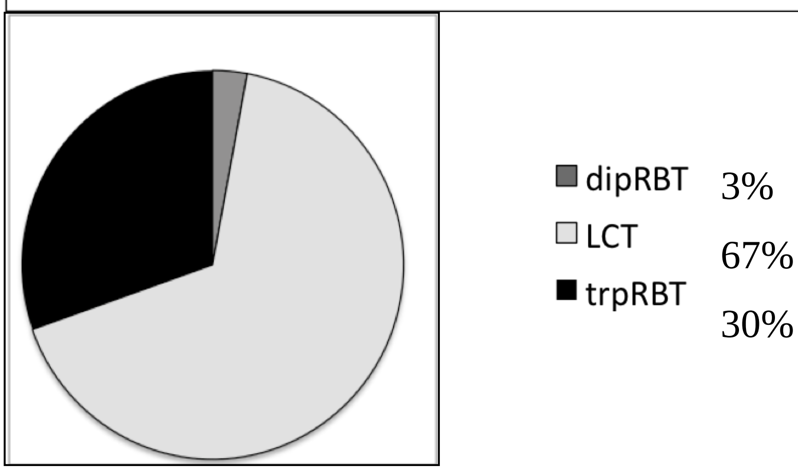

dipRBT 3%
LCT 67%
trpRBT 30%

Figure 1.

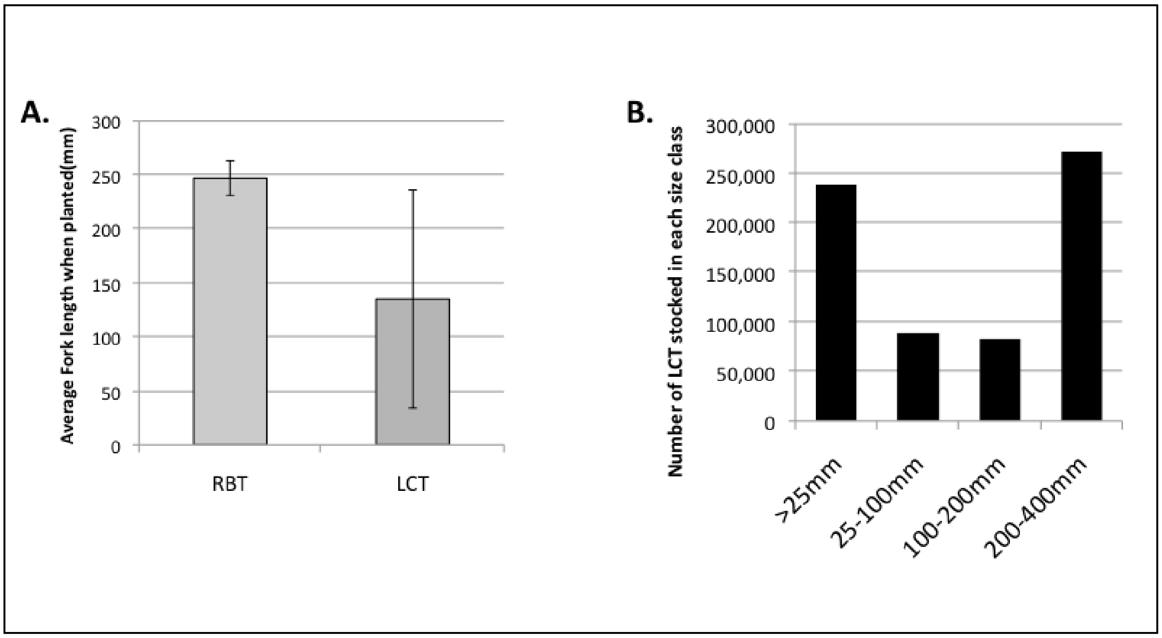

A.
Average Fork length when planted(mm)
300
250
200
150
100
50
0
RBT
LCT
B.
Number of LCT stocked in each size class
300,000
250,000
200,000
150,000
100,000
50,000
0
>25mm
25-100mm
100-200mm
200-400mm

Figure 2.

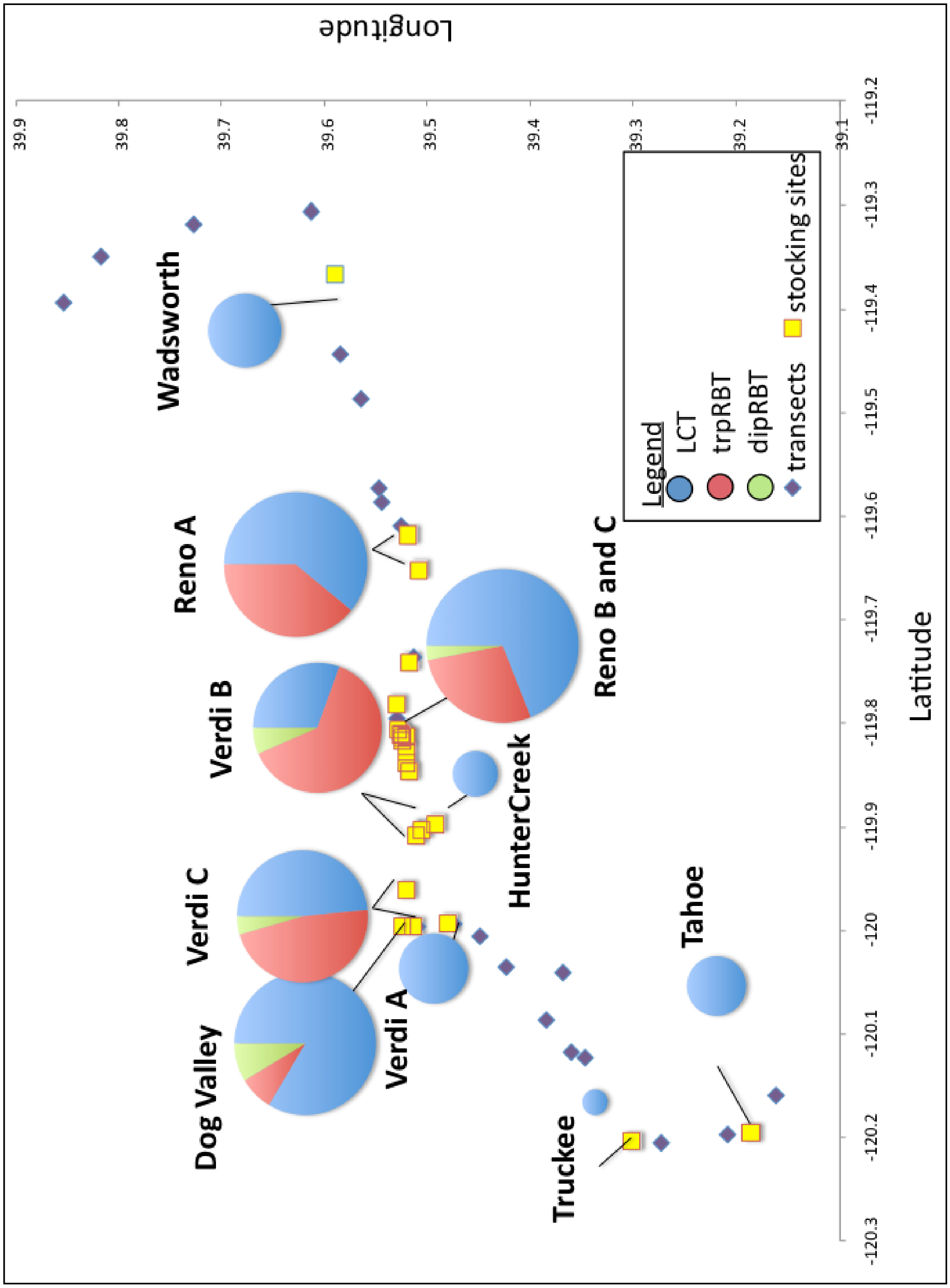

Longitude
Latitude
Wadsworth
Reno A
Verdi B
Reno B and C
Verdi C
HunterCreek
Dog Valley
Verdi A
Truckee
Tahoe
Legend
LCT
trpRBT
dipRBT
transects
stocking sites
39.9
39.8
39.7
39.6
39.5
39.4
39.3
39.2
39.1
-119.2
-119.3
-119.4
-119.5
-119.6
-119.7
-119.8
-119.9
-120
-120.1
-120.2
-120.3

Figure 3.

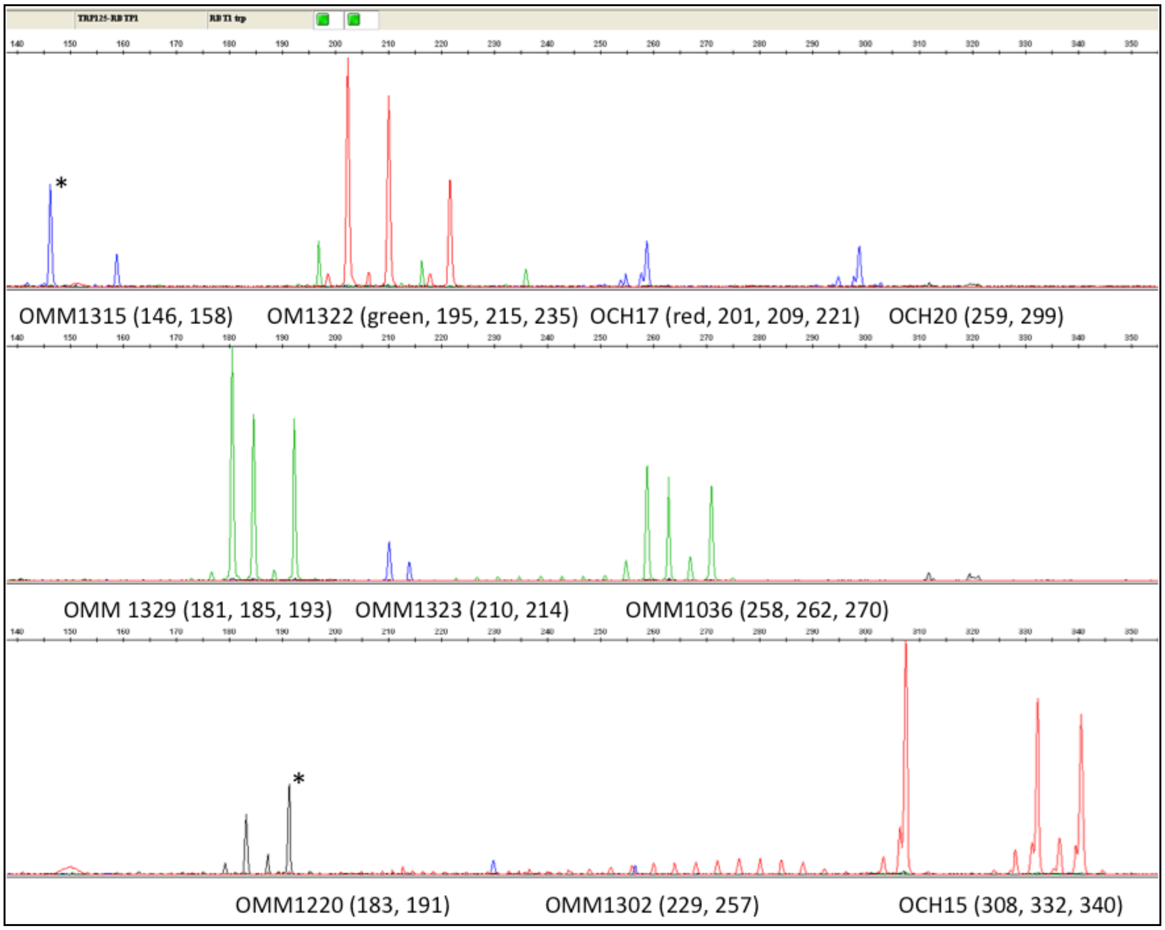

OMM1315 (146, 158)
OM1322 (green, 195, 215, 235)
OCH17 (red, 201, 209, 221)
OCH20 (259, 299)
OMM 1329 (181, 185, 193)
OMM1323 (210, 214)
OMM1036 (258, 262, 270)
OMM1220 (183, 191)
OMM1302 (229, 257)
OCH15 (308, 332, 340)

Figure 4.

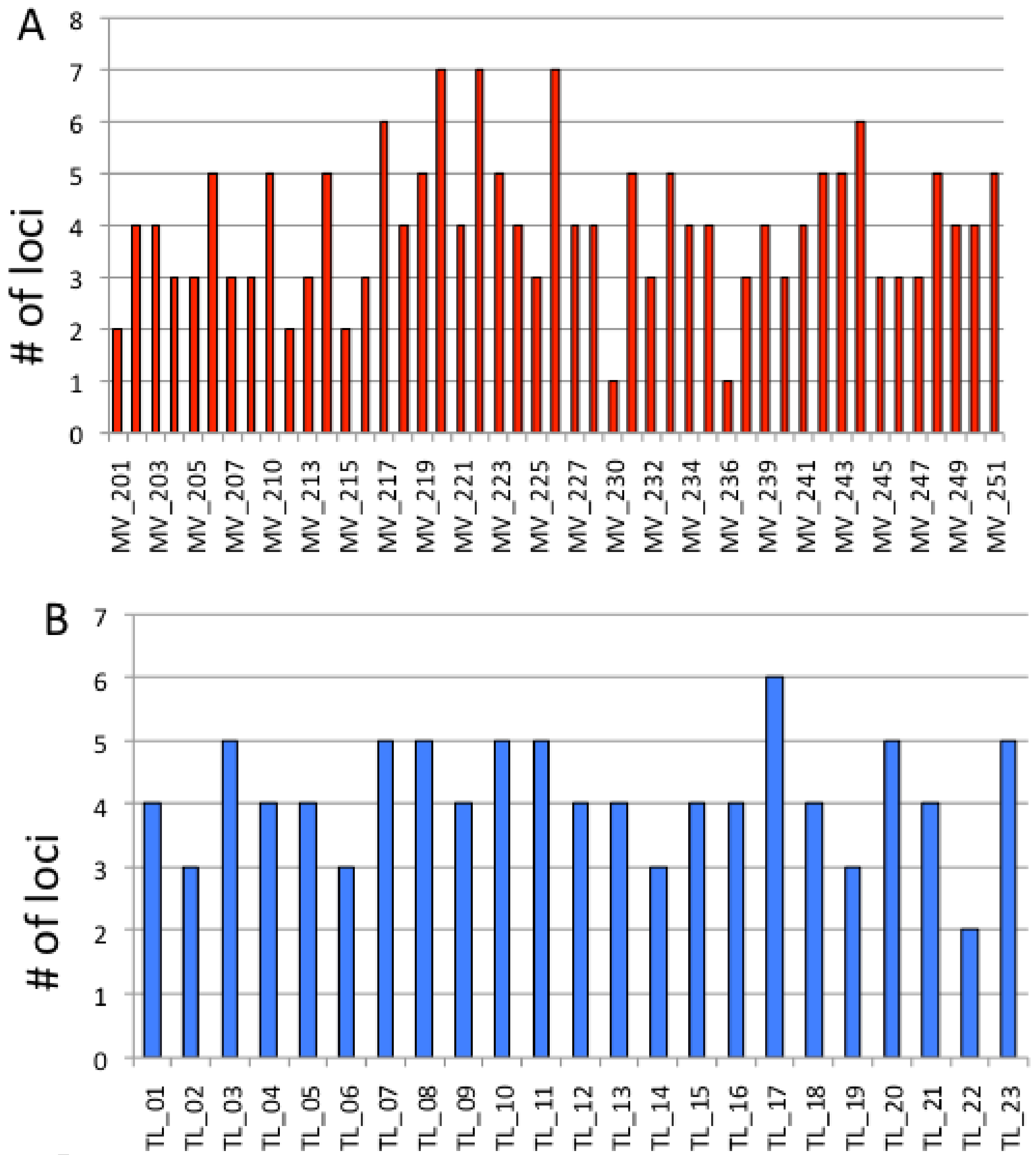
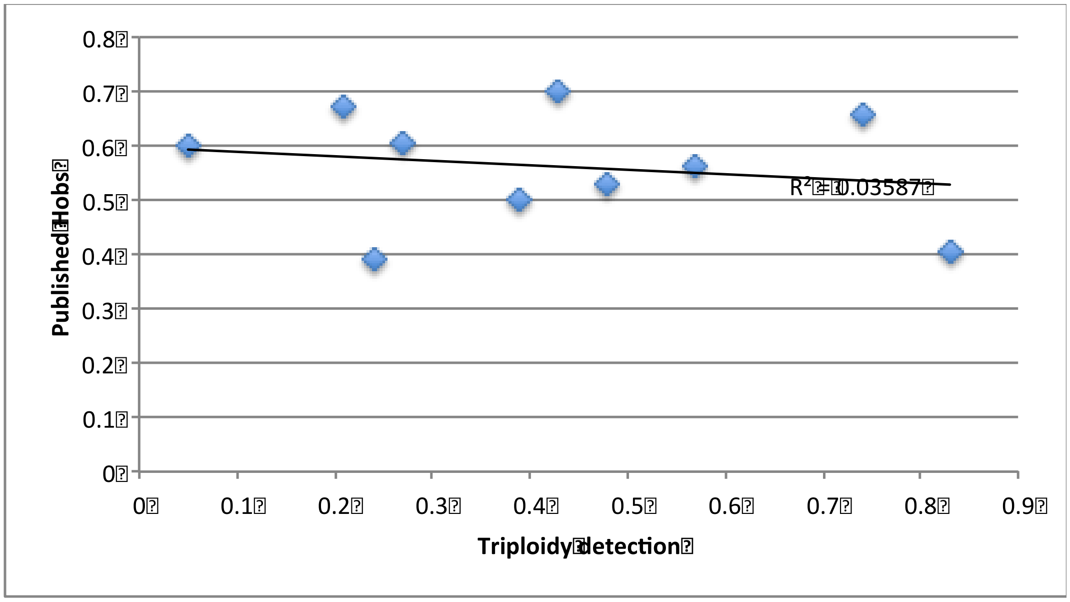

A
# of loci
MV_201
MV_203
MV_205
MV_207
MV_210
MV_213
MV_215
MV_217
MV_219
MV_221
MV_223
MV_225
MV_227
MV_230
MV_232
MV_234
MV_236
MV_239
MV_241
MV_243
MV_245
MV_247
MV_249
MV_251
B
# of loci
TL_01
TL_02
TL_03
TL_04
TL_05
TL_06
TL_07
TL_08
TL_09
TL_10
TL_11
TL_12
TL_13
TL_14
TL_15
TL_16
TL_17
TL_18
TL_19
TL_20
TL_21
TL_22
TL_23

Figure 5.


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Published Hobs
R² = 0.03587
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Triploidy detection

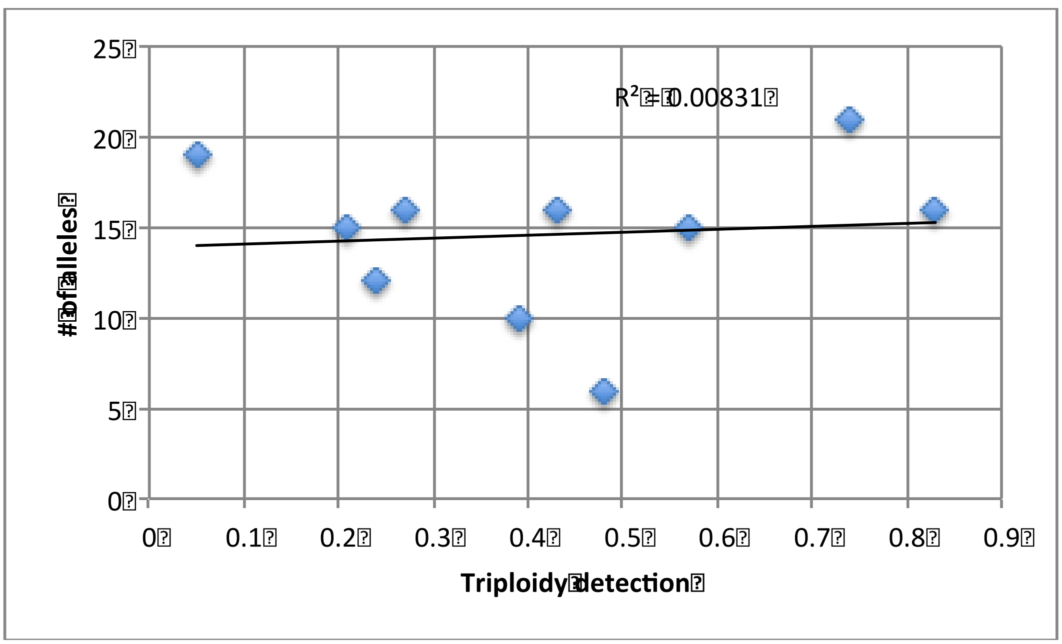

25
20
15
10
5
0
# of alleles
R² = 0.00831
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
Triploidy detection

Figure 6.

A.

B.

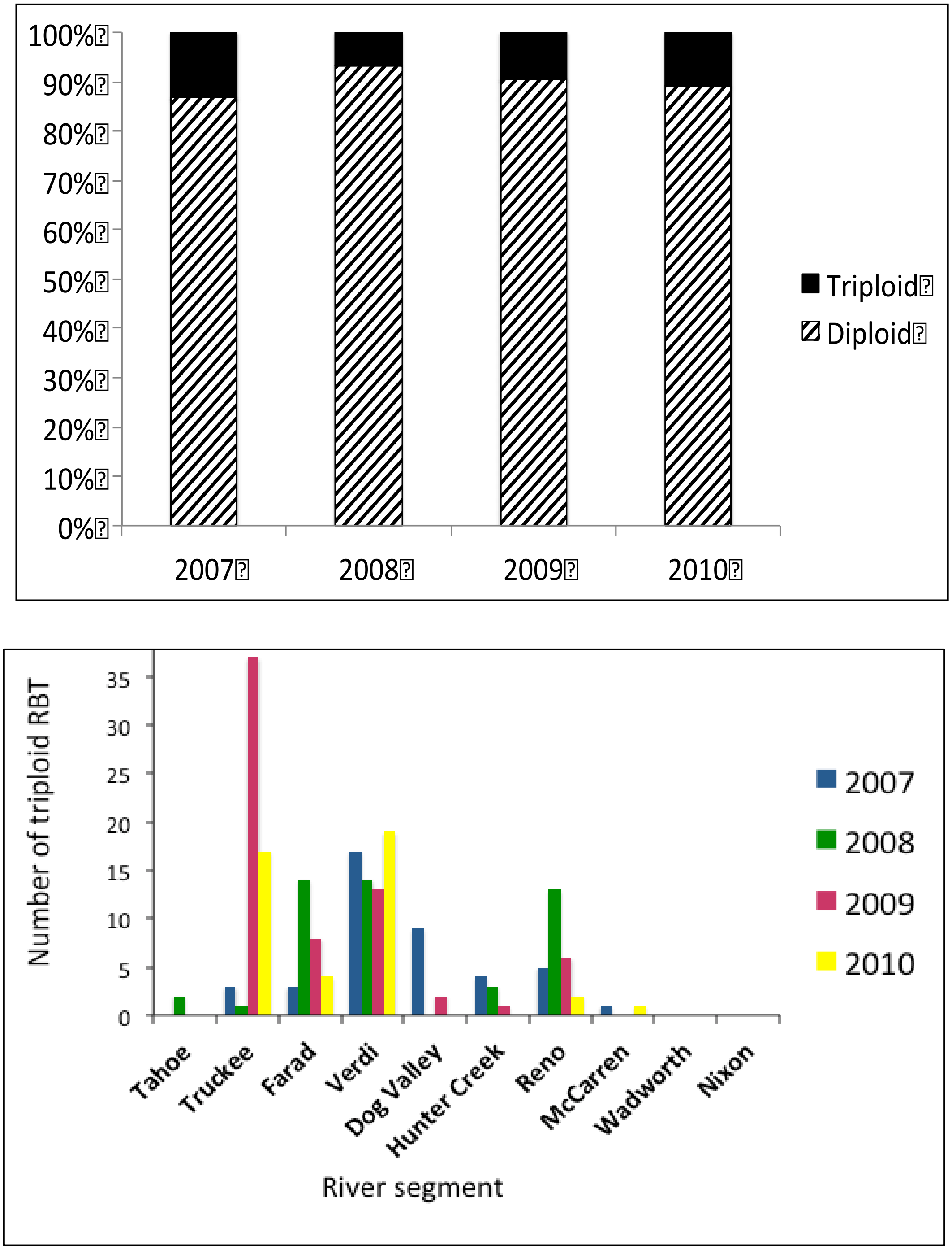
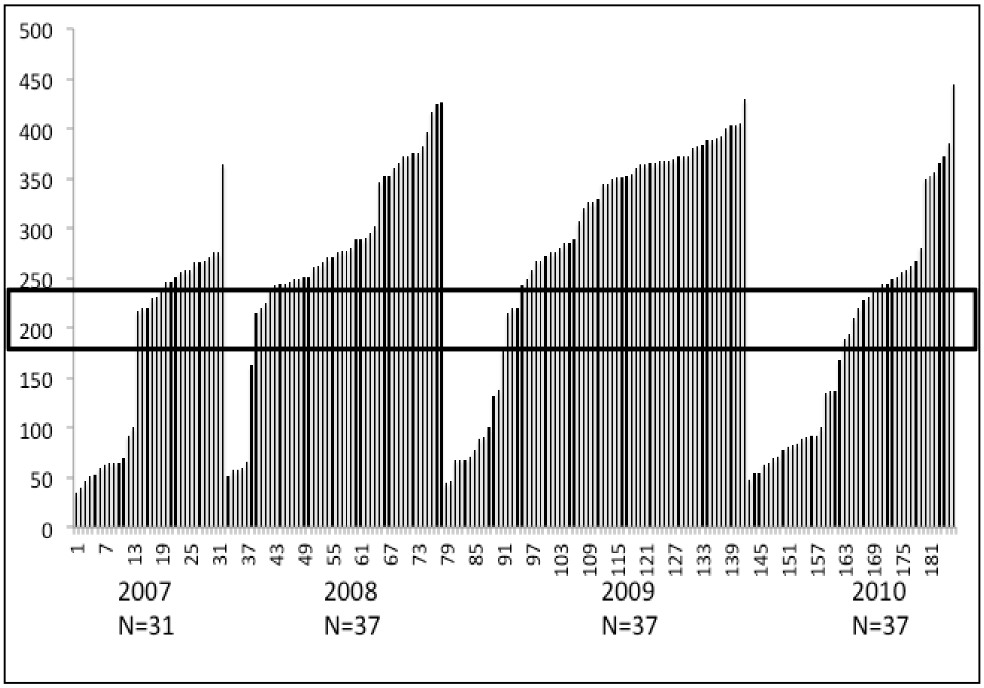

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2007
2008
2009
2010
Triploid
Diploid
Number of triploid RBT
35
30
25
20
15
10
5
0
Tahoe
Truckee
Farad
Verdi
Dog Valley
Hunter Creek
Reno
McCarren
Wadworth
Nixon
River segment
2007
2008
2009
2010

Figure 7.

A.


2007
N=31
2008
N=37
2009
N=37
2010
N=37

B.

