

University of Nevada, Reno

**Assessing the threat of non-native salmonids to Lahontan cutthroat trout recovery
in a subalpine watershed**

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in
Biology

by

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THE GRADUATE SCHOOL

We recommend that the thesis
prepared under our supervision by

JASON ALAN SMITH

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**Assessing the threat of non-native salmonids to Lahontan cutthroat trout recovery
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MASTER OF SCIENCE

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ABSTRACT

Introductions of non-native fishes complicate native fish recovery and introductions of non-native salmonids have been implicated in the decline of inland cutthroat trout subspecies. The United States Fish and Wildlife Service has been restocking Lahontan cutthroat trout *Oncorhynchus clarkii henshawi* in Fallen Leaf Lake, California annually since 2002 to stem this decline. Brown trout *Salmo trutta* pose a piscivory threat while rainbow trout *O. mykiss* and Lahontan cutthroat trout readily hybridize. Both rainbow trout and brown trout are found in Fallen Leaf Lake and the upper Glen Alpine Creek watershed which drains into the lake and therefore the presence of these species may represent significant threats to implementation of a successful reintroduction program. I investigated the genetic population structure and connectivity of naturalized rainbow and brown trout throughout the Glen Alpine Creek watershed including Fallen Leaf Lake in order to assess the threats posed by these non-native fishes. Fin clips were collected from adult rainbow trout (N=279) and brown trout (N= 350) for genetic analyses using electrofishing techniques in the stream network and gill-netting in the lakes within the watershed. Isolated DNA samples were genotyped at 13 (rainbow trout) and 10 (brown trout) polymorphic microsatellite loci. The Bayesian clustering program STRUCTURE was used to assess genetic connectivity. Using the delta k approach I found statistical support for three populations of rainbow and five populations of brown trout. Pairwise comparisons of these populations supported significant genetic differentiation and that this differentiation was associated with gradient barriers. Additionally, Mantel tests for both species showed some support for a pattern of isolation-by-distance. This study indicated genetic connectivity among rainbow and brown trout populations between the

upper Glen Alpine Creek drainage and Fallen Leaf Lake populations, and identified a self-sustaining population of rainbow trout persisting in Fallen Leaf Lake. The results of this study have management implications and suggest that a watershed level recovery approach is necessary for the long term success of the reintroduction program for Lahontan cutthroat trout in Fallen Leaf Lake.

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INTRODUCTION

Non-native salmonids have been transplanted into waters across the globe to provide recreational angling opportunities and now represent a serious extinction threat to native salmonid populations including inland cutthroat trout (*Oncorhynchus clarkii*) populations found in the western United States. Naturalized populations of non-native lake trout *Salvelinus namaycush*, rainbow trout *Oncorhynchus mykiss*, brown trout *Salmo trutta*, and brook trout *Salvelinus fontinalis*, have become established across the distributional range of cutthroat trout and impact these native salmonids through a combination of predation, competitive exclusion, and hybridization (Allendorf et al. 2001, Peterson and Fausch 2003, Vander Zanden et al. 2003, Vredenburg 2004, Cucherousset and Olden 2011). As such, these introduced species represent the largest contemporary threat to persistence of inland cutthroat trout populations (Scoppettone et al. 2012, Quist and Hubert 2004, Kruse et al. 2000).

There are approximately 14 recognized subspecies of cutthroat trout (Behnke 1992). Two are considered to be extinct (Yellowfin cutthroat trout *O. c. macdonaldi*, Alvord cutthroat trout *O. c. alvordensis*) and three have been listed as 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*), (Behnke 1992, Peacock et al. 2010, Budy et al. 2012). Most of these subspecies were historically found in large interconnected multiple order stream and lake networks (Behnke 1992, Behnke and Tomelleri 2002, Fausch 2008). Population dynamics of these subspecies include resident and migratory life histories, metapopulation dynamics, and age-based spatial segregation (Reiman and Dunham 2000, Neville et al. 2006a, Young 2011).