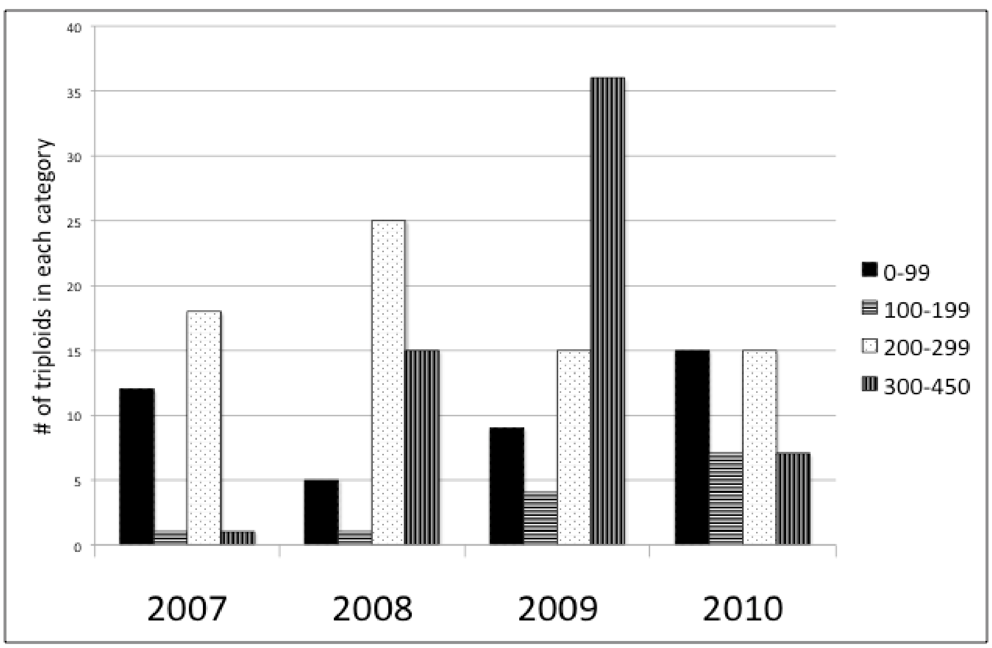

# of triploids in each category
0-99
100-199
200-299
300-450
2007
2008
2009
2010

Figure 8

LITERATURE CITED

Benfey, T. J. 1999. The physiology and behavior of triploid fishes. *Reviews in Fisheries Science*, **7**:39-67.

Benfey, T. J., I. I. Solar, G. DeJong and Donaldson, E. M. 1986. Flow-cytometric confirmation of aneuploidy in sperm from triploid rainbow trout. *Transactions of the American Fisheries Society*, **115**(6):838-840.

Bennett, S. N., and J. L. Kershner. 2009. Levels of introgression in westslope cutthroat trout populations nine years after changes to rainbow trout stocking programs in southeastern British Columbia. *North American Journal of Fisheries Management*, **29**:1271-1282.

Budy, P., G. P Thiede, A. Dean, D. Olsen, and G. Rowley. 2012. A comparative and experimental evaluation of performance of stocked diploid and triploid brook trout. *North American Journal of Fisheries Management*, **32**(6):1211-1224.

Campbell, M. R., J. Dillon, and M. S. Powell. 2002. Hybridization and introgression in a managed, native population of yellowstone cutthroat trout: Genetic detection and management implications. *Transactions of the American Fisheries Society*, **131**:364-375.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. *Nature*, **405**:234-242.

Chourrout, D. 1984. Pressure-induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids, and heterozygous and homozygous diploid gynogenetics. *Aquaculture*, **36**(1-2):111-126.

Clausen, R., and R. York. 2008. Global biodiversity decline of marine and freshwater fish: a cross-national analysis of economic, demographic, and ecological influences. *Social Science Research*, **37**:1310-1320.

Coates, K. A. 1995. Widespread polyploid forms of *Lumbricillus lineatus* (Müller) (Enchytraeidae: Oligochaeta): comments on polyploidism in the enchytraeids. *Canadian Journal of Zoology*, **73**:1727–1734.

Coffin, P.D., and W.F. Cowan. 1991. Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) Recovery Plan. U.S. Fish and Wildlife Service, Region I, Portland, Oregon.

Coleman, M. E., and V. K. Johnson. 1988. Summary of trout management at Pyramid

Lake, Nevada, with emphasis on Lahontan cutthroat trout, 1954-1987. *American Fisheries Society Symposium*, **4**:107-115.

Cordone, A. J., and T. C. Frantz. 1968. An evaluation of trout planting in Lake Tahoe. *California Fish and Game*, **54**:68-75.

Doupe, R. G., and D. W. Burrows. 2008. Thirty years later, should we be more concerned for the ongoing invasion of Mozambique tilapia in Australia? *Pacific Conservation Biology*, **14**:235-238.

Frankham, R. 2005. Conservation biology - ecosystem recovery enhanced by genotypic diversity. *Heredity*, **95**:183-183.

Fraser, T. W. K., P.G. Fjelldal, T. Hansen, and I. Mayer. 2012. Welfare considerations of triploid fish. *Reviews in Fisheries Science*, **20**(4):192-211.

Gozlan, R. E., J. R. Britton, I. Cowx, and G. H. Copp. 2010. Current knowledge on non-native freshwater fish introductions. *Journal of Fish Biology*, **76**:751-786.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool - status and strategy. *Science*, **245**:477-480.

Halverson, Anders. 2010. A less bold and spirited nation. *An entirely synthetic fish. How Rainbow Trout Bequiled America and Overran the World*, pp 1-12. New Haven and London. Yale University Press.

Harig, A. L., K. D. Fausch, and M. K. Young. 2000. Factors influencing success of greenback cutthroat trout translocations. *North American Journal of Fisheries Management*, **20**:994-1004.

Hickman, T. J., and R. J. Behnke. 1979. Probable discovery of the original pyramid lake cutthroat trout. *Progressive Fish-Culturist*, **41**:135-137.

Hitt, N., A. Frissel, ,C. Muhlfeld, and F. W. Allendorf. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarkii lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences*, **60**:1440-1451.

Houde, A. L. S., C. C. Wilson, and B. D. Neff. 2015. Competitive interactions among multiple non-native salmonids and two populations of Atlantic salmon. *Ecology of Freshwater Fish*, **24**:44-55.

Kim, D. S., Y. K. Nam, and I. S. Park. 1995. Performance of diploid and triploid hybrid leach, *Misgurnus mizolepis* female x *M. anguillicaudatus* male. *Aquaculture*, **137**:326-327.

Koenig, M. K., and Meyer, K. A. 2011. Relative performance of diploid and triploid catchable rainbow trout stocked in Idaho lakes and reservoirs. *North American Journal of Fisheries Management*, **31**(4):605-613.

Krebs, R. A., W. C. Borden, E. R. Steiner, M. S. Lyons, W. Zawiski, and B. M. Walton. 2010. Determinants of mussel diversity in Lake Erie tributaries. *Journal of the North American Benthological Society*, **9**:506-520.

Krieger, M. J. B., and L. Keller. 1998. Estimation of the proportion of triploids in populations with diploid and triploid individuals. *Journal of Heredity*, **89**:275-279.

Kozfkay, C. C., M. R. Campbell, K. A. Meyer, and D. J. Schill. 2011. Influences of Habitat and Hybridization on the Genetic Structure of Redband Trout in the Upper Snake River Basin, Idaho. *Transactions of the American Fisheries Society*, **140**:282-295.

Loxterman, J. L., E. R. Keeley, and Z. M. Njoroge. 2014. Evaluating the influence of stocking history and barriers to movement on the spatial extent of hybridization between westslope cutthroat trout and rainbow trout. *Canadian Journal of Fisheries and Aquatic Sciences*, **71**:1050-1058.

Martin, C. W., M. M. Valentine, and J. F. Valentine. 2010. Competitive interactions between invasive Nile tilapia and native fish: the potential for altered trophic exchange and modification of food webs. *Plos One*, **5:**e14395.

McCallum, M. L. 2015. Vertebrate biodiversity losses point to a sixth mass extinction. *Biodiversity and Conservation*, **24**:2497-2519.

Nam, Y. K., I. S. Park, and D. S. Kim. 2004. Triploid hybridization of fast-growing transgenic mud loach *Misgurnus mizolepis* male to cyprinid loach *Misgurnus anguillicaudatus* female: the first performance study on growth and reproduction of transgenic polyploid hybrid fish. *Aquaculture*, **231**:559-572.

Nanda, I., M. Schartl, W. Feichtinger, I. Schlupp, J. Parzefall, and M. Schmid. 1995. Chromosomal evidence for laboratory synthesis of a triploid hybrid between the gynogenetic teleost *Poecilia formosa* and its host species. *Journal of Fish Biology*, **47**:619-623.

Neville, H. M., and J. B. Dunham. 2011. Patterns of hybridization of nonnative cutthroat trout and hatchery rainbow trout with native redband trout in the Boise River, Idaho. *North American Journal of Fisheries Management*, **31**:1163-1176.

Ovenden, J. R., G. M. Macbeth, L. Pope, P. Thuesen, R. Street, and D. Broderick. 2015.

Translocation between freshwater catchments has facilitated the spread of tilapia in eastern Australia. *Biological Invasions*, **17**:637-650.

Ostberg, C. O., and R. J Rodriguez. 2004. Bi-parentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. *Molecular Ecology Notes,* **4**:26-29.

Page, K. S., M. T. Negus, M. C. Ward, and T. L. Close. 2011. Simulating effects of nonintrogressive hybridization with a stocked hatchery strain of rainbow trout on the sustainability and recovery of naturalized steelhead populations in Minnesota waters of Lake Superior. *North American Journal of Fisheries Management*, **31**:1065-1076.

Palti, Y., M. R. Fincham, and C. E. Rexroad III. 2002. Characterization and mapping of 38 polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). *Molecular Ecology Notes,* **2:**449-452.

Peacock, M. M., H. Neville, and V. S. Kirchoff. 2004. Ten species specific microsatellite loci for Lahontan cutthroat trout, *Oncorhynchus clarki henshawi*. *Molecular Ecology Notes*, **4**:557-559.

Peacock, M.M., and V.S. Kirchoff. 2007. Analysis of genetic variation and population genetic structure in Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) extant populations. *Final report to US Fish and Wildlife Service*, Region 6, Reno, Nevada.

Piferrer, F.B., , J. C. Beaumont, J.C. Falguiere, M. Flajshans, P. Haffray and L.Colombo. 2009. Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture*, **293**(1-3):125-156.

Pimm, S. L., C. N. Jenkins, R. Abell, T. M. Brooks, J. L. Gittleman, L. N. Joppa, P. H. Raven, C. M. Roberts, and J. O. Sexton. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, **344**:987.

Pritchard, V. L., J. C. Garza, and M. M. Peacock. 2015. SNPs reveal previously undocumented non-native introgression within threatened trout populations. *Conservation Genetics*, **16**:1001-1006.

Quillet, E., and J. L. Gaignon. 1990. Thermal induction of gynogenesis and triploidy in Atlantic Salmon (*Salmo salar*) and their potential interest for aquaculture. *Aquaculture*, **89**(3-4):351-364.

Reissig, M., C. Trochine, C. Queimalinos, E. Balseiro, and B. Modenutti. 2006. Impact of fish introduction on planktonic food webs in lakes of the Patagonian Plateau. *Biological Conservation*, **132**:437-447.

Rexroad, C.E. III, R. L. Coleman, A. L. Gustafson, W. K. Hershbeger, and J. Killefer . 2002. Development of rainbow trout microsatellite markers from repeat enriched libraries. *Marine Biotechnology*, **3**:12-16

Rexroad, C.E. III, and Y. Palti. 2003. Development of ninety-seven polymorphic microsatellites markers for rainbow trout. *Transactions of the American Fisheries Society*, **132:**1214-1221.

Ridout, M. S. 2000. Brief communication. Improved estimation of the proportion of triploids in populations with diploid and triploid individuals. *Journal of Heredity*, **91**(1):57-60.

Robinson, M. L., V. S. Kirchoff, and M. M. Peacock. 2009. Characterization of 13 microsatellites for Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) and cross-amplification in six other salmonids. *Molecular Ecology Resources*, **9**:134-136.

Schafhauser-Smith, D., and T. J. Benfey. 2001. The reproductive physiology of three age classes of adult female diploid and triploid brook trout (*Salvelinus fontinalis*). *Fish Physiology and Biochemistry*, **25**(4):319-333

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, **18**:233-234.

Shaibi, T., H. M. G. Lattorff, and R. F. A. Moritz. 2008. A microsatellite DNA toolkit for studying population structure in *Apis mellifera*. *Molecular Ecology Resources*, **8**:1034-1036.

Sharma, R., and S. W. Raborn. 2011. Estimating critical abundance thresholds in exploited populations: a simulation approach based on species resilience to disturbance. *Computational Ecology and Software*, **1**:189-207.

Shelton, J. M., M. J. Samways, and J. A. Day. 2015. Predatory impact of non-native rainbow trout on endemic fish populations in headwater streams in the Cape Floristic Region of South Africa. *Biological Invasions*, **17**:365-379.

Stankovic, D., A. J. Crivelli, and A. Snoj. 2015. Rainbow Trout in Europe: introduction, naturalization, and impacts. *Reviews in Fisheries Science & Aquaculture*, **23**:39-71.

Sumner, F. H. 1940. The decline of the Pyramid Lake Fishery. *Tranactions of the American Fisheries Society*, **69:**216-224.

Thibault, I., and J. Dodson. 2013. Impacts of exotic rainbow trout on habitat use by native juvenile salmonid species at an early invasive stage. *Transactions of the American Fisheries Society*, **142**:1141-1150.

Thorgaard, G. H., M. E. Jazwin, and A. R. Stler. 1981. Polyploidy induced by heat shock in rainbow trout. *Transactions of the American Fisheries Society*, **110**(4):546-550.

Tiwary, B. K., R. Kirubagaran, and A. K. Ray. 2004. The biology of triploid fish. *Reviews in Fish Biology and Fisheries*, **14**:391-402.

Verhille, C., K. Anttila, and A. P. Farrell. 2013. A heart to heart on temperature: Impaired temperature tolerance of triploid rainbow trout (*Oncorhynchus mykiss*) due to early onset of cardiac arrhythmia. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology*, **164**:653-657.

Vitousek, P. M., C. M. Dantonio, L. L. Loope, M. Rejmanek, and R. Westbrooks. 1997. Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**:1-16.

Wong, A. C., and A. L. van Eenennaam. 2007. Transgenic containment of genetically engineered fish. *Transgenic Research*, **16**:862-862.

CHAPTER 4:

Population structure and distribution of naturalized rainbow trout in the Truckee River watershed: Identifying potential eradication units to allow for reintroduction of the native threatened Lahontan Cutthroat trout

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ABSTRACT

Reintroduction of threatened or endangered species into their native habitat is dependent on a complete understanding of the size, source, and genetic structure of introduced or invasive populations inhabiting a given region. The Lahontan cutthroat trout (*Oncorhyncus clarkii henshawi*, LCT), native to the Lahontan hydrographic basin of northern Nevada, northeastern California and southeastern Oregon, has declined rapidly over the past century, during which time it was completely lost from the Truckee River within the Lake Tahoe Basin in California/Nevada. Historically, LCT was the apex predator within the Lake Tahoe basin. To provide angling opportunities, non-native rainbow trout (*Oncorhynchus mykiss*, RBT) have been stocked for decades throughout the range and are currently stocked throughout the Lake Tahoe basin and, as such, provide one of the biggest challenges to recovery of LCT. Currently, primarily non-reproductive triploid rainbow trout are stocked into the Truckee River, in hopes of allowing for Lahontan cutthroat trout recovery; however, a robust naturalized RBT population is evident and hybridization between RBT and LCT has been apparent in every site LCT have been planted in sympatry with RBT. Eradication of this introduced species is a necessary component to allow for reintroduction of the native cutthroat trout; yet, public and political pressures to maintain the recreational fishing in the Truckee River limit removal of the rainbow trout. The focus of this study was to genetically evaluate the naturalized rainbow trout found throughout the Truckee River using 11 microsatellite loci to identify population genetic structure and potential barriers

to movement in RBT. Two to 5 genotype clusters were found over the four years of the study, with increasing population genetic structure emerging during low flow years. However, there is evidence of downstream gene flow, and no isolated populations of rainbow trout or potential eradication units were obvious within the Truckee River. Planting of triploid fish does not seem to impact the reproduction and proliferation of the naturalized RBT, and eradication of the reproductive RBT throughout the Truckee River would be necessary for the successful reintroduction of the native LCT.

INTRODUCTION

Biodiversity in an ecosystem is associated with stability, productivity and overall sustainability of that ecosystem, especially when facing threats due to changing climate and anthropogenic disturbance (Kaufman et al., 1998; Chapin et al., 2000; Clausen and York., 2008; Muhlfeld et al., 2014). One of the most significant causes of biodiversity loss is biological invasion and introductions of non-native species that represent threats due to competition, predation and hybridization (Weber et al., 2003; Martin et al., 2010; Vander Zanden et al., 2010; Thibault et al., 2013; James et al., 2015). Human caused introductions have occurred both deliberately and accidentally and can have significant economic and ecological impacts (Koel et al., 2005; James et al., 2015; Stankovic et al., 2015). One notorious example of human caused introductions in the United States is the introduction of the zebra mussel (*Dreissena polymorpha*) into Oneida Lake, New York as an inadvertent stow-away on recreational aquatic vehicles (Idrisi et al., 2001). The zebra mussel rapidly expands their distribution when introduced, which dramatically increases water clarity through filtration (Idrisi et al., 2001), shifting primary production to benthic sources which impacts zooplankton production and alters the overall food web dynamics (Strayer et al., 1999; Johannsson et al., 2000). In addition, zebra mussels can alter spawning habitat for native fishes, thereby inhibiting reproduction and recruitment (Marsden and Chotkowski, 2001). Zebra mussel introduction negatively impacts the ecosystem on many levels; however, eradication efforts can be ecologically damaging and ineffective (Wimbush et al.,

2009) and often the damage wrought on the community has already been completed, so removal may cause additional destruction to the ecosystem (Simberloff, 2014).

Introductions of exotic species into a region have occurred deliberately with the intent to provide hunting or fishing opportunities; however, there are often unintended negative consequences to the native flora and fauna. The stocking of salmonid fishes into fishless lakes has been widespread for centuries as a mechanism to provide recreational angling opportunities (Halverson, 2010; Gray et al., 2014; Stankovic et al., 2014). Many populations of introduced species have become self-sustaining, dramatically altering the food webs of the ecosystems in question. Predation by introduced fish can deplete zooplankton communities, benthic invertebrates, and amphibians (Lodge, 1993; Carlisle and Hawkins, 1998; Donald et al., 2001; Knapp et al., 2001). The impact of non-native species can be devastating in island ecosystems, but is also significant in mainland regions (Saunders and Norton, 2001); it has been shown that the continental United States supports more than 2000 established introduced and invasive non-native species (Vitousek et al., 1997). These non-native species have become established in freshwater, marine, and terrestrial ecosystems. The impact of introduced species is often not recognized for many years, and it can be difficult to reverse the effects and restore the native ecosystems.

Organisms in freshwater ecosystems are especially vulnerable to a wide variety of threats, such as overfishing, habitat loss, hybridization and competition with invasive species, leading to a large number of endangered and threatened species (Clausen and York, 2008; Krebs et al., 2010; Harig et al., 2000). Habitat restoration, removal of invasive species, and supplementing extant endangered populations with hatchery raised individuals can help to protect native species. Although the survival and sustainability of existing populations can be enhanced through restoration efforts, management efforts are being challenged by situations where native fish have been extirpated from their historical range; translocations may be a reasonable alternative to reintroduce that species back into its native ecosystem. Translocation has been done for both conservation and restoration of endangered species in terrestrial, marine, and freshwater ecosystems; but the overall success rates reported have been low (Griffith et al., 1989; Dodd and Seigel, 1991; Godefroid et al., 2011), and success or failure is not always reported because it is dependent on extensive monitoring.

The results of translocations are dependent on many factors, such as habitat quality (Harig and Fausch, 2002), presence of non-natives that can compete or hybridize with the translocated species (Harig et al., 2000), or inbreeding and loss of genetic variation in the source population (Gray et al., 2014). Perceived success or failure of a reintroduction effort for freshwater fishes is loosely defined by the survival, recruitment, and reproduction of the individuals. Using publically available published studies, the factors that contribute to the success and failure of native freshwater fish reintroductions were evaluated; in some cases, simply the presence of non-native species was adequate to consider the re-introduction a failure (Harig et al., 2000; Chochran-Biederman et al., 2014), exemplifying the impact that non-native fishes can have on restoration efforts. Reintroduction success requires evaluation of the initial reasons for the decline of the species. If an introduced species has contributed to the decline, prior to any reintroduction efforts, it is necessary to remove that introduced species; in addition, reinvasion potential of the introduced species must be investigated to provide any long term success in the ability of the re-introduced species to survive in the native ecosystem (Gray et al., 2014; Weeks et al., 2011).

Molecular genetic tools such as nuclear, non-coding microsatellite loci can be utilized in order to inform eradication efforts for introduced and invading populations (Frankham et al., 2014) and identify the reinvasion potential to the restored habitat (Adams et al., 2014; Purcell and Stockwell, 2015). Genetic tests can be time consuming and costly; however, genetic evaluation of the introduced species can lead to an understanding of the routes of invasion and the dispersal of the introduced species in the landscape and is considered to be one of the most important factors to consider when attempting eradications of invasive species factors contributing to allow for reintroduction of an endangered species (Griffith et al., 1989; Dodd and Segal, 1991; Peacock et al., 2009; Cochran-Biederman et al, 2015). In recent years, the use of molecular tools such as microsatellites and mitochondrial or nuclear sequence data has become more accessible and affordable (Blanchet, 2012) and can be utilized to look at population structure of non-natives, genetic components of invasion success and to identify hybridization or other threats to the reintroduction (Allendorf and Phelps, 1980; Estoup and Guillemaud, 2010; Blanchet, 2012). Molecular data can aid in management decisions, determining the probable success of eradication efforts by measuring connectivity or identifying possible source populations for re-invasion events (Peacock et al., 2009; Estoup and Guillemaud, 2010; Blanchet, 2012; Adams et al. 2014; Purcell and Stockwell, 2015). The Coqui frog (*Eleutherodactylus coqui*), native to Puerto Rico, was introduced into the Hawaiian Islands in the late 1980s and rapidly colonized and became widespread on the island of Hawaii (Kraus and Campbell, 2002; Choi and Beard, 2012). Microsatellites and mitochondrial genetic markers used to look at the population structure analysis of the introduced Coqui frog throughout the Hawaiian Islands indicated that there were two distinct introductions in the Hawaiian Islands, first on the main island of Hawaii, which spread to Oahu, Kauai and Maui, and then a second introduction on Maui. In addition, genetic analysis of the introduced Coqui showed much lower heterozygosity and allelic richness than those found in the native range of Puerto Rico; the high invasion success of the introduced Coqui Frog on the Hawaiian Islands was not dependent on high levels of genetic variation (Peacock et al., 2009). In this case, a very small introduced population was shown to eventually spread over a large area on multiple islands. Eradication efforts have had some success on Maui, Oahu, and Kauai (Beard et al., 2009); however, given the high invasion potential displayed by the population on the Island of Hawaii, continued genetic monitoring and diligence is necessary for continued control. Genetic tools can be invaluable in developing realistic control strategies, tracking the dispersal of invasive species and identifying potential routes of reinvasion.

Often, complete eradication of an invasive species is neither cost effective nor practical, and controlled areas are often re-invaded by neighboring populations (Purcell and Stockwell, 2015; Klima and Travis, 2012). Genetic tools can be utilized to inform eradication efforts and develop "eradication units" to improve the probability of success. "Eradication units" are segments of the population that are interconnected and as such, must be eliminated simultaneously to prevent re-invasion (Robertson and Gemmell, 2004). It has been suggested that prior to implementing control measures, the genetic population structure of the target species should be assessed, and regions that show genetic isolation can be chosen as initial targets (Adams et al., 2014). Genetic isolation can indicate barriers to movement and, as such, present opportunities to remove a pest species and allow for restoration of the native habitat and reintroduction of a native species.

The Truckee River watershed, which flows from oligotrophic Lake Tahoe to endorheic Pyramid Lake, is an important fresh water source for eastern California and western Nevada and has been plagued by recurring droughts and water diversions that have dramatically altered the native flora and fauna (Cobourn, 1999). Stimulated by the placement of two of the endemic fish species, cui-ui sucker (*Chasmistes cujus)* and the Lahontan Cutthroat trout (*Oncorhyncus clarkia henshawii)* on the United States Endangered Species list in 1975, multiple restoration projects have focused on the Truckee River over the past three decades.

Attempts to restore the riparian landscape by establishing seedlings such as cottonwoods (Caicco, 1998; Mahoney and Rood, 1998) and willows (Amlin and Rood, 2002) have been successful (Rood et al., 2003). However, translocations of extirpated endangered species such as the leopard frog (*Lithobates pipiens*) and the Lahontan cutthroat trout (LCT) were hampered by the presence of vast numbers of introduced and invasive non-native species, specifically bull frogs (*Lepidium catesbeiana)(Rogers and Peacock, 2012)* and rainbow trout respectively. Currently, the United States Forest Service is conducting an ongoing restoration project that is attempting to create a self-sustaining LCT populations in the Upper Truckee River, Lake Tahoe's largest tributary (Miller and Santora, 2013). Prior to planting a pure stock of LCT, repeated rotenone applications, electro-fishing and gillnetting were necessary to remove non-native salmonids, RBT and brook trout. Genetic samples of the LCT are evaluated annually to look for hybridization and introgression with RBT, showing slight introgression in a limited region (Miller and Santora, 2013). Continued monitoring and re-stocking will be necessary to reach their goals. Even low levels of introgression of the LCT with RBT could result in considering the project a failure, (Miller and Santora, 2013; Harig et al., 2000); however, minor levels of hybridization may be acceptable if there are no "pure" populations that can be realistically established (Peacock and Kirchoff, 2004). Current activities in the Truckee River also include stocking the main stem river with LCT, other game fishes, and non-reproductive triploid RBT for angling opportunities. However, hybridization between RBT and LCT has been documented in all of the regions of the Truckee River where LCT are planted due to a robust naturalized RBT population (Chapter 2). The Truckee River has two main tributaries, Dog Valley Creek and Hunter Creek, with very different stocking histories, as Dog Valley Creek is one of the main sites for stocking of RBT over the last 20 years, and Hunter Creek has no record of being stocked with RBT. However, an established RBT population is evident in Hunter Creek, most likely supplemented by stocking in the main stem river. Tributaries can be considered as reintroduction sites if barriers exist or can be developed to allow for reproductive isolation. In an Icelandic river system, the population structure of Atlantic salmon was investigated, and researchers found that the tributaries showed the highest level of differentiation from the main stem of the system, even though they were separated by only 11 km (Gudmundsson et al., 2013). The population structure and reproduction of RBT within the Truckee River and its tributaries has not been evaluated, so it is unknown if there are regions in the Truckee River that are geographically isolated where reintroduction of LCT could lead to a healthy, established population.

This study focused on identifying the population genetic structure of the naturalized *O. mykiss* populations in the Truckee River and its two main tributaries, Dog Valley and Hunter creeks. I tested the hypothesis that in stream structures were barriers to gene flow, resulting in population structure in the naturalized trout. Substantial barriers, such as power dams found on the Truckee, can impede migration and create genetically isolated segments of the population. RBT populations in the Dog Valley and Hunter creek tributaries will likely show different population structure than the main stem; however, access to the tributaries is not significantly limited and, during higher water years, can allow for gene flow between the tributaries and the main stem river. Significant barriers to gene flow in the Truckee River can result in identification of potential "eradication units" between barriers and allow for the reintroduction of the native LCT. I used size class data to identify probable regions where reproduction is occurring. Triploid RBT have been the primary RBT stocked since 2004, in order to attempt reproductive containment of the RBT in the river. To evaluate the impact that stocking of non-reproductive triploid RBT has had on the reproduction of the naturalized RBT, effective population size was determined of the naturalized populations across time periods. This study can help to guide management decisions and evaluate the reintroduction potential of LCT into the Truckee River.

METHODS

Study system

The Truckee River flows 195 km from Lake Tahoe in California to Pyramid Lake in Nevada. Forty-one irrigation ditch diversions and dams have been identified along the 195 km river (Figure 1, Table 1). The in-stream structures have been found to have varying levels of impact on movement of native fishes within the watershed dependent on water flow (Peacock et al., 2016). Over the past several decades, the watershed has been stocked with LCT from the Independence Lake strain (a small lake in the upper Truckee River watershed), the contemporary Pyramid Lake strain (mixed stock ancestry), and the Pilot Peak strain (remnant of historical Pyramid Lake strain), as well as both diploid and triploid RBT. The Hunter Creek tributary flows into the Truckee River between the VerdiC and RenoA transects (Figure 1). Approximately 1.6 km of stream are accessible before it hits a diversion ditch that acts as a fish barrier. Dog Valley Creek enters the Truckee River near the VerdiC transect. Dog Valley Creek has been a site for stocking of RBT and LCT over the past decade, whereas Hunter Creek has no record of being stocked with RBT. Between 2005 and 2007, large numbers of LCT fry were stocked in both of these tributaries. Dog Valley Creek has more rearing habitat for young fish, whereas Hunter Creek has a greater number of large drop pools that can provide good habitat for adult fish. In the summer months, even with low water flow, the water temperature in these streams was 15-16°C (Maples et al., 2007), which is well below the upper thermal tolerance range for LCT (Robinson et al., 2008). It is likely that a number of these fish can move down into the lower section of the creek. Hunter Creek has a large barrier that can limit migration; however, the upper regions of Hunter Creek are not very accessible and stocking takes place below this barrier. In addition, sampling sites on Hunter Creek were also below this barrier when conducted by Nevada Division of Wildlife (NDOW) (2007 and 2008), and both tributaries are accessible from the Truckee River (Kim Tisdale, NDOW personal communication). In 2009, the tributaries were sampled by the United States Fish and Wildlife Service (USFWS) and were sampled both above and below the barriers.

Sample collection

Salmonid samples were collected by the United States Fish and Wildlife Service (USFWS) and Nevada Division of Wildlife (NDOW) from 2007 through 2010. USFWS collected samples from 8 primary sampling sections (n=1649). Each sampling section had 3 to 4 transects, depending on the variation in habitat types and barriers within each site (Figure 1, Table 1). Transects were 500 m long and both shore line and mid-stream regions. Raft, barge and backpack electrofishing methodologies were utilized to ensure all age classes were adequately sampled. Fin clips were placed in wax paper, dried, and placed in individual coin envelopes labeled with species (when possible), fork length, date collected and location. NDOW collected samples from five sites on the Truckee River main stem while doing their annual surveys. RBT samples collected by NDOW were primarily found in the Verdi to Reno transects (n=1820). In addition, NDOW collected samples from Hunter and Dog Valley creeks in 2007 and 2008, and collected from two transects below the diversion ditch barrier in Hunter Creek and below the California State Line in Dog Valley Creek. In 2009, the tributaries were also sampled by USFWS; for this sampling period, samples were collected from two transects above the barrier and the California State Line, in addition to the sites overlapping the NDOW collection.

Study species

RBT is a cold-water salmonid, native west of the Sierra Nevada crest extending to the west coast of North America. RBT are one of the most widely transplanted fish globally and were stocked in the Truckee River predominantly over the past century (Cordone and Franz, 1968). Rainbow trout have known stream resident, migratory, and anadromous forms and, historically, were found in rivers that drain into the Pacific Ocean from Mexico to the Aleutian Islands (Halverson, 2010). Currently, primarily triploid, non-reproductive RBT are stocked in the Truckee River. However, a large naturalized trout population is found throughout the river. RBT spawn in the early spring and are known to readily hybridize with LCT.

Stocking Analysis

Official stocking records of diploid and triploid RBT and were obtained from USFWS and NDOW for the time periods from 1990-2010. Total numbers of fishes stocked were calculated as well as averages of each salmonid species during different time periods to determine if sampling efforts were biased by stocking events. Fork length (mm) data were used to compare to the size class of the samples found.

Water discharge

I obtained flow data from United States Geological Survey (USGS; http://wdr.water.usgs.gov/wy2006/search.jsp) for monthly and total discharge (cubic meters per sec) per year from gauging stations examine stream flow dynamics along the Truckee River. Water discharge was reported separately for Dog

Valley Creek and Hunter Creek because the flow rates were more than 10 fold lower for these two tributaries.

DNA isolation and PCR amplification

DNA was isolated using DNeasy96 Blood and Tissue Kits (QIAGEN) according to the manufacturer's protocol. Double stranded DNA was quantified at the Nevada Genomics Center using a fluorescent nucleic acid stain (PicoGreen®) and read on a Labsystems Fluoroskan Ascent fluorescence plate reader, which measures only the double stranded DNA present. RBT microsatellites were selected from the literature (Rexroad et al. 2002; Palti et al., 2002; Rexroad and Palti, 2003) based on high number of alleles and ability to multiplex in PCR reactions. In addition, three LCT primers were selected on RBT and found to be highly variable and able to cross-amplify in RBT (Peacock et al., 2004; Robinson et al., 2009). All primer sequences, heterozygosity, range and number of alleles, and primer conditions are listed in Table 2. Samples were first screened using 6 bi-parentally inherited markers (Ostberg and Rodriguez, 2004) which identify them as RBT, LCT or hybrids (Chapter 2).

The primers were ordered with one of four unique M13 tails to allow for an economic method to label the PCR product with a corresponding fluorescent dye (Schuelke, 2000). I extended the basic tailed primer protocol to include 5 and 7 loci per reaction. A multiplex primer cocktail was prepared to give a final primer concentration of 0.05 μM each tailed forward primer, 0.2 μM each reverse primer, and 0.1 μM each labeled M13 primer in a 12 μl reaction. PCR reactions included 6 μl

Multiplex taq (1X final concentration)(QIAGEN), and 50 ng of DNA was used whenever possible. PCR cycle parameters included a 15 minute hot start at 95°C, followed by 41 cycles of 95°C for 30 seconds, annealing temperature for 90 seconds, and 72°C for 30 seconds. Species specific primers were chosen to have an optimal annealing temperature from 58-63°C, allowing for a touch-down PCR reaction that has 7 cycles with an annealing temperature of 65°C, 7 cycles at 61°C, 7 cycles at 58°C, and 20 cycles at 55°C in which the first 21 cycles amplified the locus specific primer and the final 20 cycles amplified the labeled M13 tail and fluorescently labeling the PCR product. PCRs were completed using an MBS Satellite 0.2G Thermal Cycler and a 96-well format (Thermo Electron Corporation). PCR products were diluted to an appropriate concentration determined by dilution tests from 1:50 to 1:200. One µl of diluted PCR product was added to 19 µl of size standard (Applied Biosystems) prepared by adding five µl of LIZ500 size standard to one ml of HiDye Formamide and 0.5 ml of molecular grade water. Fragment analysis was carried out on an Applied Biosystems 3730 Genetic Analyzer at the Nevada Genomics Center (http://www.ag.unr.edu/genomics/) and all alleles generated were scored, binned, and given allelic and genotypic designation using the ABI GeneMapper software (version 4.0).

Salmonid reproduction

Fork length (mm) data was collected for all salmonid samples analyzed in this study. Fry, fish smaller than 50 mm, were not sampled. Generally, fish between 50 and 100 mm are < 1 year old and made up a large portion of the RBT sampled. Any sample with recorded fork-length smaller than 100 ml was considered young-of-the-year (YOY). These samples were analyzed for hybridization, but were not included in evaluation of population structure or effective population size, as survival and recruitment of first year trout is known to be very low (Cochran-Biederman et al., 2015). Regions where a high proportion of samples were YOY were considered regions of high reproduction with suitable spawning habitat.

Genetic analysis

Microsatellite toolkit in Excel (Park, 2001) was used to calculate the observed and expected heterozygosity (H_o and H_e). FSTAT 2.9.3.2 (Goudet, 2001) was used to test for Hardy-Weinberg equilibrium (HWE). Loci that were out of HWE were suspected of having null alleles and were excluded from further analysis. F-statistics were also utilized to look at long term patterns of gene flow within the river (Wright, 1965).

I investigated contemporary population structure of the naturalized RBT identified in the Truckee River using a Bayesian genotype clustering method (STRUCTURE version 2.3.4; Pritchard *et al.,* 2000). The program assigns individuals to probable genotype clusters using HWE and gametic phase equilibrium between loci within groups, making no a priori assumptions of population based on sampling locations. Samples with fork length under 100mm were eliminated from this analysis. I specified a 10,000 iteration burnin followed by five 500,000 Markov chain

Monte Carlo (MCMC) replicates per *k* (number of genotype clusters) to estimate allelic distributions against which individual genotypes were compared and assigned to a cluster (Pritchard et al., 2000). I set the possible number of genotype clusters (k) equal to one through eight and used ΔK method of Evanno et al. (2005) to determine the optimal number of genotype clusters which uses the following formula in STRUCTURE HARVESTER (Earl and vonHoldt, 2012):

$$\Delta K = mean\ (|lnP(D)(k'')/sd(lnPD(k)|)$$

This formula not only takes into consideration the natural log of the probability of a certain k[LnP(D)], but also uses the increase in probability as we go from k=(n) to k=(n+1). It looks for the largest increase in probability with the smallest variation in that calculation. This has been found to be a better predictor of the true value for k than just using the increases on LnP(D) alone (Evanno et al., 2005).

Effective population size (Ne) was calculated in RBT, comparing pooled samples for each year using the linkage disequilibrium method NeEstimator (version 2.0; Do et al., 2014). This method has been found to be more precise with microsatellite data than the moment-based temporal method also available in the same program (Waples and Do, 2010). Ne was calculated for each year of the study to determine if there has been any decline in the naturalized RBT populations due to the transition toward planting of triploid fishes for angling which no longer supplements the existing population through stocking of reproductive RBT. Given that the sample size was too small in many of the sampling sites, and that population structure showed gene flow, all genotypes for adult fish sampled across sampling sites were pooled for each year for this analysis.

RESULTS

Stocking analysis.

There is a long history of stocking RBT in the Truckee River over the past century, and more than 50,000 RBT and other game fish have been stocked annually to augment sport fishing (Cordone and Franz, 1968). I specifically looked at the stocking records from 1990-2010 (Figure 2A). From 1990-1994 over 100,000 reproductive RBT were stocked each year for angling. From 1995-1999, more than 50,000 RBT were stocked annually, as were more than 75,000 of the native LCT. Hybridization between native cutthroat trout and the stocked RBT was suspected, and the potential to create a hybrid swarm was a concern. In 2000-2003, LCT stocking became much more limited, and was limited to regions where RBT were not stocked. Starting in 2004, triploid RBT were stocked instead of hatchery raised diploid RBT. Because triploid RBT are not reproductive, they can be stocked without augmenting the naturalized RBT population. Triploids were stocked as “catchables” for a put-and-take fishery with an average fork length of 250mm. During that time, LCT were also stocked in large numbers from a variety of size classes (Figure 2B).

Water discharge

To look at the overall trend in discharge on the Truckee River, I looked at the average monthly discharge data for 1945 to 2013 (Figure 3A). Population genetic structure can emerge in stocked populations if the populations become isolated due to temporal barriers created by low water flow. In the past 10 years, 2006 stands out as a high water year. I downloaded USGS discharge data for the stations that are nearest to the sample collection sites, for 2006-2010 to determine if high flow years led to a difference in the spatial distribution of genetic variation (http://wdr.water.usgs.gov/wy2006/search.jsp, Figure 3B). The tributaries showed the same pattern, (Figure 3C) despite water flow being at least ten fold lower.

Distribution of young of the year

I identified 2,012 individuals as pure diploid RBT. Although some of these samples could be remnants of past stocking events, given that primarily triploid RBT have been stocked in the river since 2004, and survival of stocked fishes from one year to the next is very low, these samples were considered to be part of the naturalized RBT population. Overall, 43% of the fish sampled had a fork length of 50-100mm and were considered young-of-the-year (YOY). In 2007, 57% or the samples collected were YOY; in 2008, that proportion fell to 47%; in 2009, the proportion fell to 26%. In 2010, reproduction rebounded to 52% of the samples collected. Very few YOY were found north of MCRD (Table 1, Figure 1, barrier 29). YOY were consistently found in VerdiC and Hunter Creek and Dog Valley (>75%). Every transect where greater than 15 trout were found had YOY, indicating high levels of reproduction in most of the Truckee River south of Derby Dam (Figure 4).

Genetic variation in Rainbow Trout

Because YOY often do not survive to become reproductive, genetic variation was calculated excluding YOY. The global observed and expected heterozygosity across all loci was high for all four years of the study (Table 3). The H_e was 0.79 ±0.0086 and the H_o was 0.73 ± 0.0089. The average number of alleles differed in 2007 (5.3 ± 1.8) , compared to the other 3 years of the study 2008(9.3 ±3.5), 2009(9.9 ± 3.9), and 2010 (9.1 ± 3.3) ($p > 0.05$). The highest effective population size was found in 2009 (Table 4). N_e was 361 in 2009 compared to N_e of only 17.1 in 2007, however the sample size in 2007 was much smaller than that sampled in the other years and the number of alleles found was lower, indicating that sampling that year may not have been representative of the complete complement of genetic variation found in the river. In 2008 and 2010, the effective population sizes were 176.1 and 136 respectively.

Genetic Population structure

FSTAT showed deviation from HWE for locus Omm1220, so that locus was removed from the data for F_{ST} and STRUCTURE analysis. I conducted pairwise F_{ST} estimates among pooled samples from the 8 major sampling sites with estimates ranging from 0.0020 to 0.0490 (Table 5). These data show very low levels of differentiation among sampling sites. Seven of the comparisons did not have statistically significant values after adjusting alpha for multiple comparisons to indicate a 5% confidence level. There were no significance differences found for the comparison of the Nixon (NIX) transect with Reno (RNO) and McCarren (MCR) transects and Wadsworth (WAD) was not differentiated from Verdi (VRD), RNO, MCR, NIX, and Dog Valley Creek (DOG).

Bayesian genotype clustering analysis results suggests 2 or 3 clusters in 2007, 4 clusters in 2008, 5 clusters in 2009 and 2 or 3 clusters in 2010 (Figure 5). In situations where the natural log of the probability of the data (LnP(D) and the analysis of ΔK gave support for more than one value for k, proportional memberships for both values of k were reported (Figure 6). The latitude and longitude of the collection sites is graphed with filled circles corresponding to the most common genotype cluster found at that site to give a representation of how the genetic variation is partitioned on the landscape. The collection sites are numbered from the outlet of Lake Tahoe (TAHa, 1) to the inlet of Pyramid Lake (NIXc, 26), upstream to downstream. In 2007, only Dog Valley Creek had membership in two of the three clusters identified (Figure 6A). All samples collected in the main stem and Hunter Creek belonged primarily to the same cluster. In 2008, Dog Valley Creek clustered with the main stem of the river, and Hunter Creek belonged in its own cluster. The main stem of the river developed structure downriver from Farad (site 6, Figure 6B), showing membership in a blue cluster in 2008. However, the red and green clusters are prevalent throughout the downstream sites. In 2009, the blue cluster dominated upstream of Farad again (Site 6, Figure 6C), but the Tahoe sites has assignment to multiple clusters. Multiple clusters were found between Farad and Verdi, between Verdi and Reno (primarily red and yellow), with the yellow cluster predominating in the McCarran transects (Figure 6C). The Nixon transects (24, n=11 and 25, n=1) also assign to multiple genotype clusters; however, NIXb which assigns the red cluster was comprised of a single individual. Interestingly in 2009, the lower Hunter

Creek and Dog Valley Creek samples share membership in all five clusters. However, sampling was done both above and below barriers in that year. In upper Dog Valley Creek, there is a purple cluster that is more segregated from the rest of the river. In 2010, the best support found in STRUCTURE was for 3 genotype clusters. From Tahoe to Farad (2-13) a blue cluster is represented and from Farad to Verdi, a green cluster, Verdi to Reno have membership in all three clusters, and the red cluster dominates downstream of McCarren. However, From Verdi to McCarran (13-16) the samples shared membership in both blue and red cluster, and from McCarran to Wadsworth (17-23) the samples were primarily the red cluster. The major divisions in population structure across all four years were consistently at Farad (site 6), Verdi (site 13) and McCarran (site 17); however, there are shifts in the degree of differentiation and there is evidence of mixing both upstream and downstream.

DISCUSSION

Using polymorphic microsatellites, I evaluated the genetic population structure of RBT in the Truckee River over the time period from 2007-2010. In this study, I was not able to find consistent population structure over the four year period to identify eradication units. High water years facilitate connectivity, making barriers from year to year transient. The year prior to the initiation of this study, 2006, was a high water year, and water discharge was low for the four years of this study (Figure 3). Although population structure was evident in the Dog Valley and Hunter Creek tributaries, even that structure was transitory and varied year to year

Once an introduced species has become naturalized in a system, successful eradication requires quantifying the census size and resilience of the existing population. Census size can be estimated using mark recapture methods, identifying individuals through DNA fingerprinting with multiple microsatellite markers. This can be difficult in situations where the population is robust; a large sample size is required in each capture session. In the case of the Truckee River, approximately 400 samples were collected from the same sampling transect in four consecutive years. Only one individual that was captured in 2008 was re-captured in a 2009, which indicates a large RBT population, however, low recapture rates make estimating census imprecise which is one of the limitations of using microsatellite data to identify census size (Putman and Carbone, 2014; Purcell and Stockwell, 2015).

Triploid trout have been stocked into the Truckee River since 2004 to provide angling opportunities without strengthening the existing RBT population in the river. Stocked triploids may interfere with the successful reproduction of the naturalized trout population, providing reproductive containment (Wong and Van Eenennaam, 2008). The N_e for 2007 was only 17.1, which would indicate the population was at risk, with an effective population size below 50 (Frankham, 1995; Frankham et al. 2014). However, during the following three years the effective population size for these trout increased to over 100, indicating stable reproduction in this population (Table 4). The robustness of the RBT population is also indicated by the number of young-of-the-year that were captured in each sampling period and each section of the river (Figure 4). The habitat and water flow of the Truckee River provides ample spawning habitat and resources to support a robust salmonid population, and the stocking of triploids does not appear to impact the RBT reproduction.

The overall F_{ST} values of the RBT found in the Truckee River show very little differentiation indicating long-term connectivity among the sites in the Truckee River. The low level of differentiation (F_{ST}) among the main stem river and the tributaries indicates that although there are abundant potential barriers in the Truckee River, these barriers are transient, and, if RBT are eradicated from any one segment of the river, they will move into the river when conditions change. The region of the river from Wadsworth to Nixon showed the lowest number of RBT and could also be a site for RBT eradication. High total dissolved solids and higher alkalinity characterize the water quality of that region, especially after four years of reduced flow. Habitat conditions in the lower regions of the river have been considered inadequate for successful reproduction (Coleman and Johnson, 1988). In addition, there was little to no RBT reproduction in that region (Figure 4). Current restoration activities are focusing on improving the conditions in the lower Truckee River as well as potentially increasing connectivity with Pyramid Lake (Tim Loux, USFWS, personal communication). Habitat conditions in the lower Truckee River have been showing signs of improvement. For the first time since 1938, in 2014 and 2015, numerous salmonid redds were discovered in the lower Truckee River, accessed from Pyramid Lake. Preliminary genetic analysis of emergent fry indicates that LCT from Pyramid Lake were the primary fish spawning (DeLong, 2014). Although sampling efforts here indicate that there are less RBT found in the lower Truckee River, if conditions continue to improve, RBT can move down into that section of the river and hybridize with the LCT. If RBT can be eradicated and barriers were stabilized, the lower Truckee River could be a site for reintroduction. STRUCTURE analysis identified 2-5 distinct population clusters; however, none of these clusters were geographically discrete over the four years of the study. Low water flows from 2007 to 2010 led to developing population structure and segregation of alleles over time, but membership among genotype clusters was shared among the major segments of the river and a high water year could disrupt any structure and eliminate the impact of barriers. It was proposed that the tributaries may be geographically isolated enough to provide eradication units and allow for reintroduction of LCT. However, the isolation of Dog Valley Creek and Hunter Creek varied from year to year. High water events allow for gene flow among the tributaries below the major barriers where the predominance of the sampling was done. Connectivity between the Truckee River and Hunter Creek is further illustrated because a large proportion of RBT were found in Hunter Creek, and there are no records of RBT being stocked in that system. Barriers upstream of collection sites may provide isolation, as they appeared to provide barriers to LCT movement and hybridization in 2009(Chapter 2), but there were insufficient samples collected to confirm this conclusion. It may be possible to construct barriers between the river and the tributaries; however, in over 50% of the studies investigated, reinvasion over barriers occurred in the first four years (Avenetti et al., 2006). Eradication of the introduced RBT would provide suitable habitat for LCT recovery; however, given the overall connectivity of the system, a large scale eradication program would need to be in effect with continued monitoring.

Recovery programs can successfully extirpate invasive species, reintroduce native species and reduce the risk of extinction of unique native species. Another strain of cutthroat trout, the greenback cutthroat trout, was thought to be extinct in the 1930s. Not unlike the Pilot Peak LCT, it was discovered in two small streams, and a multiagency recover effort was formulated to restore the trout to a portion of its native range. Fourteen of the 37 attempted translocations were successful; those that were unsuccessful either had too little habitat, unsuitable spawning habitat or, in the majority of the cases, were impacted by non-native salmonids (Harig et al., 2000). Due to recovery efforts and public support, the overall status of the greenback cutthroat trout has improved (Young and Harig, 2001). Reintroduction of Lahontan Cutthroat trout has also shown success in the Upper Truckee River restoration project (Miller and Santora, 2013). The success of this project is dependent on removal of non-native trout in that system, maintenance of quality barriers to prevent reinvasion, and continued monitoring of the LCT. Similar efforts would need to be implemented in the Truckee River for successful LCT reintroduction.

Figure Legends

Figure 1. The Truckee River Watershed. Potential barriers and water diversions identified as (X). Numbers corespond to sites listed in Table 1. Collection transects (3-4 per site) are indicated by filled circles in alternating red and blue, which depict the sampling sites.

Figure 2. Trends in Salmonid Stocking. (A) Average numbers of stocked fish were reported for each time period(±SD). Time periods were selected based on major shift seen in primary species of fish being stocked during the 20 year period shown. (B) Average fork length of RBT and LCT stocked in the Truckee River over a 20 year period.

Figure 3. Data from water gauging stations.(A) Average monthly discharge for the Truckee River from 1945-2013. Red diamond indicates the relatively high water year in 2006. (B) Total discharge in cubic meters per second for 2006-2010 measued for the USGS gauging stations on the Truckee River(C.) Mean annual discharge in cubic meters per second of the Dog Valley and Hunter Creek tributaries.

Figure 4. Spatial distribution of young of the year. Each panel represents the adult fish and young of the year found in the 26 transects of the Truckee River, and the 2 main tributaries, Dog Valley and Hunter Creek. Stocked RBT are all over 200mm in length, so abundance of fingerlings and fry indicates reproduction in the river segment.

Figure 5. Baysesian genotype clustering analysis using the program STRUCTURE (version 2.3.4) for RBT for each year. Each panel represents a

different year (2007-2010). The number of genotype clusters (k) is on the X axis and the natural log of the probability of the data is on the Y axis (left panel). Diamonds represent the average LnP(D) per k (± SD). Right panels show the Delta K determination from the same data using STRUCTURE HARVESTER and the method described by Evanno et al. (2005).

Figure 6. Population structure of naturalized Rainbow Trout. Each panel represents the proportional membership in genotype cluster for the best fit of the data. Sampling locations (open or filled circles) are graphed by latitude (Y axis) and longitude (X axis) to estimate location on the river. The color of the circle represents the major genotype cluster found at that transect for each year of the study. Open circles represent sites where no samples were found using the same effort as the other transects, with the exception of 2010, where Hunter Creek and Dog Valley were not sampled. For the inset graphs, the transects (where samples were found) are on the X axis and the average proportional membership per genotype cluster is on the Y axis.

Table 1. List of all Sampling transects **(bold)** and Barriers (numbered), elevation, latitude and longitude as indicated in Figure 1.