Recovery strategies for many of these subspecies have focused on barrier construction to prevent incursions by non-native fish species, but this results in small isolated headwater populations with a suite of increased extirpation risks (Elliot et al. 1997, Harig and Fausch 2002, Shepard et al. 2005, Peterson et al. 2008, Fausch et al. 2009). Expansion of available habitat and restoration of habitat connectivity is currently recognized as imperative for long term persistence of cutthroat trout populations (Neville et al. 2006b, Peterson et al. 2008, Peacock et al. 2010). Restoration strategies returning subspecies to historical habitat that is now occupied by non-native salmonids is a major restoration strategy. However, removal of nonnative salmonids has proven to be logistically, politically and socially challenging.

In this study I investigate the impact of non-native salmonid species on the restoration potential of a listed native salmonid into historical lacustrine habitat. Lahontan cutthroat trout, the largest of the fourteen cutthroat trout subspecies, was the apex predator in its historical distribution, the hydrographic Lahontan Basin of northern Nevada, southeastern Oregon and northeastern California (Figure 1; Behnke 1992). Extant native and naturally reproducing lacustrine populations of Lahontan cutthroat trout are currently found in only 0.4% of their original lake habitat and 10% of their original fluvial habitat (Gerstung 1988, Coffin and Cowan 1995, Dunham et al. 1997, Scoppettone et al. 2012). Three evolutionary significant units (ESUs) or geographic management units (GMUs) based upon ecological, behavioral, morphological, and genetic data are currently recognized for Lahontan cutthroat trout (Coffin and Cowan 1995). Fluvial habitat predominates in the northwestern (Quinn River, Summit Lake, and Coyote Lake basins) and the eastern GMUs (Reese and Humboldt rivers), whereas

lacustrine habitat for this subspecies is found primarily in the Truckee and Walker river watersheds of the western GMU (Truckee, Carson and Walker rivers). Behnke (1992) further proposed that the fluvial and lacustrine forms of Lahontan cutthroat trout should be recognized as separate subspecies due to morphological differences (e.g., Humboldt fish have fewer gill rakers and tend to have fewer scales in the lateral series and above the lateral line). Historically, Lahontan cutthroat trout were found in a number of lakes within the Truckee River basin as well as tributaries which drained into these lakes [Fallen Leaf, Cascade, Donner, Independence, Tahoe, Pyramid, and Winnemucca lakes (dry); Behnke 1992, Gerstung 1986, Coffin and Cowan 1995]. The challenges to recovery of lacustrine forms of Lahontan cutthroat trout in the western Lahontan Basin are numerous and include widespread introductions of non-native aquatic invertebrates, plants and fish, and habitat fragmentation, degradation, and loss.

In 2002 the United States Fish and Wildlife Service (USFWS) began a pilot re-introduction project for Lahontan cutthroat trout in Fallen Leaf Lake, a small lake connected to the much larger Lake Tahoe by a single tributary, Taylor Creek (TRRIT report 2003). The project aims were to identify and ameliorate potential limiting factors to the establishment of Lahontan cutthroat trout in altered but historical lacustrine habitat before reintroducing Lahontan cutthroat trout into the larger Lake Tahoe (Allen et al. 2006, Al-Chokhachy et al. 2009).

Fallen Leaf Lake contains a mixture of native and non-native aquatic species with established populations of introduced lake trout, rainbow trout, brown trout, and kokanee salmon *Oncorhynchus nerka* as well as mysid shrimp *Mysis diluviana*. The Glen Alpine Creek watershed that drains into Fallen Leaf Lake was historically fishless, but small

lakes in the upper drainage have been stocked with non-native trout and char species by both private and public organizations since 1877 (Lindstrom et al. 2000), and their current abundance and distribution is not well known. Although there are no programs that are currently stocking non-native fishes in the Glen Alpine Creek watershed, surveys conducted by the California Department of Fish and Wildlife (CDFW) and the United States Forest Service (USFS) have shown that introduced salmonids have persisted and established naturalized reproducing fluvial and lacustrine populations. The presence of these fishes in the upper watershed may pose a threat to Lahontan cutthroat trout recovery in Fallen Leaf Lake.