Transect/Barrier Name	Section(S) Transect (T)	Elevation (ft)	Latitude	Longitude
1. Lake Tahoe Dam	Barrier	6256	39.16691	-120.144
Tahoe A	**S8T2**	**6225**	**39.16207**	**-120.159**
Tahoe B	**S8T3**	**6079**	**39.20848**	**-120.198**
Tahoe C	**S8T1**	**5909**	**39.27361**	**-120.206**
Truckee A	**S7T3**	**5751**	**39.33366**	**-120.164**
2. Prosser Creek Dam	Barrier	5737	39.37952	-120.138
Truckee B	**S7T2**	**5676**	**39.34754**	**-120.123**
Truckee C	**S7T1**	**5598**	**39.36092**	**-120.117**
3. Martis Creek Dam	barrier	5825	39.32734	-120.115
4. Boca Dam	barrier	5565	39.39044	-120.094
Farad A	**S6T1**	**5472**	**39.38554**	**-120.086**
Farad B	**S6T2**	**5400**	**39.36848**	**-120.041**
5. Farad Power Diversion	barrier	5290	39.39693	-120.024
Farad C	**S6T4**	**5169**	**39.42332**	**-120.035**
Farad D	**S6T3**	**5076**	**39.4497**	**-120.005**
6. Fleish Power Diversion	barrier	5074	39.45219	-120.006
7. Steamboat Ditch Diversion	barrier	5017	39.46612	-120.003
Verdi A	**S5T1**	**4984**	**39.475**	**-119.994**
8. Washoe Power Diversion &	barrier	4780	39.51955	-119.962
9. Last Chance Ditch Diversion	barrier	4658	39.51477	-119.92
Verdi B	**S5T3**	**4854**	**39.50926**	**-119.996**
10. Verdi Power Div. & Coldron Ditch	barrier	4917	39.49275	-119.995
Verdi C	**S5T2**	**4637**	**39.50651**	**-119.902**
11. Lake Ditch Diversion	barrier	4612	39.50501	-119.891
12. Southside Ditch Diversion	barrier	4611	39.50928	-119.876
13. Orr Ditch Diversion	barrier	4593	39.51034	-119.874
14. Chalk Bluff Pump Station	barrier	4582	39.51049	-119.866
15. Idlewild Pond Diversion	barrier	4515	39.52275	-119.835
16. Idlewild Pond Return Drain	barrier	4497	39.52036	-119.828
Reno A	**S4T3**	**4500**	**39.52391**	**-119.819**
17. Cochran Ditch Diversion	barrier	4507	39.52406	-119.817
18. Wingfield Park Dams	barrier	4504	39.52481	-119.817
Reno B	**S4T2**	**4448**	**39.53013**	**-119.795**
19. Eastman Ditch Diversion	barrier	4481	39.52714	-119.778
20. Glendale Ditch Diversion	barrier	4480	39.52815	-119.778
21. Pioneer Ditch Diversion	barrier	4406	39.52188	-119.773
Reno C	**S4T1**	**4394**	**39.51424**	**-119.736**
22. North Truckee Drain confluence	barrier	4400	39.52126	-119.706
23. Steamboat Creek & Sparks-Reno	barrier	4360	39.52102	-119.703

24. Lagomarsino Noce Ditch Diversion	barrier	4368	39.51117	-119.669
25. Murphy Ditch Diversion	barrier	4370	39.50972	-119.667
McCarran Ranch A	**S3T3**	**4309**	**39.52563**	**-119.61**
26. McCarran Ditch Diversion	barrier	4290	39.53284	-119.599
McCarran Ranch B	**S3T2**	**4284**	**39.54498**	**-119.587**
McCarran Ranch C	**S3T1**	**4277**	**39.54703**	**-119.573**
27. Hill Ditch Diversion	barrier	4260	39.56267	-119.541
28. Tracy Power Plant	barrier	4246	39.56515	-119.522
McCarran Ranch D	**S3T4**	**4202**	**39.56469**	**-119.487**
29. Derby Dam and TCID Diversion	barrier	4212	39.58611	-119.448
Wadsworth A	**S2T1**	**4189**	**39.58506**	**-119.444**
30. Marble Bluff Dam	barrier	3844	39.8555	-119.393
31. Washburn Ditch Diversion	barrier	4129	39.58813	-119.388
32. Outlet Ditch Christensen Ranch	barrier	4155	39.58888	-119.38
WadsworthB (WadsB)	**S2T2**	**4132**	**39.59062**	**-119.368**
33. Gregory Ditch Diversion	barrier	4146	39.59719	-119.359
34. Herman Ditch Diversion	barrier	4105	39.60775	-119.318
WadsworthC (WadsC)	**S2T3**	**4099**	**39.61273**	**-119.306**
35. Pierson Ditch Diversion	barrier	4071	39.61259	-119.306
36. Cersola Ditch Diversion	barrier	4045	39.64318	-119.291
37. Olinghouse No. 3 pump	barrier	3979	39.69558	-119.288
38. Olinghouse No. 1 Pump	barrier	4050	39.64038	-119.284
39. Proctor Ditch Diversion	barrier	4062	39.63008	-119.283
40. Gardella Ditch Diversion	barrier	4006	39.66941	-119.274
NixonA	**S1T1**	**3988**	**39.72697**	**-119.319**
41. Numana Dam and Pyramid Indian	barrier	3916	39.7895	-119.35
NixonB	**S1T2**	**3903**	**39.81771**	**-119.35**
NixonC	**S1T3**	**3850**	**39.85426**	**-119.394**

Table 2. Microsatellite primers used to evaluate population structure on salmonids sampled in the Truckee River. Product size, Number of alleles (N_A), observed heterozygosity (H_O) and expected heterozygosity are reported for all RBT, LCT and hybrids (HYB) sampled and evaluated between 2007 and 2010.

			RBT (n=2011)		LCT (n=76)		HYB (n=85)		
Locus	Dye	Panel	Range (N_A)	H_O	Range (N_A)	H_O	Range (N_A)	H_O	Reference/ GenBank Ac. #
OCH15	Vic	1	286-396 (24)	0.8056	286-366 (21)	0.5541	286-366 (21)	0.8571	Peacock et al. 2004 AY374432
OCH17	Pet	1	197-293(24)	0.8442	201-281 (16)	0.4342	201-281 (16)	0.8889	Peacock et al. 2004 AY374434
OCH20	Fam	1	227-353 (23)	0.8700	227-319 (16)	0.6974	227-319 (16)	0.8765	Robinson et al. 2009 DQ979815
OMM1315	Vic	1b	122-186 (12)	0.7142	--	--	122-178(8)	0.4156	Palti et al. 2002 G73554
OMM1322	Fam	1b	196-280 (18)	0.8143	162-230 (12)	0.5714	162-230 (12)	0.8554	Palti et al. 2002 G73560
OMM1323	Fam	1b	206-222 (5)	0.6658	--	--	206-222 (5)	0.2987	Palti et al. 2002 G73561
OMM1036	Vic	1b	234-348 (24)	0.5278	--	--	234-348 (16)	0.4267	Rexroad et al. 2002 AF346686
OMM1220	Pet	2	127-259 (21)	0.7564	119(1)	--	119-215 (12)	0.4407	Rexroad and Palti 2003 AF470002
OMM1302	Ned	2	217-301 (18)	0.4298	--	--	225-297 (17)	0.4058	Palti et al. 2002 G73542
OMM1325	Pet	2	272-324 (11)	0.8366	253-297 (7)	0.4384	253-297 (7)	0.7683	Palti et al. 2002 G73564
OMM1329	Fam	2	161-227 (17)	0.8124	142-174 (10)	0.2533	142-174 (10)	0.8902	Palti et al. 2002 G73562

Table3. Population statistics excluding RBT smaller than 100 mm forklength.

2007	N	H_e	H_e SD	H_o	H_e SD	N_A	N_A SD
TAH	4	0.8593	0.0335	0.7803	0.0647	5.00	1.41
TRK	15	0.8085	0.0347	0.6998	0.0366	8.18	3.09
FAR	2	0.7879	0.0714	0.7727	0.0893	3.09	0.94
VRD	4	0.8258	0.0387	0.7500	0.0668	4.55	1.63
RNO	7	0.8052	0.0439	0.7294	0.0510	6.18	2.48
MCR	9	0.8194	0.0399	0.7403	0.0445	6.91	2.70
WAD	2	0.7576	0.0881	0.7727	0.0893	2.91	0.94
NIX	5	0.8155	0.0473	0.7591	0.0631	4.64	1.69
DOG	65	0.6492	0.0248	0.6664	0.0183	6.27	1.85
HUN	6	0.7908	0.0301	0.7606	0.0546	5.36	1.43
GLOBAL	119	0.7919	0.0452	0.7431	0.0578	5.3091	1.8184
2008	**N**	**H_e**	**H_e SD**	**H_o**	**H_e SD**	**N_A**	**N_A SD**
TAH	19	0.7783	0.0403	0.7220	0.0317	8.55	3.21
TRK	78	0.8147	0.0376	0.7327	0.0153	11.55	4.59
FAR	18	0.8102	0.0306	0.7700	0.0301	8.82	2.68
VRD	26	0.8108	0.0389	0.7289	0.0284	9.73	3.35
RNO	81	0.8058	0.0368	0.7493	0.0149	12.00	4.75
MCR	39	0.7994	0.0379	0.7428	0.0218	10.18	4.09
WAD	27	0.8219	0.0328	0.7561	0.0258	9.82	3.82
NIX	0	--	--	--	--	--	--
DOG	9	0.6760	0.0432	0.7210	0.0453	4.45	1.37
HUN	45	0.7671	0.0290	0.7752	0.0194	8.64	3.41
GLOBAL	342	0.7871	0.0364	0.7442	0.0259	9.3030	3.4743
2009	**N**	**H_e**	**H_e SD**	**H_o**	**H_e SD**	**N_A**	**N_A SD**
TAH	37	0.8004	0.0308	0.7370	0.0222	10.55	3.72
TRK	45	0.8140	0.0368	0.7523	0.0195	11.00	4.20
FAR	54	0.8166	0.0368	0.7679	0.0179	11.27	4.08
VRD	40	0.8009	0.0319	0.6769	0.0234	9.36	3.67
RNO	77	0.8017	0.0331	0.7276	0.0156	10.82	4.81
MCR	62	0.7996	0.0351	0.7516	0.0168	11.18	4.75
WAD	6	0.8026	0.0387	0.7606	0.0556	5.09	1.76
NIX	11	0.7879	0.0400	0.7655	0.0410	7.55	3.27
DOG	77	0.8073	0.0315	0.7344	0.0156	10.64	4.18
HUN	63	0.8183	0.0331	0.7243	0.0178	11.55	4.70
GLOBAL	472	0.8049	0.0348	0.7398	0.0245	9.9000	3.9128
2010	**N**	**H_e**	**H_e SD**	**H_o**	**H_e SD**	**N_A**	**N_A SD**
TAH	8	0.7981	0.0263	0.8084	0.0427	6.09	1.38
TRK	22	0.8269	0.0311	0.7605	0.0286	9.73	3.64
FAR	30	0.8080	0.0476	0.6697	0.0283	9.55	3.98
VRD	37	0.7907	0.0346	0.6458	0.0250	9.18	3.16
RNO	45	0.7973	0.0368	0.7197	0.0210	10.45	4.11
MCR	49	0.7907	0.0336	0.7289	0.0195	10.27	4.63
WAD	22	0.8096	0.0312	0.7415	0.0286	9.09	2.88
NIX	--	--	--	--	--	--	--
GLOBAL	213	0.8030	0.0345	0.7249	0.0277	9.1948	3.3952

(HUN and DOG were not sampled in 2010; -- no fish found in the sampling period)

Table 4. Effective population size (Ne) per year, per species with the 95% confidence interval around estimate, number of fish sampled >100 mm fork length (n).

	Ne	95%CI	n
2007	17.1	14.5-20	119
2008	176.1	149.4-210.1	342
2009	361	290.1-463.5	472
2010	136	110.7-172	213

Table 5. Pairwise F_{ST} estimates between sites sampled in the Truckee for RBT with fork length greater than 100mm for all samples collected between 2007 and 2010. All bolded F_{ST} estimates are statistically significant (900 permutations indicative of a p-value with a 5% confidence level of 0.00111).

(A)	TAH	TRK	FAR	VRD	RNO	MCR	NIX	WAD	DOG
TRK	**0.0286**								
FAR	**0.0270**	**0.0156**							
VRD	**0.0272**	**0.0218**	**0.0080**						
RNO	**0.0208**	**0.0252**	**0.0197**	**0.0119**					
MCR	**0.0257**	**0.0322**	**0.0327**	**0.0252**	**0.0052**				
NIX	**0.0256**	**0.0213**	**0.0153**	*0.0102*	*0.0020*	*0.0053*			
WAD	**0.0359**	**0.0268**	**0.0277**	*0.0123*	*0.0083*	**0.0138**	*0.0054*		
DOG	**0.0490**	**0.0394**	**0.036**	**0.0261**	**0.0278**	**0.0437**	**0.0340**	*0.0403*	
HUN	**0.0287**	**0.0373**	**0.0269**	**0.0262**	**0.0211**	**0.0278**	**0.0216**	**0.0324**	**0.0412**

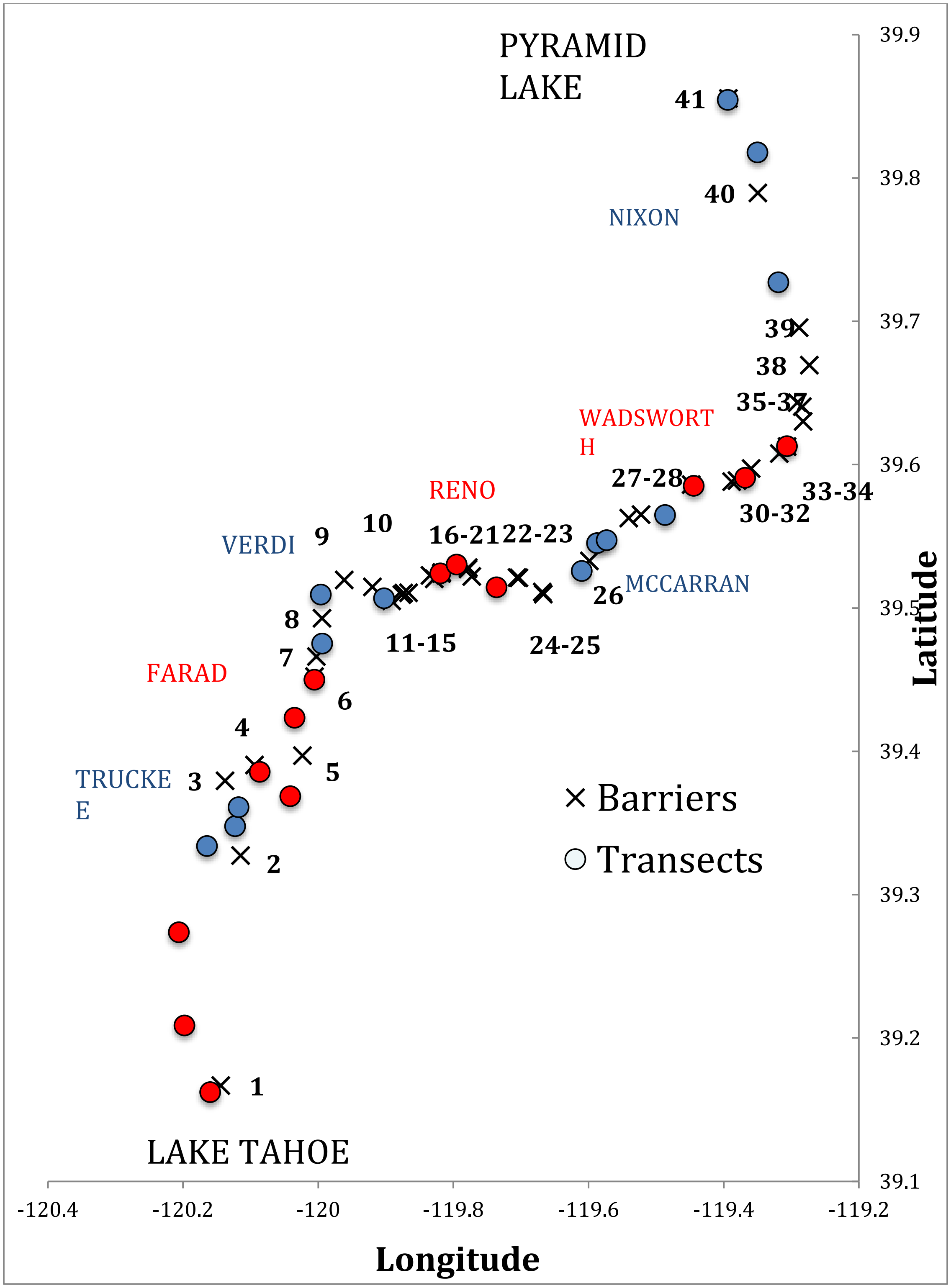

PYRAMID LAKE
41
40
NIXON
39
38
35-37
WADSWORTH
RENO
27-28
33-34
30-32
VERDI
9
10
16-21
22-23
MCCARRAN
26
8
11-15
24-25
7
FARAD
6
4
5
TRUCKEE
3
2
1
Barriers
Transects
LAKE TAHOE
39.9
39.8
39.7
39.6
39.5
39.4
39.3
39.2
39.1
-120.4
-120.2
-120
-119.8
-119.6
-119.4
-119.2
Longitude
Latitude

Figure 1.

A.

B.

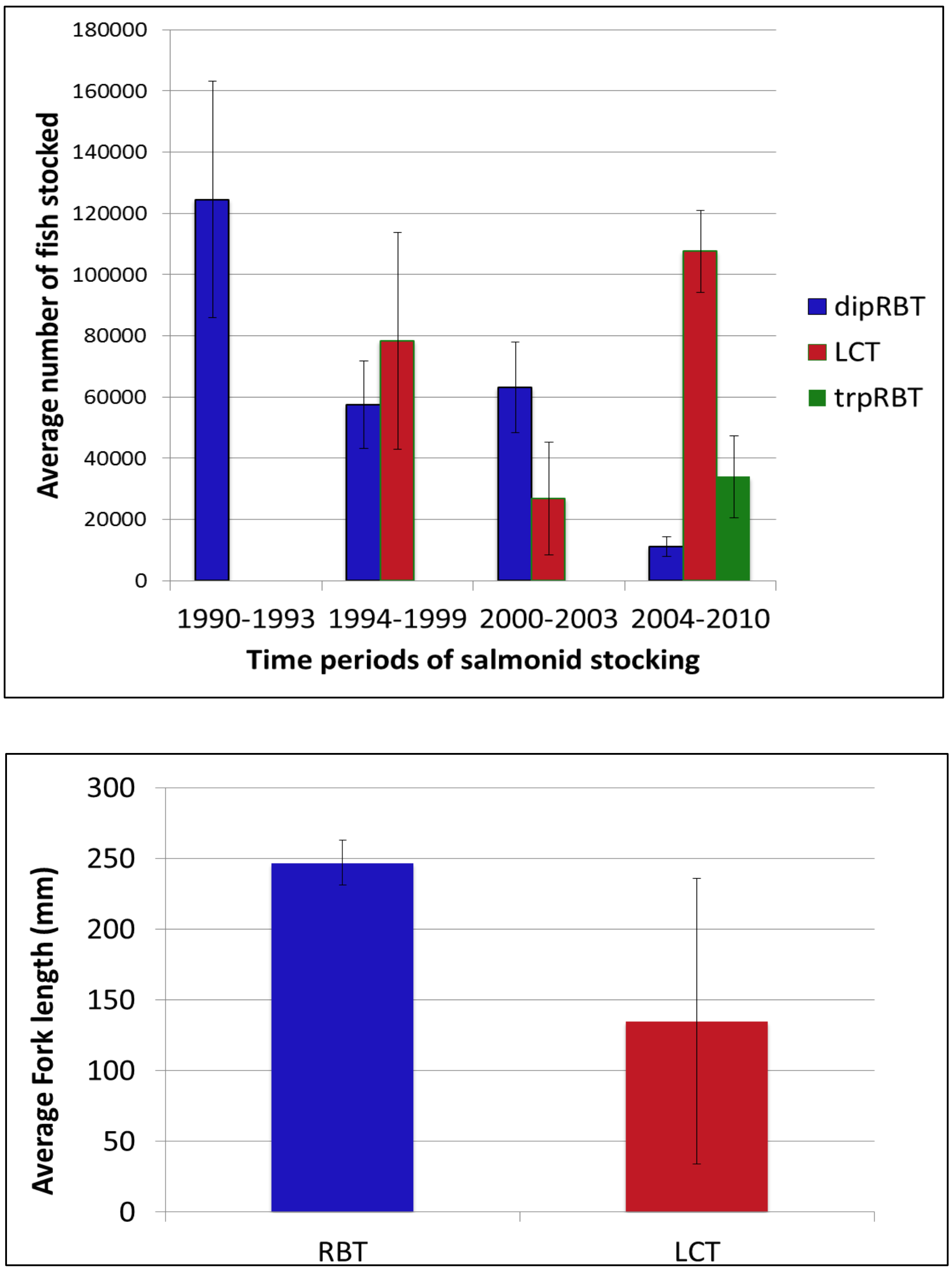
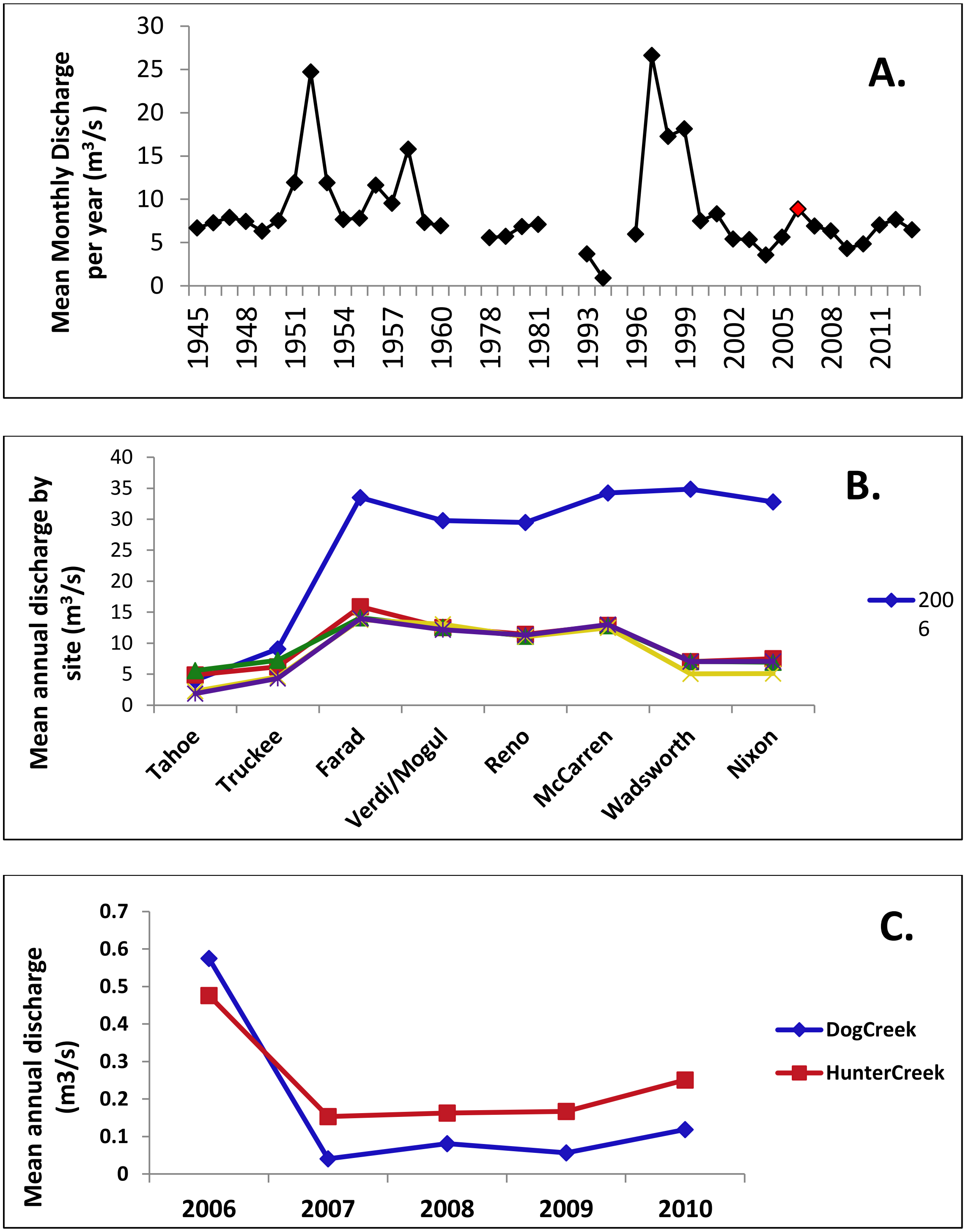

180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Average number of fish stocked
1990-1993
1994-1999
2000-2003
2004-2010
Time periods of salmonid stocking
dipRBT
LCT
trpRBT
300
250
200
150
100
50
0
Average Fork length (mm)
RBT
LCT

Figure 2.



A.
Mean Monthly Discharge per year (m³/s)
0
5
10
15
20
25
30
1945
1948
1951
1954
1957
1960
1978
1981
1993
1996
1999
2002
2005
2008
2011
B.
Mean annual discharge by site (m³/s)
0
5
10
15
20
25
30
35
40
Tahoe
Truckee
Farad
Verdi/Mogul
Reno
McCarren
Wadsworth
Nixon
2006
C.
Mean annual discharge (m3/s)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
2006
2007
2008
2009
2010
DogCreek
HunterCreek

Figure 3.

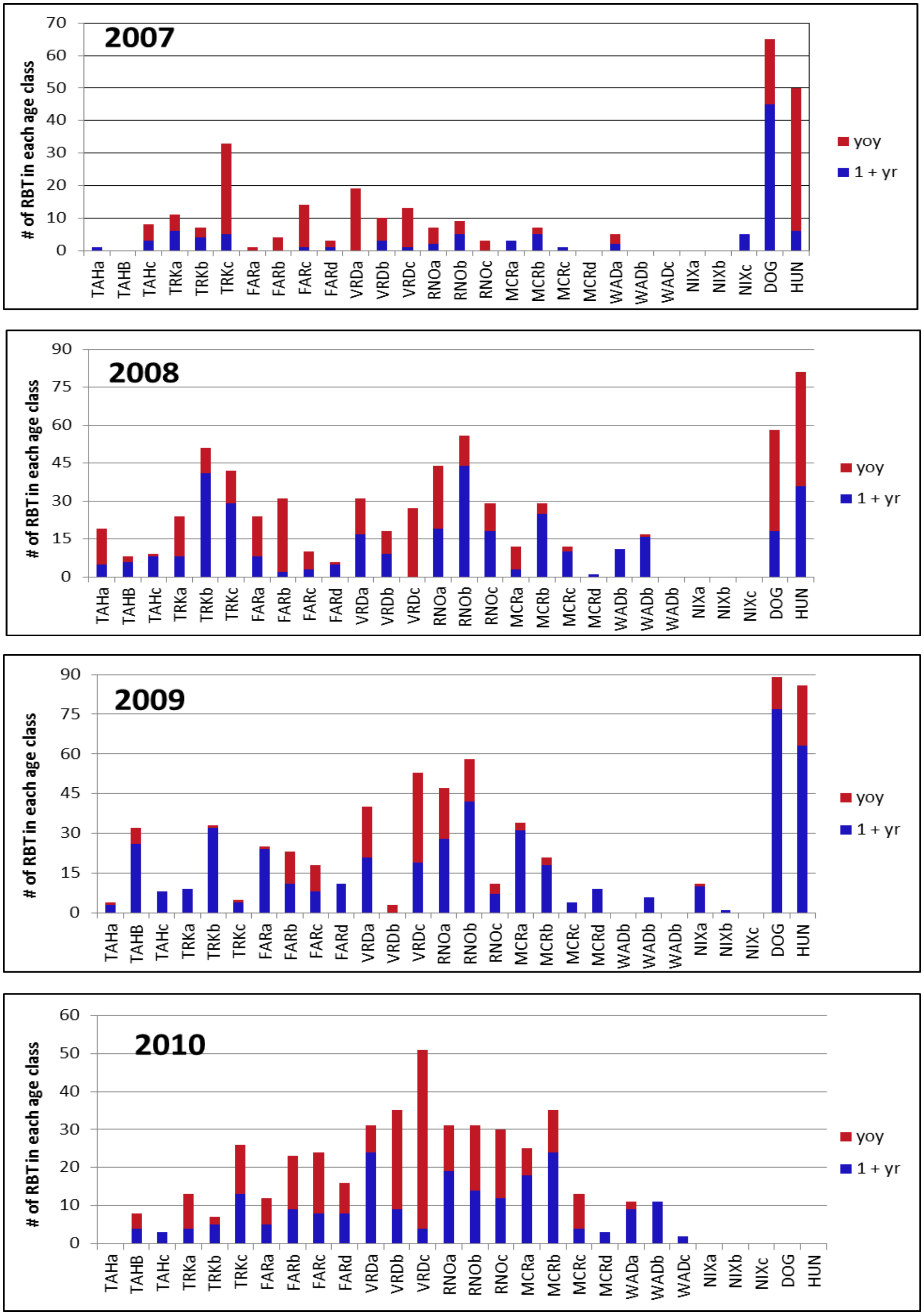

2007
2008
2009
2010
# of RBT in each age class
yoy
1 + yr
TAHa
TAHB
TAHc
TRKa
TRKb
TRKc
FARa
FARb
FARc
FARd
VRDa
VRDb
VRDc
RNOa
RNOb
RNOc
MCRa
MCRb
MCRc
MCRd
WADa
WADb
WADc
NIXa
NIXb
NIXc
DOG
HUN

Figure 4.

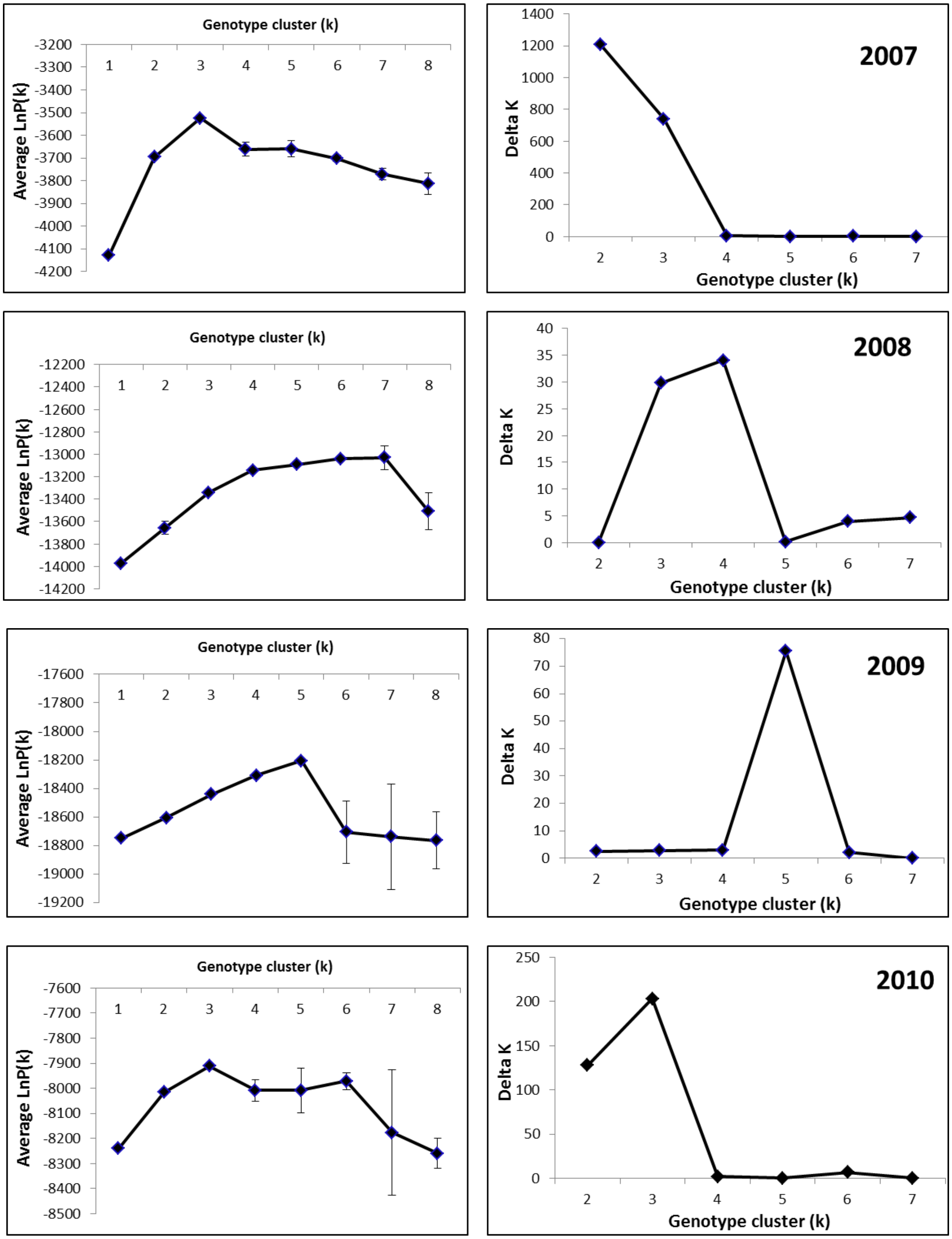
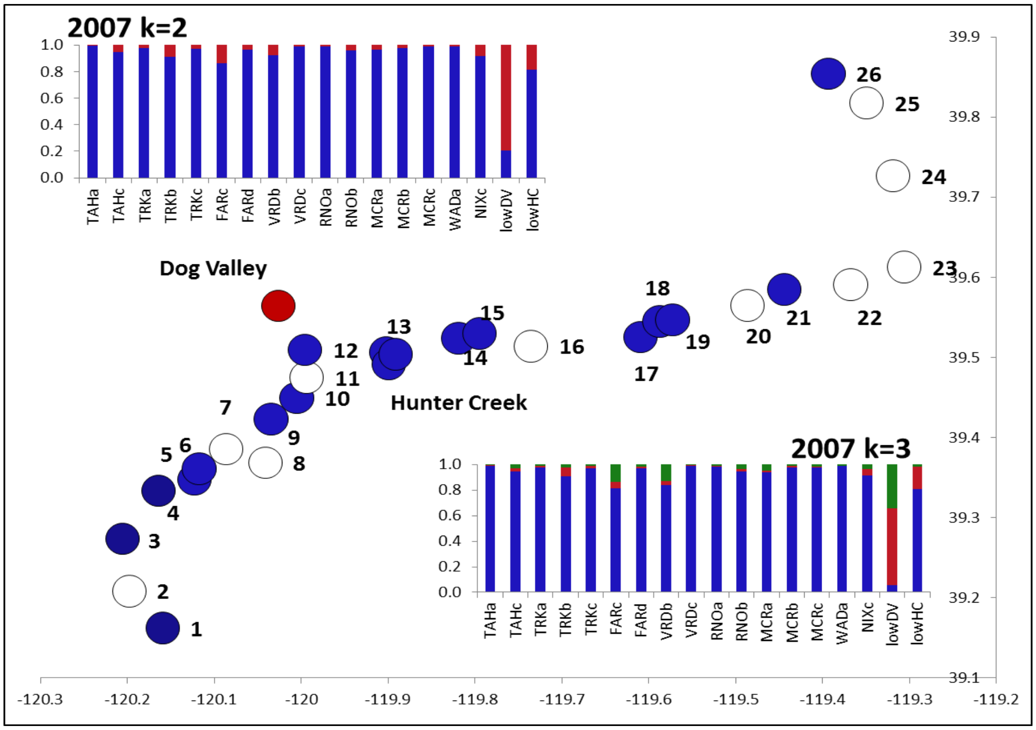


Figure 5.

A.


2007 k=2
TAHa
TAHc
TRKa
TRKb
TRKc
FARc
FARd
VRDb
VRDc
RNOa
RNOb
MCRa
MCRb
MCRc
WADa
NIXc
lowDV
lowHC
Dog Valley
Hunter Creek
2007 k=3
1
2
3
4
5
6
7
8
9
10
11
12
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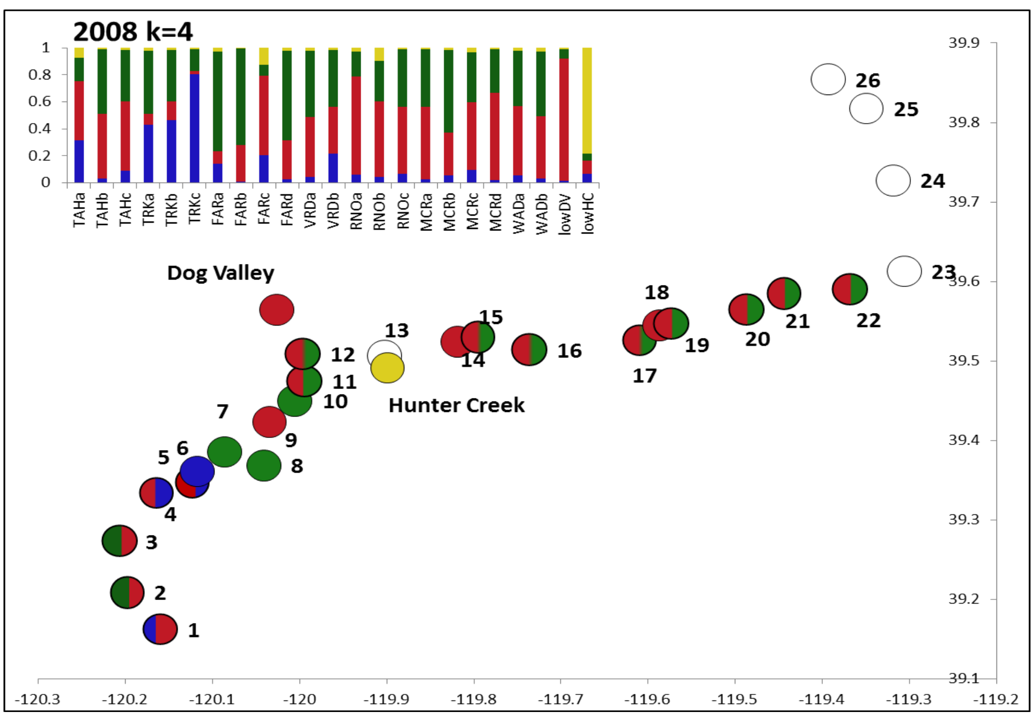
14
15
16
17
18
19
20
21
22
23
24
25
26

B.


2008 k=4
TAHa
TAHb
TAHc
TRKa
TRKb
TRKc
FARa
FARb
FARc
FARd
VRDa
VRDb
RNOa
RNOb
RNOc
MCRa
MCRb
MCRc
MCRd
WADa
WADb
lowDV
lowHC
Dog Valley
Hunter Creek
1
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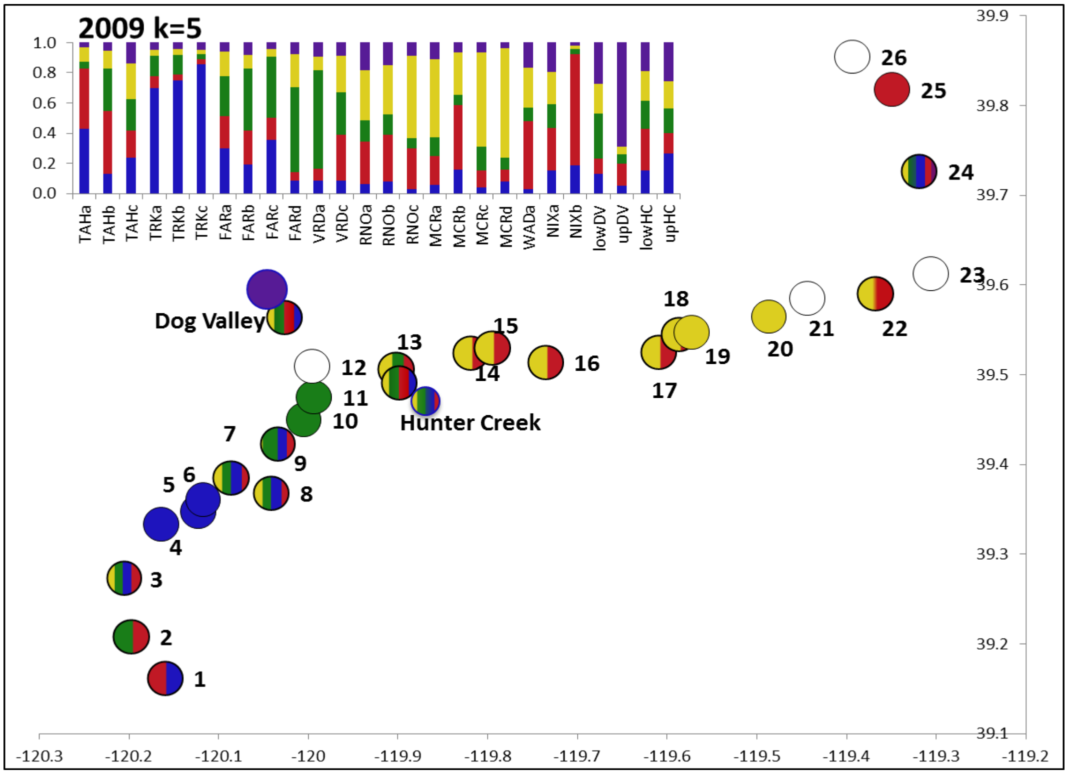
C.


2009 k=5
Dog Valley
Hunter Creek

D.

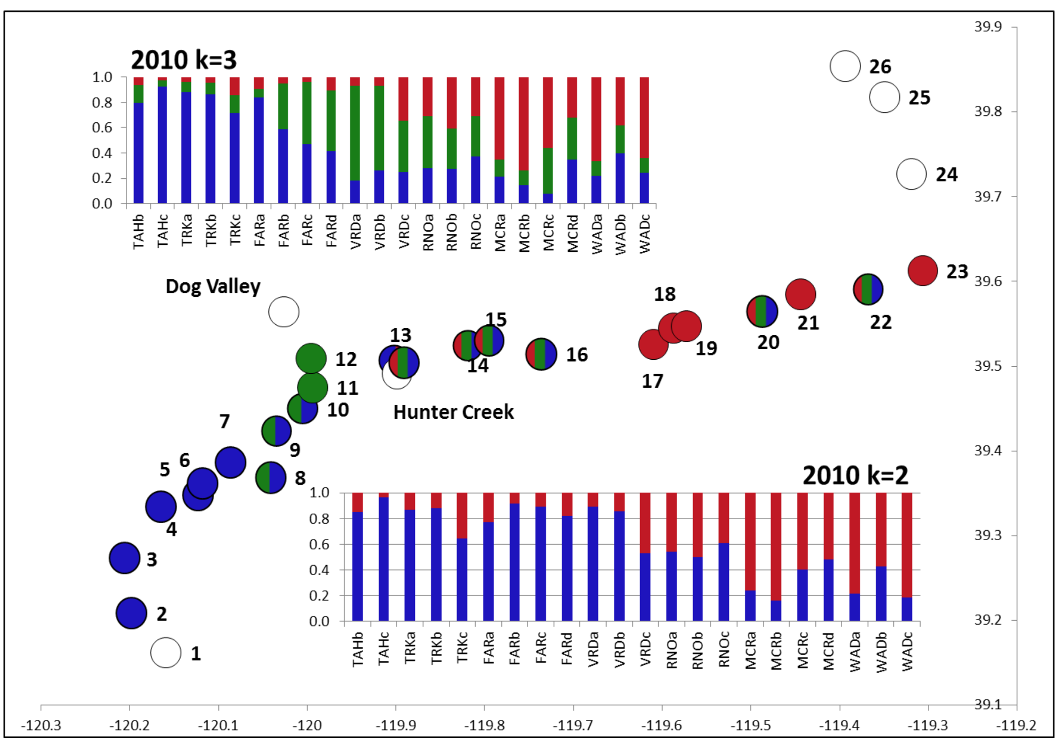

2010 k=3
Dog Valley
Hunter Creek
2010 k=2

Figure 6.

LITERATURE CITED

Adams, A. L., Y. van Heezik, K. J. M. Dickinson, and B. C. Robertson. 2014. Identifying eradication units in an invasive mammalian pest species. *Biological Invasions,* **16**:1481-1496.

Allendorf, F. W., and S. R. Phelps. 1980. Loss of Genetic Variation in a Hatchery Stock of Cutthroat Trout. *Transactions of the American Fisheries Society,* **109**:537-543.

Amlin, N. M., and S. B. Rood. 2002. Comparative tolerances of riparian willows and cottonwoods to water-table decline. *Wetlands*, **22**:338-346.

Avenetti, L. D., A. T. Robinson, and C. J. Cantrell. 2006. Short-term effectiveness of constructed barriers at protecting Apache trout. *North American Journal of Fisheries Management,* **26**:213-216.

Blanchet, S. 2012. The use of molecular tools in invasion biology: an emphasis on freshwater ecosystems. *Fisheries Management and Ecology,* **19**:120-132.

Caicco, S. L. 1998. Current status, structure, and plant species composition of the riparian vegetation of the Truckee River, California and Nevada. *Madrono,* **45**:17-30.

Carlisle, D. M., and C. P. Hawkins. 1998. Relationships between invertebrate assemblage structure, 2 trout species, and habitat structure in Utah mountain lakes. *Journal of the North American Benthological Society*, **17**:286-300.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. *Nature*, **405**:234-242.

Choi, R. T., and K. H. Beard. 2012. Coqui frog invasions change invertebrate communities in Hawaii. *Biological Invasions*, **14**:939-948.

Clausen, R., and R. York. 2008. Global biodiversity decline of marine and freshwater fish: A cross-national analysis of economic, demographic, and ecological influences. *Social Science Research,* **37**:1310-1320.

Cobourn, J. 1999. Integrated watershed management on the Truckee River in Nevada. *Journal of the American Water Resources Association,* **35**:623-632.

Cochran-Biederman, J. L., K. E. Wyman, W. E. French, and G. L. Loppnow. 2015. Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology,* **29**:175-186.

Coleman, M. E., and V. K. Johnson. 1988. Summary of trout management at Pyramid Lake, Nevada, with emphasis on Lahontan cutthroat trout, 1954-1987. *American Fisheries Society Symposium*, **4:107**-115.

Cordone, A. J., and T. C. Frantz. 1968. An Evaluation of trout planting in Lake Tahoe. *California Fish and Game,* **54**(2):68-89.

DeLong, J. 2014. Cutthroats spawn at Pyramid Lake. *Reno Gazette-Journal*

Do, C., R. S. Waples, D.Peel, G. M.Macbeth, B. J.Tillett, and J. R. Ovenden. 2014. NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources*. **14:**209-214.

Dodd, C. K., and R. A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles- Are they conservation strategies that work. *Herpetologica,* **47**:336-350.

Donald, D. B., R. D. Vinebrooke, R. S. Anderson, J. Syrgiannis, and M. D. Graham. 2001. Recovery of zooplankton assemblages in mountain lakes from the effects of introduced sport fish. *Canadian Journal of Fisheries and Aquatic Sciences,* **58**:1822-1830.

Earl, D. A., and B. M. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**(2):359-361.

Estoup, A., and T. Guillemaud. 2010. Reconstructing routes of invasion using genetic data: why, how and so what? Molecular Ecology **19**:4113-4130.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology **14**:2611-2620.

Frankham, R. 1995. Conservation genetics*. Annual Review of Genetics,* **29**:305-327.

Frankham, R., C. J. A. Bradshaw, and B. W. Brook. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation* **170**:56-63.

Godefroid, S., C. Piazza, G. Rossi, S. Buord, A.-D. Stevens, R. Aguraiuja, C. Cowell, C. W. Weekley, G. Vogg, J. M. Iriondo, I. Johnson, B. Dixon, D. Gordon, S. Magnanon, B. Valentin, K. Bjureke, R. Koopman, M. Vicens, M. Virevaire, and T. Vanderborght. 2011. How successful are plant species reintroductions? *Biological Conservation,* **144**:672-682.

Goudet, J. 2001. *FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices* (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Gray, Q. Z., D. J. Fraser, and J. W. A. Grant. 2014. Extirpation for Conservation: Applying Predictors of Extinction Risk to Eradicate Introduced Trout Populations for Lake Restoration. *Ecological Restoration,* **32**:59-67.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool- Status and Strategy. *Science,* **245**:477-480.

Gudmundsson, L. A., S. Gudjonsson, G. Marteinsdottir, D. L. Scarnecchia, A. K. Danielsdttir, and C. Pampoulie. 2013. Spatio-temporal effects of stray hatchery-reared Atlantic salmon *Salmo salar* on population genetic structure within a 21 km-long Icelandic river system. *Conservation Genetics* **14**:1217-1231.

Halverson, A. 2010. A less bold and spirited nation. An entirely synthetic fish. How rainbow trout beguiled America and overran the world. pp 1-12. New Haven and London. Yale University Press.

Harig, A. L., K. D. Fausch, and M. K. Young. 2000. Factors influencing success of greenback cutthroat trout translocations. *North American Journal of Fisheries Management,* **20**:994-1004.

Harig, A. L., and K. D.Fausch 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications,* **12**(2):535-551.

Idrisi, N., E. L. Mills, L. G. Rudstam, and D. J. Stewart. 2001. Impact of zebra mussels (*Dreissena polymorpha*) on the pelagic lower trophic levels of Oneida Lake, New York. *Canadian Journal of Fisheries and Aquatic Sciences,* **58**:1430-1441.

James, J., F. M. Slater, I. P. Vaughan, K. A. Young, and J. Cable. 2015. Comparing the ecological impacts of native and invasive crayfish: could native species' translocation do more harm than good? *Oecologia,* **178**:309-316.

Johannsson, O. E., R. Dermott, D. M. Graham, J. A. Dahl, E. S. Millard, D. D. Myles, and J. LeBlanc. 2000. Benthic and pelagic secondary production in Lake Erie after the invasion of Dreissena spp. with implications for fish production. *Journal of Great Lakes Research,* **26**:31-54.

Kaufman, J. H., D. Brodbeck, and O. R. Melroy. 1998. Critical biodiversity. *Conservation Biology*, **12**:521-532.

Klima, K., and S. E. Travis. 2012. Genetic population structure of invasive nutria

(Myocastor coypus) in Louisiana, USA: is it sufficient for the development of eradication units? *Biological Invasions*, **14**:1909-1918.

Knapp, R. A., P. S. Corn, and D. E. Schindler. 2001. The introduction of nonnative fish into wilderness lakes: Good intentions, conflicting mandates, and unintended consequences. *Ecosystems,* **4**:275-278.

Koel, T. M., P. E. Bigelow, P. D. Doepke, B. D. Ertel, and D. L. Mahony. 2005. Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. *Fisheries*, **30**:10-19.

Kraus, F., and E. W. Campbell, III. 2002. Human-mediated escalation of a formerly eradicable problem: The invasion of Caribbean frogs in the Hawaiian Islands. Biological Invasions, **4**:327-332.

Krebs, R. A., W. C. Borden, E. R. Steiner, M. S. Lyons, W. Zawiski, and B. M. Walton. 2010. Determinants of mussel diversity in Lake Erie tributaries. *Journal of the North American Benthological Society,* **29**:506-520.

Lodge, D. M. 1993. Biological invasions- lessons for ecology. *Trends in Ecology & Evolution*, **8**:133-137.

Mahoney, J. M., and S. B. Rood. 1998. Streamflow requirements for cottonwood seedling recruitment - an integrative model. *Wetlands,* **18**:634-645.

Maples, M. 2008. Field Trip Report: Truckee River tributaries electroshocking survey. Nevada Division of Wildlife, Reno, NV.

Marsden, J. E., and M. A. Chotkowski. 2001. Lake trout spawning on artificial reefs and the effect of zebra mussels: fatal attraction? *Journal of Great Lakes Research,* **27**:33-43.

Martin, C. W., M. M. Valentine, and J. F. Valentine. 2010. Competitive interactions between invasive Nile Tilapia and native fish: The potential for altered trophic exchange and modification of food webs. *Plos One* **5**:1-6.

Miller, R., and M. Santora. 2013. Upper Truckee River Lahontan cutthroat trout restoration project, 2013 *Annual Report. USDA Forest Service, Lake Tahoe Basin Management Unit.* BO#: 2008-F-0434-R001. 1-26.

Muhlfeld, C. C., R. P. Kovach, L. A. Jones, R. Al-Chokhachy, M. C. Boyer, R. F. Leary, W. H. Lowe, G. Luikart, and F. W. Allendorf. 2014. Invasive hybridization in a threatened species is accelerated by climate change. Nature Climate Change **4**:620-624.

Ostberg, C. O., and R. J. Rodriguez. 2004. Bi-parentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. *Molecular Ecology Notes*, **4**:26-29.

Palti, Y., M. R. Fincham, and C. E. Rexroad III. 2002. Characterization and mapping of 38 polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). *Molecular Ecology Notes,* **2:**449-452.

Peacock, M. M., K. H. Beard, E. M. O'Neill, V. S. Kirchoff, and M. B. Peters. 2009. Strong founder effects and low genetic diversity in introduced populations of Coqui frogs. *Molecular Ecology*, **18**:3603-3615.

Peacock, M. M., M. Gustin, V. Kirchoff, M. L. Robinson, E. Hekkala, C. Pizzarro-Barraza and T. Loux. *In press 2016*. Native non-game fishes in the Truckee River: are barriers and patterns of population genetic structure related? *Science of the Total Environment. STOTEN-D-15-05108*

Peacock, M. M., and V. S. Kirchoff. 2004. Assessing the conservation value of hybridized cutthroat trout populations in the Quinn River drainage, Nevada. *Transactions of the American Fisheries Society,* **133**:309–325.

Peacock, M. M., H. Neville and V. S. Kirchoff. 2004. Ten species specific microsatellite loci for Lahontan cutthroat trout, *Oncorhynchus clarki henshawi*. *Molecular Ecology Notes***, 4**:557-559.

Pritchard, J. K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**:945–959.

Purcell, K. M., and C. A. Stockwell. 2015. An evaluation of the genetic structure and post –introduction dispersal of a non-native invasive fish to the North Island of New Zealand. *Biological Invasions,* **17**:625-636.

Putman, A. I., and I. Carbone. 2014. Challenges in analysis and interpretation of microsatellite data for population genetic studies. *Ecology and Evolution***, 4**:4399-4428.

Rexroad, C. E. III, R. L. Coleman, A. L. Gustafson, W. K. Hershbeger and J. Killefer . 2002. Development of rainbow trout microsatellite markers from repeat enriched libraries. *Marine Biotechnology,* **3**:12-16.

Rexroad, C.E. III, and Y. Palti 2003. Development of ninety-seven polymorphic microsatellites markers for rainbow trout. *Transactions of the American Fisheries Society*, **132:**1214-1221.

Robertson, B. C., and N. J. Gemmell. 2004. Defining eradication units to control invasive pests. *Journal of Applied Ecology*, **41**:1042-1048.

Robinson, M.L., V.S. Kirchoff, and M.M. Peacock. 2009. Characterization of 13 microsatellites for Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) and cross-amplification in six other salmonids*. Molecular Ecology Resources,* **9**:134-136.

Robinson, M. L., L. Gomez-Raya, W. M. Rauw, and M. M. Peacock. 2008. Fulton's body condition factor K correlates with survival time in a thermal challenge experiment in juvenile Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*). *Journal of Thermal Biology,* **33**:363-368.

Rogers, S. D., and M. M. Peacock. 2012. The disappearing northern leopard frog (*Lithobates pipiens*): conservation genetics and implications for remnant populations in western Nevada. *Ecology and Evolution*, **2**:2040-2056.

Rood, S. B., C. R. Gourley, E. M. Ammon, L. G. Heki, J. R. Klotz, M. L. Morrison, D. Mosley, G. G. Scoppettone, S. Swanson, and P. L. Wagner. 2003. Flows for floodplain forests: A successful riparian restoration. *Bioscience*, **53**:647-656.

Saunders, A., and D. A. Norton. 2001. Ecological restoration at Mainland Islands in New Zealand. *Biological Conservation* **99**:109-119.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology,* **18**:233-234.

Simberloff, D. 2014. Biological invasions: What's worth fighting and what can be won? *Ecological Engineering*, **65**:112-121.

Strayer, D. L., N. F. Caraco, J. J. Cole, S. Findlay, and M. L. Pace. 1999. Transformation of freshwater ecosystems by bivalves - a case study of zebra mussels in the Hudson River. *Bioscience,* **49**:19-27.

Stankovic, D., A. J. Crivelli, and A. Snoj. 2015. Rainbow Trout in Europe: Introduction, Naturalization, and Impacts. *Reviews in Fisheries Science & Aquaculture*, **23**:39-71.

Vander Zanden, M. J., G. J. A. Hansen, S. N. Higgins, and M. S. Kornis. 2010. A pound of prevention, plus a pound of cure: early detection and eradication of invasive species in the Laurentian Great Lakes. *Journal of Great Lakes Research,* **36**:199-205.