In this study I expand upon the USFWS restoration study to include the upper Glen Alpine Creek watershed in order to examine the threat posed by non-native salmonids found in waters which drain into Fallen Leaf Lake. Here I focus on rainbow and brown trout as rainbow trout readily hybridize with cutthroat trout and represent a significant threat due to genetic assimilation (Allendorf et al. 2001, Sieler and Keeley 2009) and brown trout are highly piscivorous and prey upon cutthroat trout (McHugh et al. 2006, Jensen et al. 2008). It is important to understand the physical connection of upper watershed populations of brown and rainbow trout to populations of these fishes in Fallen Leaf Lake. If the upper drainage is isolated from Fallen Leaf Lake then the naturalized non-native salmonid populations in the upper watershed are not likely to move into the lake and impact the restoration of Lahontan cutthroat trout. Alternatively, if there is movement of these fish species from upper watershed populations into Fallen Leaf Lake then they present a threat and could negatively impact the restoration of Lahontan cutthroat trout. Genetic methods can be used to test this connection between

fish populations in the upper Glen Alpine Creek drainage and those in Fallen Leaf Lake. Genetic approaches that analyze patterns of gene-flow and combine traditional population genetics with landscape genetic methods (see Manel et al. 2003 for a good review) are especially useful for this question. These approaches can identify previously unknown geographic movement barriers, uncover cryptic breeding patterns, and clarify genetic connectivity between and among populations of organisms.

Immigration by rainbow and brown trout from the upper watershed could represent a potential source of ongoing non-native salmonid introduction into Fallen Leaf Lake thereby compromising the cutthroat trout reintroduction effort. I use a combination of traditional fisheries sampling techniques and population genetic approaches to: (1) characterize the distribution of rainbow and brown trout in the upper Glen Alpine Creek watershed, (2) assess the connectivity among rainbow and brown trout populations in the upper watershed with Fallen Leaf Lake populations, and (3) determine the extent to which lower Glen Alpine Creek is used for spawning by rainbow trout.

METHODS

Field Methods

Study area.— I conducted this study in the Glen Alpine Creek watershed located in Desolation Wilderness, El Dorado County, California, which drains into Fallen Leaf Lake in the Lake Tahoe basin (Figure 2). I sampled brown trout and rainbow trout individuals in Fallen Leaf Lake for inclusion in the genetic analyses. Fallen Leaf Lake is a 567 hectare meso-oligotrophic lake located at an elevation of 1953 meters (m) with an average depth of 53 m, and a maximum depth of about 120 m (Allen et al. 2006, Al-

Chokhachy et al. 2009). A dam on the northern end of the lake allows limited downstream movement of fishes, but prevents upstream movement into Fallen Leaf Lake. Glen Alpine Creek is the primary inlet for Fallen Leaf Lake and enters at the southwestern end of the lake (Figure 2). Glen Alpine Falls, located approximately 500 m upstream of Fallen Leaf Lake, is a 22 m tall cascade waterfall that forms a natural barrier to upstream movement of fishes. The stream reach between Fallen Leaf Lake and Glen Alpine Falls provides the only available spawning habitat for stream-spawning salmonids present in Fallen leaf Lake, and has a substrate composed primarily of cobble and bedrock (Allen et al. 2006, Al-Chokhachy et al. 2009). Glen Alpine Creek drains approximately 41 square kilometers of snowmelt fed wilderness area which contains numerous small lakes that are interconnected by flashy ephemeral streams (Allen et al. 2006). These streams have many natural gradient barriers (mostly waterfalls) that limit upstream salmonid movement (Figure 3). In addition, under low flow conditions sections of the watershed can completely disconnect, or only exhibit subterranean flow. This increases waterway subdivision and creates additional barriers to fish movement.