Vitousek, P. M., C. M. Dantonio, L. L. Loope, M. Rejmanek, and R. Westbrooks. 1997. Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology,* **21**:1-16.

Weber, E. D., and K. D. Fausch. 2003. Interactions between hatchery and wild salmonids in streams: differences in biology and evidence for competition.

Canadian Journal of Fisheries and Aquatic Sciences, **60**:1018-1036.

Weeks, A. R., C. M. Sgro, A. G. Young, R. Frankham, N. J. Mitchell, K. A. Miller, M. Byrne, D. J. Coates, M. D. B. Eldridge, P. Sunnucks, M. F. Breed, E. A. James, and A. A. Hoffmann. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications,* **4**:709-725.

Wimbush, J., M. E. Frischer, J. W. Zarzynski, and S. A. Nierzwicki-Bauer. 2009. Eradication of colonizing populations of zebra mussels (Dreissena polymorpha) by early detection and SCUBA removal: Lake George, NY. *Aquatic Conservation*, **19**:703-713.

Wright, Sewall. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution*, **19:**395-420.

CHAPTER 5:

Survival and reproduction of Lahontan Cutthroat trout in the Truckee River: implications for the utilizing the Pilot Peak strain for reintroduction

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ABSTRACT

The Lahontan cutthroat trout (*Oncorhyncus clarkii henshawi*, LCT) are listed as threatened under the U. S. Endangered Species Act. Historically, LCT was the apex predator within the Lake Tahoe basin and was extirpated from Lake Tahoe, the Truckee River, and Pyramid Lake due to water diversions, pollution, competition and hybridization with non-native species in the 1930s and 1940s. The historical Pyramid Lake strain of LCT was thought to be extinct. The Pyramid Lake fishery was rebuilt using LCT from Independence Lake, the Carson River and Summit Lake, creating the contemporary Pyramid Lake LCT. In the Truckee River, rainbow trout (*Oncorhynchus mykiss,* RBT) was stocked to provide angling opportunities, and there is now a robust naturalized population throughout much of the Truckee River. RBT can readily hybridize with LCT. A strain of LCT discovered in 1977 in a small stream in Pilot Peak, Utah, considered to be a descendent from the original Pyramid Lake strain, is being propagated by the Lahontan National Fish Hatchery. Recovery efforts by the United States Fish and Wildlife Service are underway to reintroduce LCT back into their native habitat in the Truckee River; both the contemporary Pyramid Lake LCT and the Pilot Peak LCT were stocked and hybridization is evident. The focus of this study is to genetically evaluate the LCT, the RBT and LCT/RBT hybrids found in the Truckee River using 6 microsatellite loci to assess the differences between the LCT stocked strains in survival and participation in reproduction. Three genotype clusters were found and the Truckee River LCT clustered with the Pilot Peak strain of LCT. The hybrids clustered predominantly

with the RBT cluster, with slight contribution from both the Pyramid Lake and Pilot Peak genotype clusters. These data indicate that the Pilot Peak strain has higher survival in the Truckee River, and, if RBT can be successfully eradicated, the Pilot Peak strain should be stocked and would provide the highest probability at reintroduction.

INTRODUCTION

Translocations, the intentional movement of and release of organisms across landscapes, have occurred for many centuries. However, in recent decades, the focus of translocations to enhance or maintain biodiversity has increased in frequency (Weeks, 2011; Seddon et al., 2014). With increasing species extinction rates and habitat losses due to climate change and anthropogenic alterations, translocating threatened or endangered species has become a powerful tool available to conserve biodiversity (Griffith, 1989; Seddon et al., 2014). Translocations can occur to protect endangered species due to impending habitat destruction (Struebig et al., 2015), to reinforce existing populations or to reintroduce endangered species to their native habitat (Seddon et al., 2014) and occurs across all taxonomic groups (Seddon et al., 2005). High public and governmental support is required to allow for reintroduction conservation efforts (Struebig et al., 2015), and bias towards charismatic mammalian species is apparent both in terms of financial support and perceived value of the conservation effort (Cain et al., 2011). In an evaluation of reintroduction projects published in *Conservation Biology* and *Biological Conservation* between1987-2001 Seddon et al. (2005) found a dramatic bias in reintroduction projects among plants, vertebrates, and invertebrates when considering the relative number of described at-risk species within each group. Vertebrate species made up the largest portion (61% of projects versus 4% of at-risk species), and the vertebrate group primarily was focused on mammals and birds, while underrepresenting fish reintroductions (4% of projects versus 50% of at-risk vertebrates, Seddon et al., 2005). This organismal bias fails to consider the IUCN listing status of the species in question and, for translocations specifically, an endangered species listing does not appear to be a requirement; many of the species being reintroduced are not listed or are listed as Least Concern (Seddon et al. 2005). This can lead to an extremely unbalanced distribution of resources for conservation projects. For instance, orangutan conservationists have moved multiple individuals from dangerous habitats, threatened by poaching or deforestation, to more suitable habitats, in hopes of long term protection. However, the annual cost per animal is up to $14,000, resulting in an overall cost of several million dollars per year (Meijaard et al., 2012), and the long term projection for success is unknown (Struebig et al., 2015). Many factors, such as the potential success of the reintroduction, the impact of that reintroduction on the ecosystem stability, and the economic cost of maintaining the "established" population should have a predominant role in determining which organisms should be the recipients of reintroduction efforts (Seddon et al., 2014). It is necessary for reintroduction efforts to be well planned and executed, taking into consideration the potential survival of the organisms being translocated, and the impact on the community ecology of the introduction site. In addition, post reintroduction monitoring needs to be done more consistently to evaluate the benefit of these efforts for global conservation (Griffith et al., 1989; Seddon et al., 2007; Cochran-Biederman et al., 2015).

Genetic evaluation of reintroductions can be utilized in order to evaluate the genetic viability of the translocated populations (Frankham et al., 2014). Genetic tests evaluating strain survival and ability to adapt to a putative translocation site can be time consuming and costly, but genetic assessment can elucidate important characteristics contributing to successful reintroductions of endangered species (Griffith et al., 1989; Dodd and Segal, 1991; Cochran-Biederman et al, 2015). In recent years, the use of molecular tools such as microsatellites and mitochondrial or nuclear sequence data has become more accessible and affordable (Blanchet, 2012). These molecular markers can be utilized to look at population structure of non-natives, identify hybridization or other threats to reintroduction and look at the genetic viability of the source population(s) available for reintroduction (Allendorf and Phelps, 1980; Estoup and Guillemaud, 2010; Blanchet, 2012). The genetic evaluation of the source stock used for the reintroduction is important because many reintroduction projects utilize broodstock or small founder populations that have been reared in a hatchery, potentially leading to lower genetic diversity (Allendorf and Phelps, 1980) and a decline in adaptability (Weber and Fausch, 2003). Over time, captive stocks can become less viable and unable to thrive in a wild environment (Sedinger et al., 2012). Genetic diversity and local adaptation of the source stock are valuable predictors of survival, recruitment and reproduction necessary to establish a wild population from a captive stock (Cochran-Biederman et al., 2015).

The Lahontan cutthroat trout, *Oncorhynchus clarkii henshawi,* (LCT) are currently listed as threatened under the United States Endangered Species Act and translocations of LCT into native habitats where the trout has been extirpated are underway (Coffin and Cowan, 1995). This trout evolved in pluvial Lake Lahontan during the last glacial period (12,000-100,000 ybp). Lake Lahontan occupied 13,000 km^2 of what is now northwestern Nevada and northeastern California. With the desiccation of Lake Lahontan about 8000 years ago, lacustrine populations of LCT were still abundant throughout the Lahontan Basin of California and Nevada , found in Pyramid Lake, Walker Lake, Independence Lake, Lake Tahoe and Summit Lake (Behnke and Zarn, 1976; Hickman and Behnke, 1979). Pyramid Lake was a highly productive lake with the full array of Lahontan basin fish fauna, and the LCT were arguably the largest and most piscivorous trout found in North America, with reports of LCT reaching 28 kg (Sumner, 1940). Fluvial populations were found in the Truckee, Carson, Walker and Humboldt River systems (Behnke and Zarn, 1976). Abundant stocking of RBT into the lakes and tributaries of the Tahoe basin occurred in the late 1800s; however, the LCT was able to thrive and resist hybridization with RBT (Behnke and Zarn, 1976). With the construction of Derby Dam in 1904, 30 miles above Pyramid Lake, the LCT in Pyramid Lake were unable to access much of their spawning habitat and connectivity with the Truckee River was limited. The inability of fish to pass over the Derby dam resulted in extirpation of the Pyramid Lake fishery in 1939. The LCT population in the Truckee River declined rapidly and LCT were extirpated from Lake Tahoe basin in the early 1940s (Sumner, 1940; Cordone and Frantz, 1968). The Pyramid Lake Pauite tribe rebuilt the fishery in Pyramid Lake using LCT from Independence Lake, the Carson River and Summit

Lake, an out-of-basin lake population. However, the trout never regained the great size achieved by the historic Pyramid Lake strain of LCT.

Over the past few decades, translocations of LCT for angling opportunities and half-hearted attempts at reintroduction of LCT into the Truckee River have not resulted in re-establishing an LCT population in the river (Cordone and Frantz, 1968; Coffin and Cowan, 1995; Coleman and Johnson, 1988). For reintroduction efforts to be successful, it is imperative to eradicate non-native species and stop reinvasion, especially if that species is responsible for the initial decline of the species in question (Griffith et al., 1989; Young and Harig, 2001).

Another factor to consider for achieving successful reintroduction is the strain of the trout being stocked, as different strains of LCT show differential survival, recruitment and reproduction success in different habitats (Bigelow et al., 2010; Sedinger et al., 2012). For example, Walker Lake, a terminal alkaline lake in the western portion of the LCT range, once supported an LCT fishery that declined due to dams and water diversions, and by 1949 when spawning habitat was no longer available was no longer viable. The fishery was maintained for more than five decades by stocking LCT derived from other populations (Bigelow et al., 2010). Since 2009, the lake has become progressively more saline, smaller in size, and more alkaline due to climactic warming and water diversions; therefore, trout survival is limited. Yearling LCT from the Pyramid Lake strain and the Pilot Peak strain of LCT were planted in Walker Lake from 1998-2009 by the United States Fish and Wildlife Service (USFWS). The planted trout were tagged, and creel surveys were used to report captures of tagged fish. Annual survival of LCT dropped over the years of the study; however, USFWS found that the Pilot Peak strain survived significantly better than the contemporary mixed stock Pyramid Lake strain (Sedinger et al., 2012). This was attributed to the ancestry of the Pilot Peak strain which was genetically identified as the original Pyramid Lake strain (Peacock and Kirchoff 2007) meaning that the Pilot Peak strain evolved in the alkaline habitat of Pyramid Lake and has the genetic variation and local adaptability to survive in that habitat.

The Tahoe basin is characterized by fluctuating water levels due to drought cycles. Water levels are further impacted by dams and water diversions. The lower Truckee River and Pyramid Lake have seen increases in total dissolved solids, salinity and alkalinity over the 20th century, largely due to water diversions such as Derby dam in the lower watershed but exacerbated by fluctuating water flow (Bartlett and Warwick, 2009). With climate change forecasts, these trends in the Truckee River are expected to continue. The Pilot Peak strain of LCT is thought to be longer-lived and have the adaptability to survive in the increasingly saline and more alkaline conditions. Since 2006 the USFWS in partnership with the Pyramid Lake Paiute trip have been planting the Pilot Peak strain into Pyramid Lake. Molecular genetic techniques can be used to identify the different strains of LCT in the Truckee River (e.g., contemporary Pyramid Lake or Pilot Peak), in order to assess differences in survivorship and reproduction, thereby informing conservation and reintroduction efforts for LCT.

Here I am utilizing six highly variable nuclear microsatellite markers that amplify in both LCT and RBT to differentiate between the Pilot Peak, Independence Lake and Pyramid Lake LCT sampled in the Truckee River from 2007 to 2010. Mitochondrial sequence variation among these strains at the ND2 locus had insufficient variation to distinguish between the three strains stocked (Chapter 2). Because these markers amplify in both LCT and RBT, they can also be used to investigate not only source strain of the LCT sampled that survived in the Truckee River, but also can indicate which strain(s) are able to spawn in the river and contribute to hybridization. My goals were to assess the differences between the three strains of LCT trout stocked in survival and participation in reproduction. This information can inform management decisions and assist in the recovery efforts for LCT by indicating which strain(s) have the highest survivorship and local adaptability to acclimate and be reintroduced in the Truckee River.

METHODS

Study system and Stocking history

The Truckee River flows 195 km from Lake Tahoe in California to Pyramid Lake in Nevada and has two main tributaries, Hunter Creek and Dog Valley Creek, which are being considered for reintroduction of LCT. The Hunter Creek tributary flows into the Truckee River between the VerdiC and RenoA transects, and Dog Valley Creek enters the Truckee River near the VerdiC transect (Figure 2). Over the past several decades, the watershed has been stocked with LCT from the Independence Lake strain, the contemporary Pyramid Lake strain (PYR, mixed stock ancestry), and the Pilot Peak strain (PP) as well as both diploid and triploid rainbow trout. Both creeks have a robust naturalized trout fishery of both RBT and brook trout. Dog Valley Creek has more rearing habitat for young fish, whereas Hunter Creek has a greater abundance of larger drop pools that can provide good habitat for adult fish. Official stocking records of diploid and triploid RBT and LCT (Pilot Peak, Independence Lake and Pyramid Lake strains) were obtained from USFWS and NDOW for the time periods from 1990-2010. Total numbers of fishes stocked were calculated as well as averages of each salmonid species during different time periods to determine if sampling efforts were biased by stocking events.

Study species

LCT is listed as threatened under the United States Endangered Species Act (U.S. Office of the Federal Register, 1975; Coffin and Cowan, 1995), and is found in less than 10% of its historic fluvial habitat and 0.4% of its historic lake habitat. This subspecies of cutthroat trout was extirpated from Lake Tahoe, the Truckee River, and Pyramid Lake during the 1940s. Pyramid Lake supported a strain of LCT that reached a greater body size than any other trout native to North America, with the official world record trout weighing 18.6 kg (Coleman and Johnson, 1988). The historical Pyramid Lake strain of LCT was thought to have gone extinct in the late 1930s, and a trout fishery was established by the Pyramid Lake Pauite tribe in the 1950s in Pyramid Lake from a combination of several wild stocks of LCT. Currently, the Pyramid Lake strain is thought to be primarily a descendent from Summit Lake

LCT (Coleman and Johnston, 1988; Peacock and Kirchoff, 2007). Beginning in 2006, the Pyramid Lake Paiute tribe also stock trout from the Lahontan National Fish Hatchery (LNFH), which are propagated from a strain of LCT discovered in 1977 is a small stream in Pilot Peak, Utah. The Pilot Peak strain is considered to be a descendent from the original Pyramid Lake strain, supported by morphological and genetic comparisons to historic samples (Hickman and Behnke, 1979; Peacock and Kirchoff, 2007). LCT spawn in the early spring, so there is temporal overlap with RBT spawning; hybridization with RBT is one of the biggest threats to LCT recovery.

Sample collection

Salmonid samples were collected by the United States Fish and Wildlife Service (USFWS) and Nevada Division of Wildlife (NDOW) from 2007 through 2010. USFWS collected samples from 8 primary sampling sections (n=1649). Each sampling section had 3 to 4 transects, depending on the variation in habitat types and barriers within each site (Figure 1, Table 1). Transects were 500 m long and both shore line and mid-stream regions. Raft, barge and backpack electrofishing methodologies were utilized to ensure all age classes were adequately sampled. Fin clips were placed in wax paper, dried, and placed in individual coin envelopes labeled with species (when possible), fork length, date collected and location. NDOW collected samples from the five sites on the Truckee River main stem while doing their annual surveys. RBT samples collected by NDOW were primarily sampled in the Verdi to Reno transects (n=1820). In addition, NDOW collected samples from Hunter and

Dog Valley creeks in 2007 and 2008, and collected from two transects below the diversion ditch barrier in Hunter Creek and below the California State Line in Dog Valley Creek. In 2009, the tributaries were also sampled by USFWS; for this sampling period, samples were collected from two transects above the barrier and beyond the California State Line, in addition to the sites overlapping the NDOW collection. Throughout the past couple decades, Pyramid Lake contemporary LCT, Independence Lake LCT and Pilot Peak LCT have been stocked into the Truckee River. DNA from these stocked strains collected for a previous study were included as putative source populations. Twenty-four samples were included from each LCT strain.

DNA isolation and PCR amplification

DNA was isolated using DNeasy96 Blood and Tissue Kits (QIAGEN) according to the manufacturer's protocol. Double stranded DNA was quantified at the Nevada Genomics Center using a fluorescent nucleic acid stain (PicoGreen®) and read on a Labsystems Fluoroskan Ascent fluorescence plate reader, which measures only the double stranded DNA present. Samples were first screened using 6 bi-parentally inherited markers (Ostberg and Rodriguez, 2004), which identify them as RBT, LCT or hybrids (Chapter 2). RBT microsatellites were selected from the literature (Rexroad et al. 2002; Palti et al., 2002; Rexroad and Palti, 2003) based on high number of alleles and ability to multiplex in PCR reactions. I used OMM1036 (Rexroad et al., 2002), OMM1220 (Rexroad and Palti, 2003), OMM1302, OMM1315, OMM1322, OMM1323, OMM1325, and, OMM1329 (Palti et al., 2002). In addition, three LCT primers were selected to be highly variable and cross-amplify in RBT, OCH15 and OCH17 (Peacock et al., 2004) and OCH 20 (Robinson et al., 2009). Eleven microsatellite primers were ordered with one of four unique M13 tails to label the PCR product with a corresponding fluorescent dye (Schuelke, 2000). Multiplex reactions included 4 and 7 loci per reaction. Primer sequences, observed and expected heterozygosity for RBT, LCT and hybrids found in the system are reported in Chapter 4. A multiplex primer cocktail was prepared to give a final primer concentration of 0.05 μM each tailed forward primer, 0.2 μM each reverse primer, and 0.1 μM each labeled M13 primer in a 12 μl reaction. PCR reactions included 6 μl Multiplex taq (1X final concentration, QIAGEN), and 20-50 ng of DNA. PCR cycle parameters included a 15 minute hot start at 95°C, followed by 41 cycles of 95°C for 30 seconds, a touch down annealing temperature for 90 seconds, and 72°C for 30 seconds. The annealing temperature had 7 cycles at 65°C, 7 cycles at 61°C, 7 cycles at 58°C, and 20 cycles at 55°C. The first 21 cycles amplified the locus specific primer and the final 20 cycles amplified the labeled M13 tail with a fluorescently M13 primer, labeling the PCR product. PCR reactions were completed using an MBS Satellite 0.2G Thermal Cycler and a 96-well format (Thermo Electron Corporation). PCR products were diluted to an appropriate concentration determined by dilution tests from 1:50 to 1:200. One μl of diluted PCR product was added to 19 μl of size standard (Applied Biosystems) prepared by adding five μl of LIZ500 size standard to one ml of HiDye Formamide and 0.5 ml of molecular grade water. Fragment analysis was carried out on an Applied Biosystems 3730 Genetic Analyzer at the Nevada Genomics Center (http://www.ag.unr.edu/genomics/) and all alleles generated were scored, binned, and given allelic and genotypic designation using the ABI GeneMapper software (version 4.0).

Genetic analysis

Microsatellites were run on all salmonids sampled, regardless of designation as RBT, LCT or hybrid. Of the 11 loci that were utilized, four did not amplify in LCT (OMM1315, OMM1323, OMM1036, and OMM1302) and one was fixed in LCT (OMM1220 had one LCT allele at 119 bp in all samples). Only the six consistent variable loci that amplified in both RBT and LCT were used to evaluate the source population found in the LCT and hybrids in this system. Six of the microsatellites used for RBT genetic analysis consistently amplified in LCT, so the LCT stocked strains and the sampled LCT were analyzed with OCH15, OCH17, OCH20, OMM1322, OMM1325, and OMM1329 and compared to the Truckee River samples identified as LCT and hybrids. Sixty-three Truckee River samples identified as RBT were included as well. Microsatellite toolkit in Excel (Park 2001) was used to calculate the observed and expected heterozygosity (H_o and H_e).

I investigated the source population of the LCT and hybrids sampled in the Truckee River using a Bayesian genotype clustering method (STRUCTURE version 2.3.4; Pritchard *et al.,* 2000). The program assigns individuals to probable genotype clusters using HWE and gametic phase equilibrium between loci within groups, making no apriori assumptions of population based on sampling locations. I specified a 10,000 iteration burnin, followed by five 500,000 Markov chain Monte Carlo (MCMC) replicates per *k* (number of genotype clusters) to estimate allelic distributions against which individual genotypes were compared and assigned to a cluster (Pritchard et al., 2000). I set the possible number of genotype clusters (k) equal to one through ten and used ΔK method of Evanno *et al.* (2005) to determine the optimal number of genotype clusters in STRUCTURE HARVESTER (Earl and vonHoldt, 2012):

RESULTS AND DISCUSSION

Stocking of salmonids over the past 20 years included stocking of multiple strains of RBT, multiple strains of LCT and, starting in 2004, non-reproductive triploid RBT. The trends of stocking from 1990-2010 are shown in Figure 2A. In the time period from 1990-1993, over 100,000 RBT were stocked for angling opportunities; primarily catchable RBT were stocked with an average fork length of 243 mm (±16mm) (Figure 2B). Starting in 1994, there was a push to stock native trout in the watershed, and for the next several years, both LCT and RBT were stocked. Hybridization of LCT and RBT was suspected, and, to reduce the hybridization potential, fom 2000-2003, LCT stocking became more selective to regions where RBT were not currently being stocked; however, a large naturalized RBT population is found throughout the Truckee River, so hybridization was still probable. Starting in 2004, again in hopes of preventing hybridization and allowing for LCT recovery, NDOW began stocking primarily non-reproductive triploid RBT, and, from 2004 to 2010, over 100,000 LCT were stocked, including both fry and adults. Fry were stocked because they can stock large numbers of fry, which may be more adaptable in the watershed and able to acclimate; the adult fish are more likely to survive and potentially contribute to reproduction. Prolonged stocking of both adult fish and fry in the watershed over a number of years increases the probablility of successful reintroduction (Cochran-Biederman et al., 2015, Todd and Lintermans, 2015).

The genetic diversity of the stocked source and the local adaptation of the strain stocked have been shown to be important predictors in the successful translocations (Bigelow et al., 2010, Sedinger et al., 2012, Cochran-Biederman et al., 2015). There are three LCT strains that have been recorded as being stocked into the Truckee River: Independence Lake, Pyramid Lake and Pilot Peak. Since 2004, primarily Pyramid Lake and Pilot Peak have been stocked (Figure 3A). LCT were stocked in regions where RBT were stocked (Verdi, Dog Valley, Reno) and in areas where no RBT were stocked (TahoeB, Hunter Creek, Wadsworth)(Figure 3B). In addition, the size class for stocking of LCT varied dramatically ranging from 20 mm to 400 mm, but the averageLCT size stocked was 134.62 mm ±100mm. Most of the LCT stocked were either under 100 mm (fry and fingerlings) or over 200 mm (catchables) (Figure 4B). The larger fish, 200 mm and above, were stocked in popular fishing spots and were most likely readily fished out of the river (Kim Tisdale, NDOW personal communication). The only recorded stocking in Hunter

Creek were Pilot Peak fry, stocked in 2007. Between 2004 and 2010, Dog Valley Creek was stocked with 47,668 fry and 3,538 adults of the Pilot Peak strain and 70,600 fry and 6,134 adult LCT from the contemporary Pyramid Lake strain. More than 35,000 Independence Lake adult LCT were stocked into the Truckee River between 2003 and 2004; this strain was not stocked during the sampling period.

LCT were found primarily in the Verdi transects, Reno transects and in Dog Valley and Hunter creeks. They were found in these location in all four years of the study (Figure 5A and B). A size class histogram (Figure 5C) shows that YOY were found in all four years of the sudy; however, fry were stocked from 2005-2009. The pure LCT YOY found in 2010 could represent reproduction of pure LCT in the river. Survival and recruitment of LCT is also indicated by the presence of large LCT (300 mm) in Hunter Creek, as only fry were stocked in Hunter Creek.

I characterized genetic variation in RBT in addition to the three strains of LCT stocked in the Truckee River (Table 1). The YOY were included in this analysis because of overall low sample size (n=76). For the three strains of LCT, the H_e was lowest for the Pilot Peak strain, 0.44 and the H_o was 0.45, highest for Independence Lake ($H_e = 0.66$; $H_o = 0.63$) and intermediate for Pyramid lake ($H_e = 0.46$; $H_o = 0.63$). There is significant overlap in the range of the alleles for all three strains, but the number of alleles was significantly lower in Pilot Peak ($N_A = 2.67 \pm 0.82$) compared to Pyramid (6.3 ±3.44) and Independence (6.5 ± 3.89) ($p < 0.5$). For the salmonids sampled in the river, the highest heterozygosity was found in the hybrids ($H_e = 0.88$; $H_o = 0.86$), followed by the RBT ($H_e = 0.82$; $H_o = 0.82$) and then the LCT

(H_e = 0.60 ; H_o = 0.51). The number of alleles and allelic range for the salmonids sampled is reported in Table 6.

I also tested the assignment of LCT sampled in the river to multiple stocked strains of LCT found in the river using Bayesian genotype cluster analysis. The best fit for the data was three genotype clusters (k=3)(Figure 6). Structure analysis of the three stocked strains showed the Pilot Peak is genetically very different from the Independence Lake and contemporary Pyramid Lake strains. The Pyramid Lake strain and the Independence Lake strains custered in a single cluster (Blue) and the Pilot Peak Strain created a red cluster, not surprisingly as Independence Lake LCT were part of the contemporary Pyramid Lake broodstock. In the salmonid samples collected from the Truckee River, the RBT clustered in a single group (green). The LCT sampled in the river clustered primarily with the Pilot Peak strain, with very little membership in the PYR/IND strains. The hybrids included any of the first generation hybrids as well as any hybrids that showed introgression. They clustered primarily with the RBT, which is consistent with the higher genetic variation seen in the RBT strains as well as the fact that backcrossing of hybrids occurred with pure RBT and not with LCT (Chapter 2). The hybrids also show a slight signal from both the PYR/IND cluster (Blue) and Pilot Peak (Red). The data presented here were not able to differentiate between Pyramid Lake and Independence Lake LCT. However, a suite of SNP markers are in development that will distiguish among all three LCT strains. Analyzing the hybrid trout found in the Truckee River with this suite of SNP markers will clarify the amount these LCT strains contribute to reproduction in the Truckee River.