Study species.– **Brown trout** (Walbaum 1792) are native to Europe, North Africa, and West Asia and were first introduced into the United States in 1884 (Behnke 2002). This is an ecologically diverse species that spawns in fluvial habitat in the fall and exhibits resident, migratory and anadromous life histories. Feeding strategies vary with age as do most salmonids, and brown trout are known to feed on a variety of benthic organisms, and zooplankton. Brown trout are considered to be extremely piscivorous as adults, often switching over to piscivory at an earlier age and smaller size class than other salmonids.

Rainbow trout (Walbaum 1792) is a cold water fish species native to the west coast of North America. The historic range of rainbow trout is in waters which drain to the Pacific Ocean from Mexico to the Aleutian Islands and extending to the Kamchatka Peninsula (Behnke 1992, 2002). They are one of the most widely transplanted fish species in the world and have been successfully translocated to every continent with the exception of Antarctica (Behnke 2002). As a result rainbow trout have been widely transplanted into the native waters of cutthroat trout (also *Oncorhynchus*) to which they are closely related. As with cutthroat trout, rainbow trout are spring spawners in fluvial habitat and thus represent a significant hybridization risk for cutthroat trout subspecies. Rainbow trout exhibit a high degree of plasticity in their life history strategies with known stream resident, migratory, and anadromous forms.

Sample collection.– Using a combination of electrofishing and gill netting techniques I surveyed 28 stream and lake sampling locations in the Glen Alpine Creek watershed (Figure 4; Dunham et al 2009, Meeuwig et al. 2008). The sampling design was intended to facilitate characterization of non-native fish distribution in the watershed as well as the collection of tissue samples to assess the extent of genetic connectivity among brown trout and rainbow trout populations in the upper watershed and Fallen Leaf Lake.

Stream network sampling.– I conducted stream sampling over two years between 7/21 and 9/11 in 2010 and 8/24 to 10/16 in 2011. I sampled a total of 5.11 km of stream network in 2010 and 6.97 km in 2011. I used a Smithroot LR-24 backpack electrofishing unit and a single pass sampling scheme with two netters in unblocked sections (Dunham et al. 2009, Snyder 2003). I sampled during base flow conditions, which were similar between years. I electrofished in a downstream to upstream direction

and stopped when either one of two conditions occurred: (1) a barrier to upstream movement was encountered or (2) when collected fishes were showing signs of stress. No fish were moved above barriers. I recorded shocking time in seconds, fish species encountered, total length in mm, fork length in mm, and wet weight in grams for each fish. I measured a minimum of 100 individuals when greater than 100 individuals of a particular species were encountered within a sampling section. I tagged brown trout and rainbow trout over 150 mm in total length using Visual Implant Alpha Tags (Northwest Marine Technology, Shaw Island Washington) in 2010 in order to assess movement over barriers. All individual fish I measured were returned to the stream section from which they were collected. I calculated catch per unit effort (i.e., relative abundance) of brown trout and rainbow trout as fish per minute for each electrofishing section separately as:

$$\text{Catch per unit effort} = \frac{C}{F},$$

where C is the number of fish caught and f is the unit of effort expended, in this case, the shocking time in number of minutes spent electrofishing.

Upper watershed lakes sampling.— Sampling was conducted in the upper lakes from 7/28 to 9/11 in 2011. I sampled nine of the 13 largest lakes that drain into Glen Alpine Creek (see Figure 3). There were four lakes (Le Conte, Margery, Lucille, and Jabu) that I did not sample, as they were part of an ongoing fish removal study by the United States Forest Service in an attempt to reestablish the mountain yellow legged frog *Rana sierrae* (Figure 3). I sampled lakes using three lightweight monofilament sinking gill nets that were 36 m long, 1.5 m deep, and constructed of six panels; 10, 12.5, 18.5, 25, 33, and 38 mm bar mesh. The first net was set beginning at a randomly generated

shoreline starting point and the other two nets were distributed equidistantly around the perimeter of the lake. I set the nets using float tubes and oriented them perpendicular to the shoreline with the net end containing the smallest mesh anchored to the shore. The number of gill nets, soak time, and depth varied between lakes due to sampling methodology, logistical constraints, and lake morphometry (Table 1). In order to sample both crepuscular periods I set nets in the late afternoon or early evening and pulled them the next morning beginning shortly after sunrise. I rotated subsequent net sets on the same lake clockwise 100 m along the shore and reset as before. I placed nets for a maximum of three days. If no rainbow trout or brown trout had been caught by the second day of sampling, I assumed they were not present in that lake and did no further sampling except at Half Moon Lake (Figure 3). Despite the fact that no rainbow or brown trout were found in Half Moon Lake, I continued sampling for three days because this lake was known to have been repeatedly stocked with rainbow trout and anecdotal evidence suggested the possible persistence of rainbow trout in the lake. If no fish of any species were collected after two days I considered a lake to be fishless. I recorded net location, net soak time, fish species present, total length in mm, fork length in mm, and wet weight in grams. When I encountered greater than 100 individuals of a particular species within a lake I measured a minimum of 100 individuals. I enumerated all individual fish and then returned them to the lake. I calculated brown trout and rainbow trout catch per unit effort (i.e., relative abundance) separately for each gill net using the number of fish caught divided by the number of hours the net was set. I calculated mean catch per unit effort for brown trout and rainbow trout by lake.

Fallen Leaf Lake sampling.– Brown trout and rainbow trout genetic samples were collected in Fallen Leaf Lake using gill nets between 2009 and 2011 by USFWS and University of Nevada, Reno (UNR) researchers. I used these samples to assess genetic connectivity, but did not incorporate them into the relative abundance study as the collection protocol in Fallen Leaf Lake differed from the one used in the upper drainage lakes.

Assessing rainbow trout reproduction.– To examine the extent to which lower Glen Alpine Creek was used by rainbow trout for reproduction, I surveyed lower Glen Alpine Creek in June and July 2011 for the presence of redds. I used a double observer rock picking technique to systematically sample the approximately 500 m long stream reach below Glen Alpine Falls in order to locate and enumerate salmonid redds. This technique uses two shoulder to shoulder snorkelers snorkeling across the stream perpendicular to stream flow while gently disturbing the streambed substrate by hand searching the exposed gravels for the presence of eggs, or alevin, which would indicate a redd. Upon reaching the opposite stream bank both snorkelers shifted one shoulder width upstream and repeated the process, systematically surveying the entire reach. I assigned redds an identification number, photographed the stream location, and recorded Universal Transverse Mercator (UTM) coordinates. I visualized redd locations for spatial analyses using ArcMap 10 software (ESRI).

Genetic Methods

Genetic sample collection.– I collected small fin clip samples (~25 mm²) from either the upper lobe of the caudal fin or from the anal fin of all rainbow trout and brown

trout sampled at all locations. The fin clips were air dried, placed in wax paper, and stored within coin envelopes for transport to the lab for genetic analysis. I recorded sample date, species if known, an identification number assigned for each individual fish, total length in mm, fork length in mm, and weight in grams on the coin envelopes. Juveniles, defined here as fish less than 100 mm in length, were excluded from the genetic connectivity analyses, but were genotyped for species identification.

Genetic data collection.– I extracted DNA from dried fin clips using QIAGEN DNeasy® 96 Blood and Tissue kits (QIAGEN Inc., Valencia California) according to the manufacturer's protocol. I quantified the extracted DNA using a Thermo Scientific Fluoroskan Ascent Fluorometer and normalized the samples to a concentration of 10 ng/μL. I genotyped rainbow trout individuals thirteen polymorphic microsatellite loci (Table 2): OCH9, OCH10, OCH17 (Peacock et al. 2004), OCH20 (Robinson et al. 2009), OMM1036, OMM1037 (Rexroad et al. 2002), OMM1220, OMM1286 (Rexroad and Palti 2003), OMM1302, OMM1315, OMM1323, OMM1325, OMM 1329 (Palti et al. 2002). I genotyped brown trout individuals at ten polymorphic microsatellite loci (Table 3): BRUN13, BRUN14 (Norwegian Salmon Genome Project registered as BHMS155 and BHMS111 respectively (Wollebaek et al. 2010), Ssa85, Ssa197 (O'Reilly et al. 1996), SsaD157, SsaD190 (King et al. 2005), Str15, Str60, Str73 (Estoup et al. 1993), Strutta58 (Poteaux et al. 1999).