Implications for LCT recovery

The results of this study suggest that the Pilot Peak strain has higher survivorship in the Truckee River compared to either the contemporary mixed stock Pyramid Lake or Independence Lake strains. Although I cannot rule out that some of the LCT sampled were from recent stocking events, the preponderance of pure LCT sampled in the Truckee River were Pilot Peak LCT from the USFWS Lahontan National Fish Hatchery (LNFH). These results suggest that future LCT introductions into the Truckee River should focus on using the Pilot Peak strain, as was recommended by Sedinger et al. (2012). Despite the fact that genetic breeding in captivity can lead to loss of genetic variation and that the captive strains have been found, in some species, to become adapted to the captive environment (Frankham, 1995), this does not seem to be the case here. This strain appears well suited for this environment, notwithstanding both the lower allelic variation and heterozygosity compared to other strains of LCT (Table 6). The LNFH is maximizing the existing genetic variation in a captive breeding program that uses variation at microsatellite markers to decrease inbreeding and increase heterozygosity. Genetic evaluation of the brood stock is done each year to ensure that the existing variation in the Pilot Peak LCT strain is maintained. Although partial reintroduction of LCT could be possible in segments of the river if artificial barriers are constructed, the long-term effectiveness of these barriers is questionable. Public support is needed to initiate a large-scale reintroduction effort if LCT reintroduction is to be possible in the Truckee River. One of the previous shortcomings of LCT recovery utilizing the contemporary Pyramid Lake strain is the small average adult size of the LCT strains compared to the historic Pyramid Lake fish. Since 2006, the Pilot Peak strain of LCT has been stocked in Pyramid Lake and has been monitored through creel studies. Although the official world record for this fish was 18.6 kg (41 lbs) in 1925, and current fish have not yet reached that size, reports have noted Pilot Peak LCT captures of more than 9.0 kg (20 lbs) in the last three years, exceeding the size of LCT fished in Pyramid Lake in the previous decades (Schweber, 2013). In addition, for the first time in 76 years, LCT from Pyramid Lake spawned in the lower Truckee River in 2014 (DeLong, 2014) as well as in 2015. Genetic analysis of the 2014 fry confirmed these fish to be progeny from the Pilot Peak LCT (DeLong, 2014). Preliminary analysis of the 2015 fry also show that the Pilot Peak LCT from Pyramid Lake are preferentially spawning in the lower regions of the Truckee River (data not shown) due in part to restoration efforts from USFWS to reconnect Pyramid Lake LCT to the spawning habitat in the lower Truckee River. The survival and reproduction the Pilot Peak strain indicated by this study, can garner significant support for a large-scale RBT eradication and re-introduction of LCT into the Truckee River. Future translocations of LCT should utilizing the Pilot Peak strain in this system for the highest probability of re-introduction.

Figure Legends

Figure 1. The Truckee River Watershed. Potential barriers and water diversions identified as (X). Numbers correspond to sites listed in Table 1. Transects (3-4 per sampling section) are indicated by filled circles in alternating red and blue, which depict the sampling sites.

Figure 2. Trends in Salmonid Stocking. (A) Average numbers of stocked fish were reported for each time period(±SD). Time periods were selected based on major shift seen in primary species of fish being stocked during the 20 year period shown. (B) Average fork length of RBT and LCT stocked in the Truckee River over a 20 year period.

Figure 3. Distribution of LCT and RBT stocked along the Truckee River. Independence Lake LCT were stocked in 2003 and 2004. Pilot Peak and Pyramid Lake LCT were both stocked throughout the river from 2005-2007. Pilot Peak was the only LCT stocked in 2008-2010.

Figure 4. Strains of LCT and size class stocked throughout the Truckee River. (A) Recorded stocking of LCT by strain for each year. Independence Lake strain was only stocked in 2003, and primarily the Pilot Peak strain was stocked in 2004. From 2005-2007, both Pilot Peak strain and Pyramid lake strain were stocked**.** (B) Size class distribution of LCT stocked from 2004 to 2010. Large numbers of Pilot Peak LCT fry were stocked in 2005 and 2006, focusing mainly on the tributaries as potential sites for LCT reintroduction. Large LCT were stocked, primarily in the main stem river for fishing opportunities.

Figure 5. LCT sampled from 2007-2010. (A) The number of LCT sampled is on the Y axis and the sampling transect arranged from Lake Tahoe to Nixon is on the X axis. Most of the LCT recovered were in the region from Verdi through Reno. (B) Total number of LCT recovered each year. Total numbers of LCT found at all sites are on the Y axis and the years are on the X axis. More pure LCT were found in 2007. (C) Forklength data for individual LCT measured in each year of the study. All years have a size class distribution that includes YOY, and fish with fork length up to 400 mm. Although some large fish were stocked, they were stocked in regions with abundant fishing. Large fish were also found in Hunter Creek, where only fry were stocked.

Figure 6. Determination of the Ln(PD) of genotype cluster and Delta K for LCT. The number of genotype clusters (k) is on the X axis and the natural log of the probability of the data is on the Y axis (left panel). Diamonds represent the average LnP(D) per k (± SD). Right panels show the Delta K determination from the same data using STRUCTURE HARVESTER and the method described by Evanno et al. (2005). The best fit of the data was k=3.

Figure 7. Proportional membership in genotype clusters of sampled salmonids and stocked strains of LCT. Pilot Peak strain stands out as being genetically distinct from the Pyramid Lake strain and the Independence Lake strain. Most of the LCT sampled in the river between 2007 and 2010 clustered with the Pilot Peak strain. The LCT-RBT hybrids showed primarily a RBT signature, but shared some of the stocked LCT signatures in this analysis.

Table 1. Statistics of microsatellite loci used to examine source populations of LCT and Hybrids sampled in the Truckee River. Product size and number of alleles (N_A), annealing observed heterozygosity(H_O) and expected heterozygosity (H_E) for sampled Truckee River RBT, LCT and HYBRIDS, as well as in stocked LCT strains from Pyramid Lake Hatchery, (PYR), Pilot Peak strain from Lahontan National Fish Hatchery (PP) and Independence Lake strain (INL).

A.	Independence Lake (n=22)			Pilot Peak Hatchery n=24			Pyramid Lake n=23		
Locus	H_O	H_E	Range (N_A)	H_O	H_E	Range (N_A)	H_O	H_E	Range (N_A)
OCH15	0.77	0.88	326-402 (12)	0.71	0.69	330-358 (4)	0.82	0.84	326-402 (11)
och17	1.00	0.82	217-289 (7)	0.46	0.36	237-265 (2)	0.50	0.83	217-285 (8)
OCH20	0.81	0.88	300-352 (10)	0.75	0.66	300-325 (3)	0.48	0.72	308-360 (9)
OMM1322	0.57	0.64	200-216 (4)	0.25	0.34	196-206 (2)	0.73	0.60	200-212 (4)
OMM1325	0.56	0.68	272-288 (4)	0.38	0.36	272-280 (3)	0.06	0.65	272-288 (3)
OMM1329	0.09	0.09	161-207(2)	0.21	0.19	153-161 (2)	0.19	0.18	153-207 (3)
			N_A (ave ±sd)			N_A (ave ±sd)			N_A (ave ±sd)
Global	0.63	0.66	6.5±3.89	0.45	0.43	2.67 ± .82	0.46	0.63	6.3 ± 3.44

	LCT (n=76)			LCT/RBT Hybrids (n=85)			Truckee RBT n= 63		
Locus	H_O	H_E	Range (N_A)	H_O	H_E	Range (N_A)	H_O	H_E	Range (N_A)
OCH15	0.55	0.76	326-374 (13)	0.86	0.93	286-378 (20)	0.78	0.92	286-362 (19)
och17	0.45	0.52	229-285 (9)	0.89	0.91	169-289 (19)	0.95	0.85	201-281 (14)
OCH20	0.71	0.72	300-356 (10)	0.88	0.93	242-356 (25)	0.84	0.88	242-320 (17)
OMM1322	0.59	0.66	196-272 (10)	0.86	0.92	196-280 (18)	0.82	0.88	196-272 (12)
OMM1325	0.45	0.56	272-284 (4)	0.77	0.73	272-320 (8)	0.58	0.55	284-324 (6)
OMM1329	0.29	0.37	153-207 (4)	0.89	0.86	161-209 (13)	0.92	0.87	161-209 (14)
			N_A (ave ±sd)			N_A (ave ±sd)			N_A (ave ±sd)
Global	0.51	0.60	11.83 ± 5.04	0.86	0.88	17.33 ±6.02	0.82	0.82	13.5 ±4.28

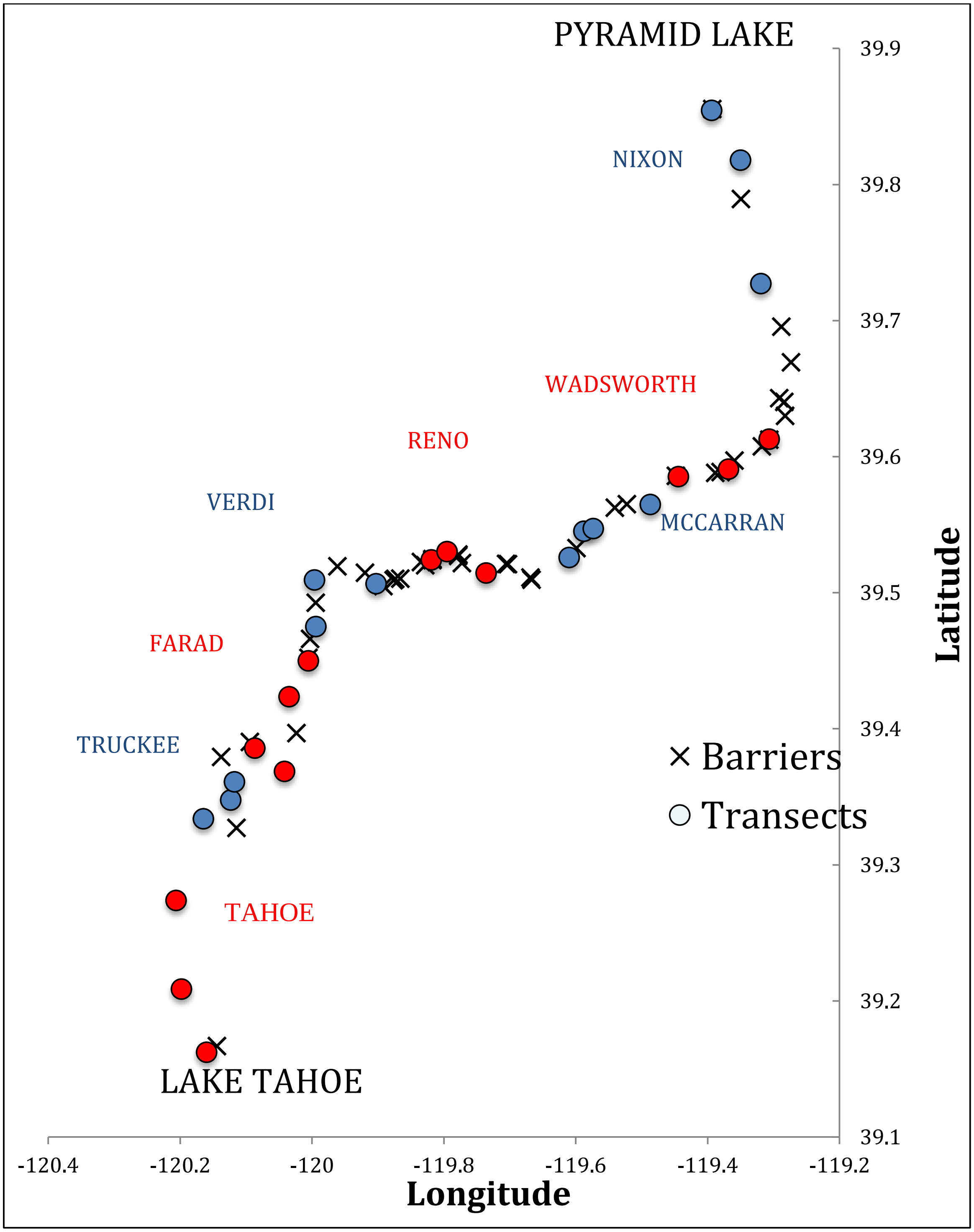
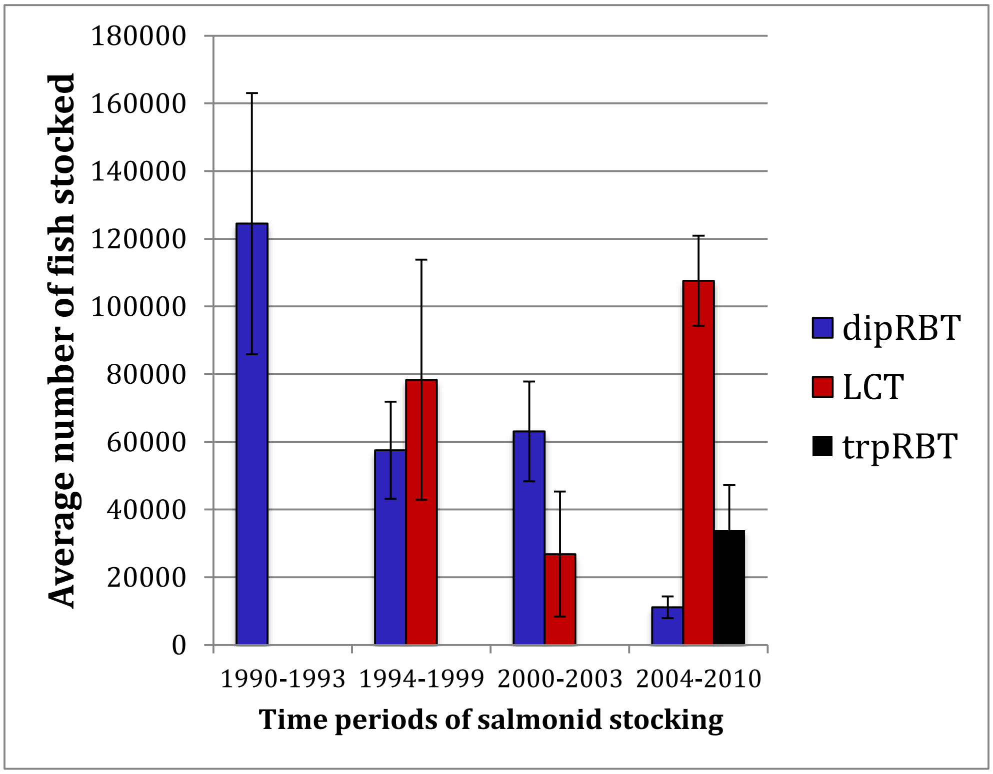

PYRAMID LAKE
NIXON
WADSWORTH
RENO
VERDI
MCCARRAN
FARAD
TRUCKEE
TAHOE
LAKE TAHOE
Barriers
Transects
39.9
39.8
39.7
39.6
39.5
39.4
39.3
39.2
39.1
Latitude
-120.4
-120.2
-120
-119.8
-119.6
-119.4
-119.2
Longitude

Figure 1.

A.


180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Average number of fish stocked
1990-1993
1994-1999
2000-2003
2004-2010
Time periods of salmonid stocking
dipRBT
LCT
trpRBT

B.

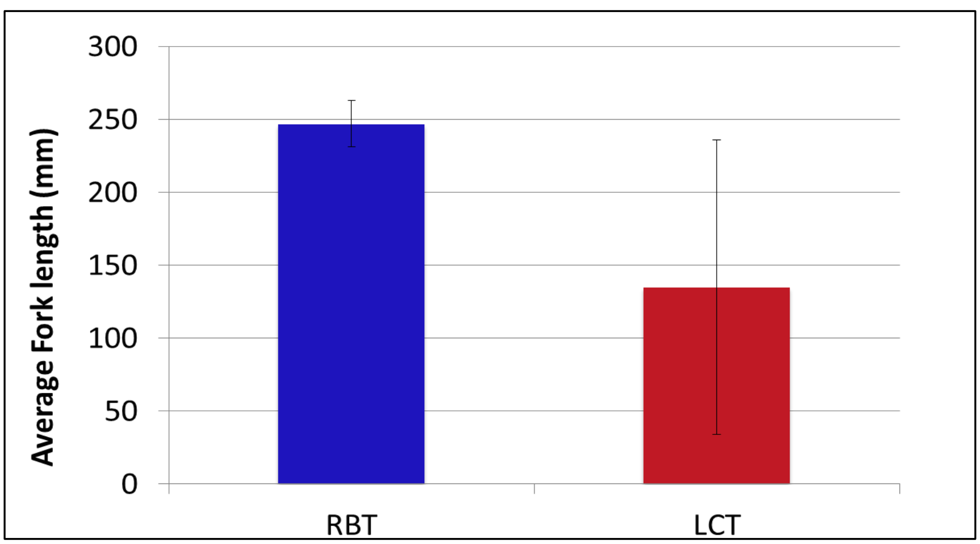
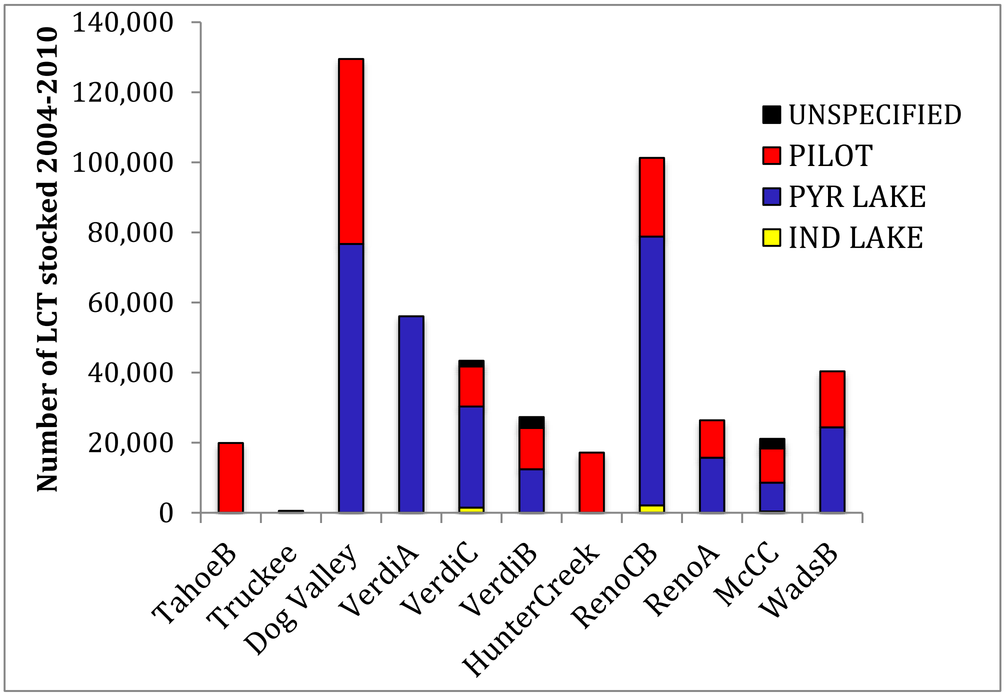

300
250
200
150
100
50
0
Average Fork length (mm)
RBT
LCT

Figure 2.

A.


Number of LCT stocked 2004-2010
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
UNSPECIFIED
PILOT
PYR LAKE
IND LAKE
TahoeB
Truckee
Dog Valley
VerdiA
VerdiC
VerdiB
HunterCreek
RenoCB
RenoA
McCC
WadsB

B.

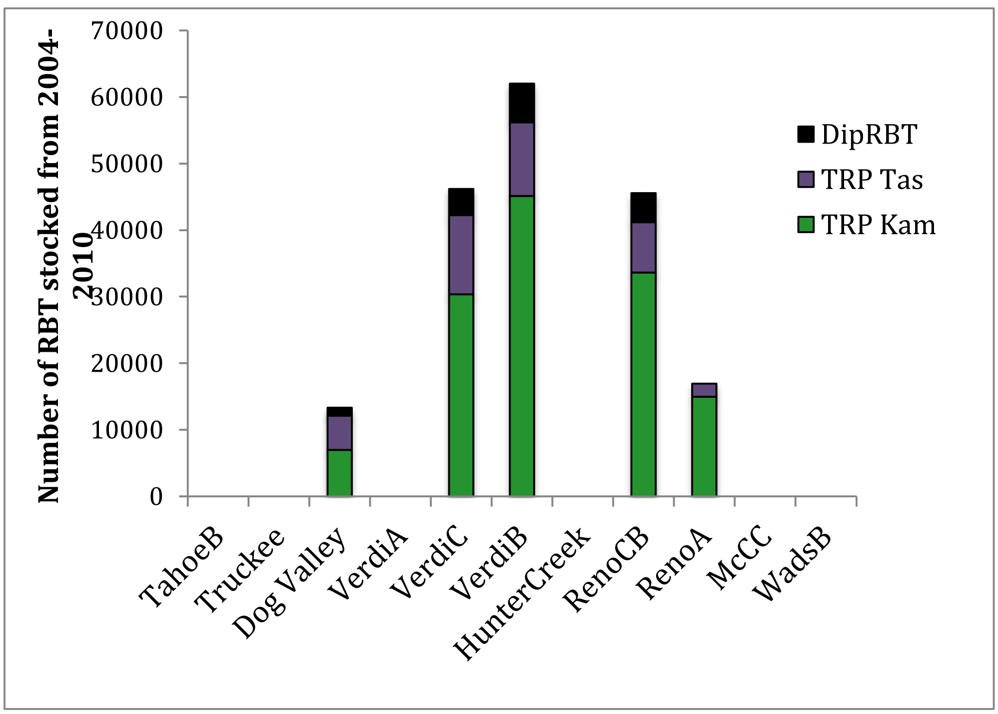
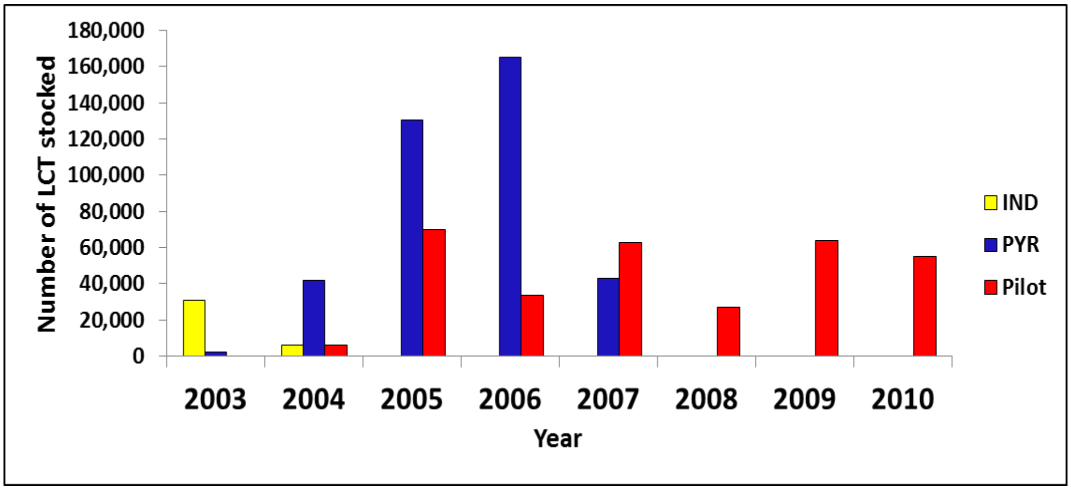

Number of RBT stocked from 2004-2010
70000
60000
50000
40000
30000
20000
10000
0
DipRBT
TRP Tas
TRP Kam
TahoeB
Truckee
Dog Valley
VerdiA
VerdiC
VerdiB
HunterCreek
RenoCB
RenoA
McCC
WadsB

Figure 3.

A.


Number of LCT stocked
180,000
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
2003
2004
2005
2006
2007
2008
2009
2010
Year
IND
PYR
Pilot

B.

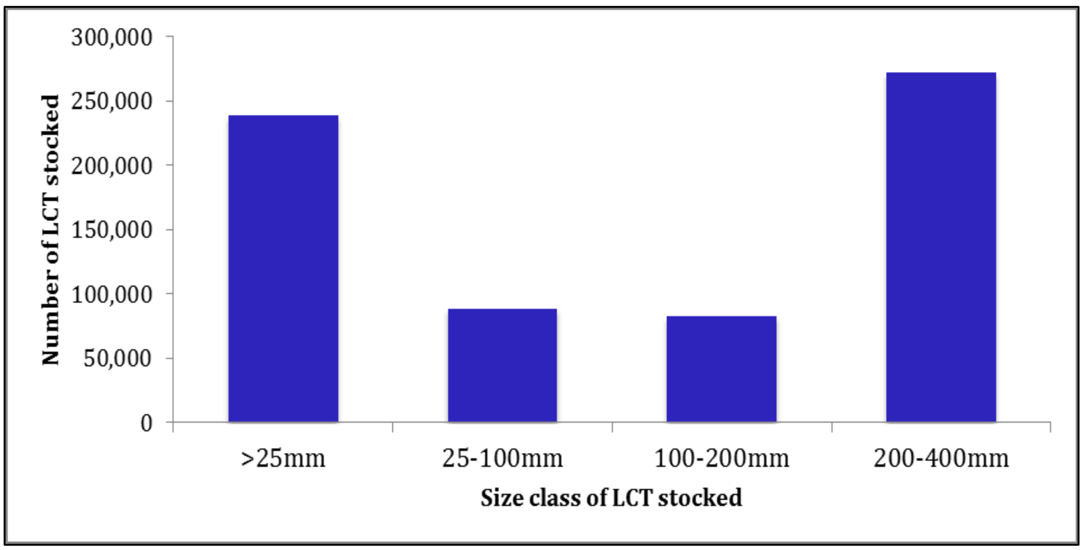
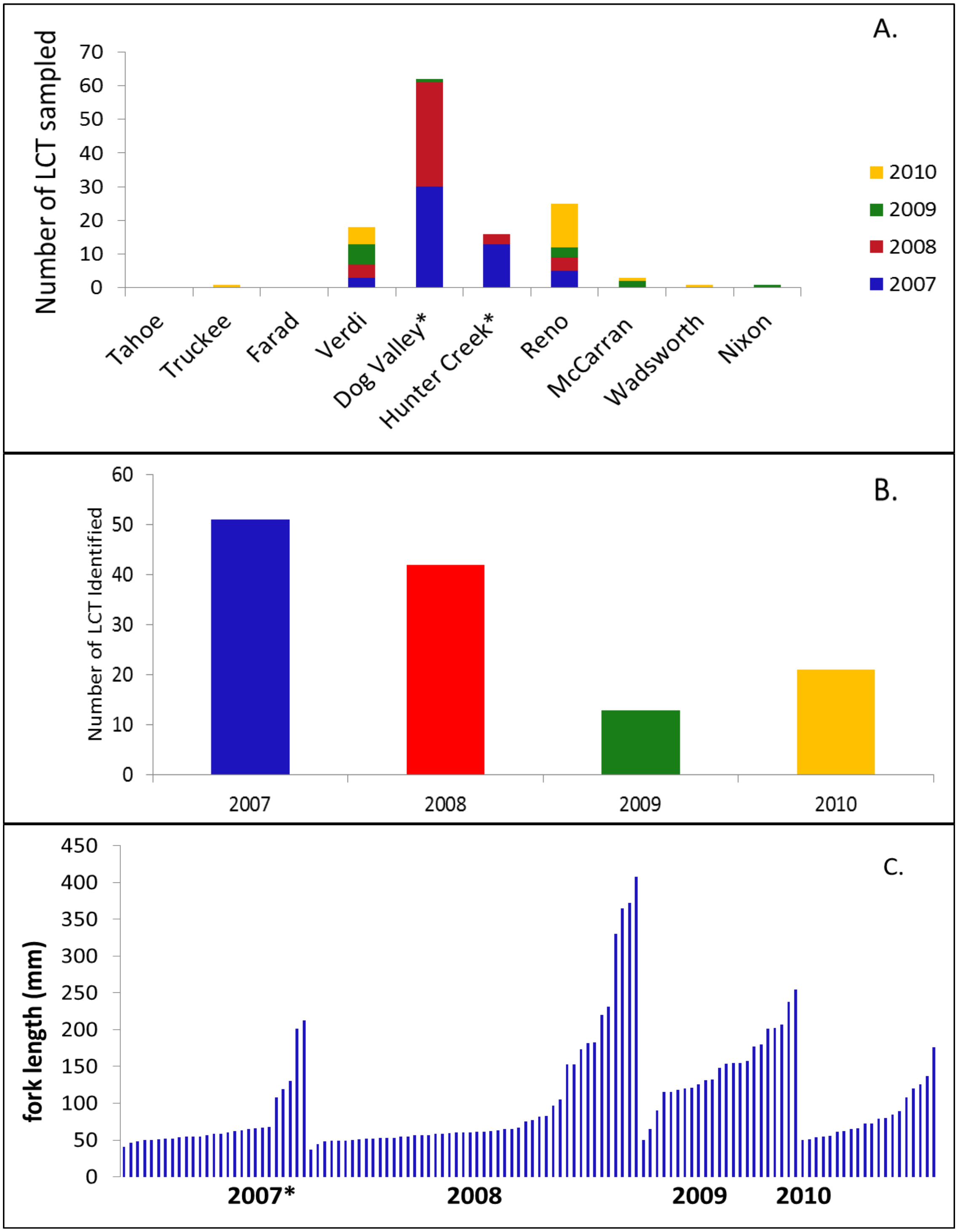
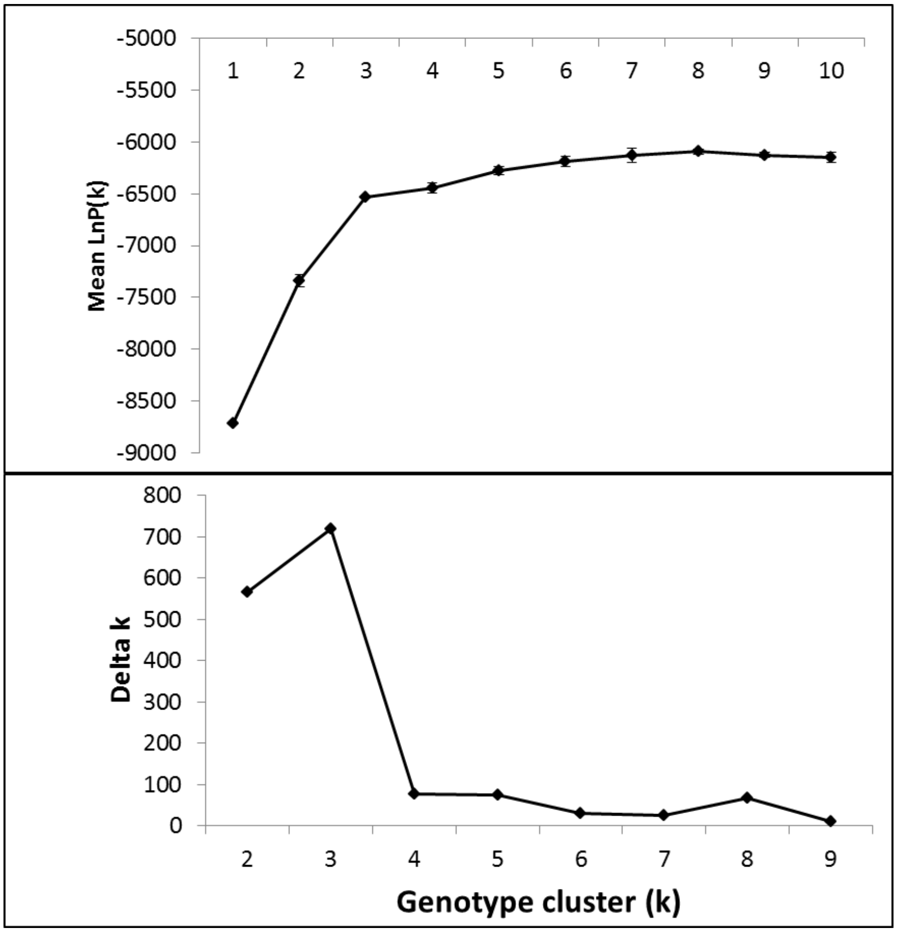

Number of LCT stocked
300,000
250,000
200,000
150,000
100,000
50,000
0
>25mm
25-100mm
100-200mm
200-400mm
Size class of LCT stocked

Figure 4.



*Not all individuals were measured in the 2007 sampling period,

Figure 5.



-5000
-5500
-6000
-6500
-7000
-7500
-8000
-8500
-9000
1
2
3
4
5
6
7
8
9
10
Mean LnP(k)
800
700
600
500
400
300
200
100
0
Delta k
2
3
4
5
6
7
8
9
Genotype cluster (k)

Figure 6

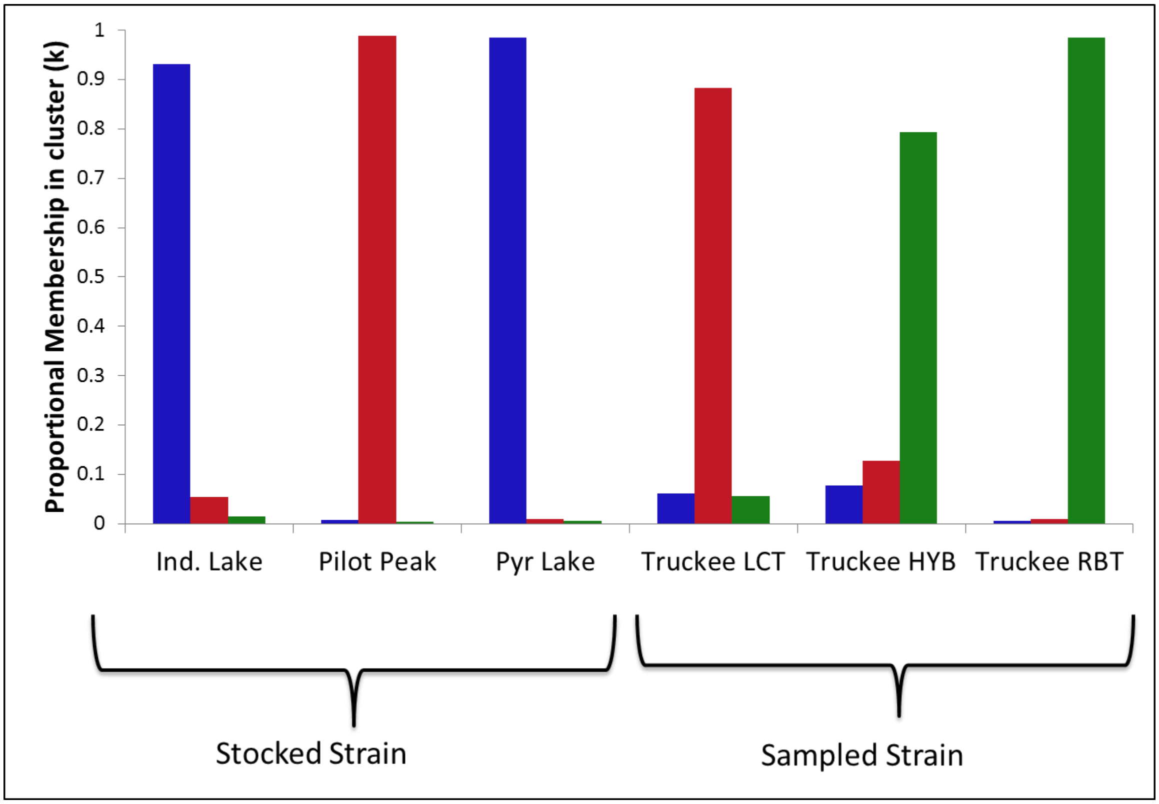

Proportional Membership in cluster (k)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Ind. Lake
Pilot Peak
Pyr Lake
Truckee LCT
Truckee HYB
Truckee RBT
Stocked Strain
Sampled Strain

Figure 7.

LITERATURE CITED

Allendorf, F. W., and S. R. Phelps. 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. *Transactions of the American Fisheries Society,* **109**:537-543.

Bartlett, J., and J. J. Warwick. 2009. Assessing the impacts of nutrient load uncertainties on predicted Truckee river water quality. *Journal of Environmental Engineering-Asce***, 135**:636-644.

Behnke, R. J., and M. Zarn. 1976. Biology and management of threatened and endangered western trouts. General Technical Report RM-28. Fort Collins, CO: U.S. Department of Agriculture. 23-30

Bigelow, J. P., W. M. Rauw, and L. Gomez-Raya. 2010. Acclimation improves short-term survival of hatchery Lahontan cutthroat trout in water from saline, alkaline Walker Lake, Nevada. *Journal of Fish and Wildlife Management,* **1**:86-92.

Blanchet, S. 2012. The use of molecular tools in invasion biology: an emphasis on freshwater ecosystems. *Fisheries Management and Ecology,* **19**:120-132.

Cain, C. M., T. M. Livieri, and B. J. Swanson. 2011. Genetic evaluation of a reintroduced population of black-footed ferrets (*Mustela nigripes*). *Journal of Mammalogy,* **92**:751-759.

Cochran-Biederman, J. L., K. E. Wyman, W. E. French, and G. L. Loppnow. 2015. Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology,* **29**:175-186.

Coffin, P.D., and W.F. Cowan. 1995. Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) Recovery Plan. United States Fish and Wildlife Service, Region I, Portland, Oregon.

Coleman, M. E., and V. K. Johnson. 1988. Summary of trout management at Pyramid Lake, Nevada, with emphasis on Lahontan cutthroat trout, 1954-1987. *American Fisheries Society Symposium,* **4:**107-115.

Cordone, A. J., and T. C. Frantz. 1968. An Evaluation of trout planting in Lake Tahoe. *California Fish and Game,* **54**(2):68-89.

DeLong, J. 2014. Cutthroats spawn at Pyramid Lake. *Reno Gazette-Journal*

Dodd, C. K., and R. A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles- are they conservation strategies that work. *Herpetologica,* **47**:336-350.

Earl, D. A., and B. M. vonHoldt. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**(2):359-361.

Estoup, A., and T. Guillemaud. 2010. Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology* **19**:4113-4130.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**:2611-2620.

Frankham, R. 1995. Conservation genetics*. Annual Review of Genetics,* **29**:305-327.

Frankham, R., C. J. A. Bradshaw, and B. W. Brook. 2014. Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. *Biological Conservation*, **170**:56-63.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool- status and strategy. *Science,* **245**:477-480.

Hickman, T. J., and R. J. Behnke. 1979. Probable discovery of the original Pyramid Lake Cutthroat Trout. *Progressive Fish-Culturist*, **41**:135-137.

Kirchoff, V. S. 2016. Hybridization dynamics of Lahontan cutthroat trout (LCT) and rainbow trout (RBT) and implications of LCT reintroduction to their native habitat. Chapter 2.

Meijaard, E., S. Wich, M. Ancrenaz, and A. J. Marshall. 2012. Not by science alone: Why orangutan conservationists must think outside the box. Pages 29-44 in R. S. Ostfeld, and W. H. Schlesinger, editors. Year in Ecology and Conservation Biology. Blackwell Science Publ, Oxford.

Ostberg, C. O., and R. J. Rodriguez. 2004. Bi-parentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. *Molecular Ecology Notes*, **4**:26-29.

Palti, Y., M. R. Fincham, and C. E. Rexroad III. 2002. Characterization and mapping of 38 polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). *Molecular Ecology Notes,* **2:**449-452.

Peacock, M. M., and V. S. Kirchoff. 2007. Analysis of genetic variation and population genetic structure in Lahontan cutthroat trout (*Oncorhynchus clarkii* *henshawi*) extant populations. *Final report to US Fish and Wildlife Service*, Region 6, Reno, Nevada.

Peacock, M. M., H. Neville, and V. S. Kirchoff. 2004. Ten species specific microsatellite loci for Lahontan cutthroat trout, *Oncorhynchus clarki henshawi*. *Molecular Ecology Notes*, **4**:557-559.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**:945–959.

Rexroad, C. E. III, R. L. Coleman, A. L. Gustafson, W. K. Hershbeger and J. Killefer . 2002. Development of rainbow trout microsatellite markers from repeat enriched libraries. *Marine Biotechnology,* **3**:12-16.

Rexroad, C.E. III, and Y. Palti 2003. Development of ninety-seven polymorphic microsatellites markers for rainbow trout. *Transactions of the American Fisheries Society*, **132:**1214-1221.

Robinson, M. L., V. S. Kirchoff, and M. M. Peacock. 2009. Characterization of 13 microsatellites for Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) and cross-amplification in six other salmonids. *Molecular Ecology Resources,* **9**:134-136.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology,* **18**:233-234.

Schweber, N. 2013. 20 Pounds? Not too bad, for an extinct fish. *Pyramid Lake Journal, The New York Times.*

Seddon, P. J., D. P. Armstrong, and R. F. Maloney. 2007. Developing the science of reintroduction biology. *Conservation Biology*, **21**:303-312.

Seddon, P. J., C. J. Griffiths, P. S. Soorae, and D. P. Armstrong. 2014. Reversing defaunation: Restoring species in a changing world. *Science*, **345**:406-412.

Seddon, P. J., P. S. Soorae, and F. Launay. 2005. Taxonomic bias in reintroduction projects. *Animal Conservation*, **8**:51-58.

Sedinger, J. S., E. J. Blomberg, A. W. VanDellen, and S. Byers. 2012. Environmental and population strain effects on survival of Lahontan cutthroat trout in Walker Lake, Nevada: a bayesian approach. *North American Journal of Fisheries Management,* **32**:515-522.

Struebig, M. J., M. Fischer, D. L. A. Gaveau, E. Meijaard, S. A. Wich, C. Gonner, R. Sykes, A. Wilting, and S. Kramer-Schadt. 2015. Anticipated climate and land-cover changes reveal refuge areas for Borneo's orangutans. *Global Change Biology*,

21:2891-2904.

Sumner, F. H. 1940. The decline of the Pyramid Lake Fishery. *Tranactions of the American Fisheries Society*, **69:**216-224.

Todd, C. R., and M. Lintermans. 2015. Who do you move? A stochastic population model to guide translocation strategies for an endangered freshwater fish in south-eastern Australia. *Ecological Modelling* **311**:63-72.

Weber, E. D., and K. D. Fausch. 2003. Interactions between hatchery and wild salmonids in streams: differences in biology and evidence for competition. *Canadian Journal of Fisheries and Aquatic Sciences,* **60**:1018-1036.

Weeks, A. R., C. M. Sgro, A. G. Young, R. Frankham, N. J. Mitchell, K. A. Miller, M. Byrne, D. J. Coates, M. D. B. Eldridge, P. Sunnucks, M. F. Breed, E. A. James, and A. A. Hoffmann. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications,* **4**:709-725.

Young, M. K., and A. L. Harig. 2001. A critique of the recovery of greenback cutthroat trout. *Conservation Biology,* **15**:1575-1584.

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

Introduced species in freshwater ecosystems: utility of genetic tools to inform management promoting species recovery of threatened native trout

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The biodiversity of native trout in western North America is declining and wildlife management agencies have limited options when attempting to restore native trout populations into regions where they have been eradicated. Habitat restoration, removal of invasive species, and supplementing extant endangered populations with hatchery raised individuals can help to protect native species. The Truckee River watershed is a large fragmented habitat with multiple water diversions and barriers that limit fish movement. A large variety of introduced species have become naturalized in this river and the impact of these species on the reintroduction potential of the native trout must be evaluated. Here I have taken a first step in evaluation of the RBT population in the Truckee River and have determined that although it is possible to reintroduce LCT, RBT present in the system will be an impediment to that recovery. Translocations for conservation and restoration of endangered species can protect that species from extinction, but the overall success rates reported have been low (Griffith et al., 1989; Dodd and Seigel, 1991; Godefroid et al., 2011). The results of translocations are dependent on many factors such as habitat quality (Harig and Fausch, 2002), presence of non-natives that can compete or hybridize with the translocated species (Harig et al., 2000), or inbreeding and loss of genetic variation in source population (Gray et al., 2014), all of which need to be considered in the Truckee River. The initial decline of the LCT in this system was due to a combination of factors. First, brown trout and RBT and other game fishes were planted for recreational angling. Historically, when the Truckee was a large interconnected system, LCT dominated the watershed and were able to resist hybridization with LCT (Coleman, 1988). The presence of the introduced species alone was not enough to endanger the population. Secondly, industrialization and logging created pollution and decreased available spawning habitat (Halverson, 2010). This weakened the LCT population, and created an environment where the introduced species were able to compete for the diminishing spawning area, starting to displace the native fish because LCT do not prefer to live in high densities unlike brook trout and brown trout. The final alteration of the Truckee that resulted in LCT extirpation was the creation of Derby Dam in 1906, and multiple complete barriers all along the watershed (Sumner, 1940). LCT from Pyramid Lake, which migrated up the Truckee River to spawn, could no longer successfully reproduce and the population eventually collapsed.

Reintroduction success requires evaluation of the initial reasons for the decline of the species. If an introduced species has contributed to the decline, prior to any reintroduction efforts, it is necessary to remove that introduced species; in addition, reinvasion potential of the introduced species must be investigated to provide any long term success in the ability of the re-introduced species ability to survive in the native ecosystem (Gray et al., 2014; Weeks et al., 2011). Translocation guidelines indicate that prior to attempting a relocation, you have to evaluate the social and economic interests in the region. Recreational angling provides substantial economic support to the Truckee River region. To maintain economic stability, reintroduction of LCT would have be able to satisfactorily replace the angling of non-natives. Given the recent popularity of Pilot Peak LCT fishing in Pyramid Lake, if this LCT strain were able to initially recolonize the lower regions of the Truckee River, beyond Derby Dam, it is plausible that it could gradually replace the non-native fishery. This would enhance the fishing in the Truckee

River providing a unique native trout that could be potential draw for the angling industry.

This study evaluated the hybridization dynamics of LCT with RBT in the Truckee River using a suite of molecular genetic tools that indicate not only the level of hybridization but also showed that LCT were able to successfully compete for spawning habitat. This result suggests that if the threat of hybridization were to be removed, LCT would be able to find suitable habitat and refugia to sustain in the river. The stocking of triploid RBT is done to try to limit hybridization. While I was able to show that the triploid RBT had little impact on hybridization, stocking of triploids is an acceptable alternative for state gaming agencies to maintain angling opportunities; however, if removal of the naturalized RBT population is a goal, it is imperative that steps be taken to disrupt the RBT reproduction in the river. Currently, flooding the system with LCT in good spawning habitat primarily results in hybridization.

To improve the chance of successful reintroduction into the Truckee River, managers need to take a systematic approach. First, they must identify regions of the river that have limited introduced species or are isolated by barriers to allow for removal of that introduced species. Often complete eradication of an invasive species is neither cost effective nor practical and controlled areas are often re-invaded by neighboring populations (Purcell and Stockwell, 2015; Klima and Travis, 2012). In the Truckee River, the lower regions of the river have very limited introduced species, however, the habitat is currently marginal. Current restoration efforts are improving habitat and spawning of Pilot Peak LCT from Pyramid Lake has been seen in the lower river (DeLong, 2014). Utilizing this section of the river is a low risk first step in creating a sustainable LCT fishery out of Pyramid Lake. It is equally important when translocating a population that an appropriate source of native fish be utilized. Here I have shown that the Pilot Peak strain has better survival and contributes more to reproduction (hybridization) than the Pyramid lake strain. Sedinger et al. (2012) found a similar result in Walker Lake, suggesting that the Pilot Peak strain be the focus of reintroduction.

Here I also used genetic tools to look at the population structure of the RBT to and to identify potential threats to the reintroduction (Allendorf and Phelps, 1980; Estoup and Guillemaud, 2010; Blanchet, 2012). At this time, was not able to find any clear "eradication units," segments of the population that are interconnected and as such, must be eliminated simultaneously to prevent re-invasion (Robertson and Gemmell, 2004). The best habitat for reintroduction is clearly the region of the river where reproduction (and therefore hybridization was the highest. This was seen in the tributaries, Dog Valley and Hunter creeks. Analysis of population structure showed connectivity of these tributaries to the main stem; however, there are populations above putative barriers that appear more isolated that were only sampled in 2009. The results of that years sampling period suggested that it could be possible to isolate the tributaries above the current stocking and sampling transects, but further research needs to be done to investigate this.

Implications for LCT recovery

The results of this study suggest that the Pilot Peak strain has higher survivorship in the Truckee River compared to either the contemporary mixed stock Pyramid Lake or Independence Lake strains. Although I cannot rule out that some of the LCT sampled were from recent stocking events, the preponderance of pure LCT sampled in the Truckee River were Pilot Peak LCT from the USFWS Lahontan National Fish Hatchery (LNFH). These results suggest that future LCT introductions into the Truckee River should focus on using the Pilot Peak strain, as was recommended by Sedinger et al. (2012). Despite the fact that genetic breeding in captivity can lead to loss of genetic variation and that the captive strains have been found, in some species, to become adapted to the captive environment (Frankham, 1995), this does not seem to be the case here. This strain appears better suited for this environment, notwithstanding both the lower allelic variation and heterozygosity compared to other strains of LCT (Table 6). The LNFH is maximizing the existing genetic variation in a captive breeding program that uses variation at microsatellite markers to decrease inbreeding and increase heterozygosity. Genetic evaluation of the brood stock is done each year to ensure that the existing variation in the Pilot Peak LCT strain is maintained.

An option available to management agencies is to stock only native trout and non-reproductive triploid RBT for angling opportunities in hopes that the native trout can recover. To increase the probability of success, simultaneously reducing the non-native populations when possible can allow for the LCT to be able to better compete. Removal of non-native brook trout from spawning streams around Independence Lake has improved the survival and recruitment for LCT fry (Scoppettone, 2012). Low water years were shown here to interfere with RBT connectivity. Given the cyclic nature of water flow in the Truckee River, managers can utilize low water flow to remove large numbers of RBT prior to planting LCT. Continued monitoring, and continued stocking of LCT would be necessary to limit hybridization.

Although partial reintroduction of LCT could be possible in segments of the river if artificial barriers are constructed, the long-term effectiveness of these barriers is questionable. Public support is needed to initiate a large-scale reintroduction effort if LCT reintroduction is to be possible in the Truckee River. One of the previous shortcomings of LCT recovery utilizing the contemporary Pyramid Lake strain is the small average adult size of the LCT strains compared to the historic Pyramid Lake fish. Since 2006, the Pilot Peak strain of LCT has been stocked in Pyramid Lake and has been monitored through creel studies. Although the official world record for this fish was 18.6 kg (41 lbs) in 1925, and current fish have not yet reached that size, reports have noted Pilot Peak LCT captures of more than 9.0 kg (20 lbs) in the last three years, exceeding the size of LCT fished in Pyramid Lake in the previous decades (Schweber, 2013). In addition, for the first time in 76 years, LCT from Pyramid Lake spawned in the lower Truckee River in 2014 (DeLong, 2014) as well as in 2015. Genetic analysis of the 2014 fry confirmed these fish to be progeny from the Pilot Peak LCT (DeLong, 2014). Preliminary analysis of the 2015 fry also show that the Pilot Peak LCT from Pyramid Lake are preferentially spawning in the lower regions of the Truckee River (data not shown) due in part to restoration efforts from USFWS to reconnect Pyramid Lake LCT to the spawning habitat in the lower Truckee River. The survival and reproduction the

Pilot Peak strain indicated by this study, can garner significant support for a large-scale RBT eradication and re-introduction of LCT into the Truckee River. Continued efforts at controlling non-native fishes, creating available habitat and improving the habitat currently available can allow for LCT recovery.

LITERATURE CITED

Allendorf, F. W., and S. R. Phelps. 1980. Loss of genetic-variation in a hatchery stock of cutthroat trout. *Transactions of the American Fisheries Society*, **109**:537-543.

Blanchet, S. 2012. The use of molecular tools in invasion biology: an emphasis on freshwater ecosystems. *Fisheries Management and Ecology*, **19**:120-132.

Coleman, M. E., and V. K. Johnson. 1988. Summary of trout management at Pyramid Lake, Nevada, with emphasis on Lahontan cutthroat trout, 1954-1987. *American Fisheries Society Symposium* **4**:107-115.

DeLong, J. 2014. Cutthroats spawn at Pyramid Lake. *Reno Gazette-Journal.*

Dodd, C. K., and R. A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles- Are they conservation strategies that work. *Herpetologica,* **47**:336-350.

Estoup, A., and T. Guillemaud. 2010. Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology*, **19**:4113-4130.

Frankham, R. 1995. Conservation genetics*. Annual Review of Genetics,* **29**:305-327.

Godefroid, S., C. Piazza, G. Rossi, S. Buord, A.-D. Stevens, R. Aguraiuja, C. Cowell, C. W. Weekley, G. Vogg, J. M. Iriondo, I. Johnson, B. Dixon, D. Gordon, S. Magnanon, B. Valentin, K. Bjureke, R. Koopman, M. Vicens, M. Virevaire, and T. Vanderborght. 2011. How successful are plant species reintroductions? *Biological Conservation,* **144**:672-682.

Gray, Q. Z., D. J. Fraser, and J. W. A. Grant. 2014. Extirpation for Conservation: Applying Predictors of Extinction Risk to Eradicate Introduced Trout Populations for Lake Restoration. *Ecological Restoration,* **32**:59-67.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool - status and strategy. *Science*, **245**:477-480.

Halverson, A. 2010. A less bold and spirited nation. An entirely synthetic fish. How rainbow trout beguiled America and overran the world. pp 1-12. New Haven and London. Yale University Press.

Harig, A. L., K. D. Fausch, and M. K. Young. 2000. Factors influencing success of greenback cutthroat trout translocations. *North American Journal of Fisheries Management*, **20**:994-1004

Harig, A. L., and K. D. Fausch. 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications* **12**:535-551.

Klima, K., and S. E. Travis. 2012. Genetic population structure of invasive nutria (Myocastor coypus) in Louisiana, USA: is it sufficient for the development of eradication units? *Biological Invasions*, **14**:1909-1918.

Purcell, K. M., and C. A. Stockwell. 2015. An evaluation of the genetic structure and post –introduction dispersal of a non-native invasive fish to the North Island of New Zealand. *Biological Invasions,* **17**:625-636.

Robertson, B. C., and N. J. Gemmell. 2004. Defining eradication units to control invasive pests. *Journal of Applied Ecology*, **41**:1042-1048.

Schweber, N. 2013. 20 Pounds? Not too bad, for an extinct fish. *Pyramid Lake Journal, The New York Times.*

Scoppettone, G. G., P. H. Rissler, S. P. Shea, and W. Somer. 2012. Effect of Brook Trout Removal from a Spawning Stream on an Adfluvial Population of Lahontan Cutthroat Trout. *North American Journal of Fisheries Management*, **32**:586-596.

Sedinger, J. S., E. J. Blomberg, A. W. VanDellen, and S. Byers. 2012. Environmental and Population Strain Effects on Survival of Lahontan Cutthroat Trout in Walker Lake, Nevada: A Bayesian Approach. *North American Journal of Fisheries Management*, **32**:515-522.

Sumner, F. H. 1940. The decline of the Pyramid Lake Fishery. *Tranactions of the American Fisheries Society*, **69:**216-224.

Weeks, A. R., C. M. Sgro, A. G. Young, R. Frankham, N. J. Mitchell, K. A. Miller, M. Byrne, D. J. Coates, M. D. B. Eldridge, P. Sunnucks, M. F. Breed, E. A. James, and A. A. Hoffmann. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications,* **4**:709-725.