To facilitate economical labeling of PCR product with a corresponding fluorescent dye I ordered microsatellite loci primer pairs with M13 tails (Schuelke 2000). I designed primers to have an optimal annealing temperature from 60-63°C. I optimized PCR reactions to include 2-6 loci per reaction, and prepared a multiplex primer cocktail

to give a final primer concentration of 0.25 μM of each tailed forward primer, 0.5 μM of each reverse primer, and 0.25 μM of each M13 fluorescently labeled primer in a reaction volume of 12 μl . I carried out PCR reactions on a 96 well Techne Touchgene thermoelectronic block using 6 μl Multiplex taq (QIAGEN), and 20-50 ng of DNA. The PCR conditions I used included an initial denaturation of 94°C for 15 minutes followed by 41 cycles of 94°C for 30 seconds, touchdown annealing temperature for 90 seconds, and 72°C for 30 seconds. The annealing temperatures I used were as follows: 65°C for 7 cycles, 61°C for 7 cycles, 58°C for 7 cycles, and 20 cycles at 55°C in which the first 21 cycles are amplifying specific primers and the final 20 cycles for adding the fluorescently labeled M13 tail to the PCR product. I used fluorescently labeled forward primers for loci that did not amplify successfully using the M13 labeled primers and the above protocol. The PCR reaction conditions were the same as above except the final concentration of labeled forward primer was 0.25 μM and the final concentration of reverse primer was 0.37 μM . I diluted the PCR product in deionized water to an appropriate intensity determined by dilution tests, and added 1 μL to 19 μL of GeneScan 500 LIZ size standard with Hi-Dye Formamide (Applied Biosystems, Inc. [ABI], Perkin-Elmer Corporation). Fragment analysis was carried out on a Perkin Elmer Applied Biosystems 3730 Genetic Analyzer (Nevada Genomics Center, <http://www.ag.unr.edu/genomics/>). I scored alleles using the ABI GeneMapper software (Version 3.7). In order to minimize genotyping error I only used matching allele calls that were independently verified by two lab personnel in this study.

Genetic data analyses.— I used Micro-checker (version 2.2.3, Van Oosterhout et al. 2004) to test for null alleles and allelic dropout per locus per sampling location and FSTAT 2.9.3.2 (Goudet 2001) to test for Hardy-Weinberg equilibrium (HWE), calculate allelic richness (R_S) for all loci, and estimate genetic differentiation (F_{ST}) among populations identified using Bayesian genotype clustering analysis (see below). I conducted assignment tests using the Bayesian genotype clustering program STRUCTURE (version 2.3.2 Pritchard et al. 2000) which assigns individuals into k groups based upon assumptions of HWE. The initial range of possible genotype clusters was based on the number of sampling locations. I used an admixture model in STRUCTURE with a burn in period of 500,000 iterations followed by ten 500,000 Markov Chain Monte Carlo replicates per k . To determine the optimal k , I used the Δk method of Evanno et al. (2005). This method calculates the largest change in the natural logarithm of the probability of the data ($\log_e P[D]$) between each pair of k and $k-1$ for all tests of k . I overlaid the spatial distribution of the populations identified using STRUCTURE onto a map of gradient barriers in the watershed created in ArcMap 10 (ESRI). As an additional test of genetic population differences I used Microsatellite Toolkit in Excel to estimate gene diversities (H_E , H_O) per locus per population and I used analysis of variance (ANOVA) to separately test for differences in the average levels of these heterozygosities among populations. I did not compare H_E to H_O within populations.

I used the linkage disequilibrium method in NeEstimator to calculate the effective population size (N_e) for each genotype cluster identified using STRUCTURE (Peel et al. 2004). This method uses Burrows composite measure of disequilibrium (Campton 1987)

and the correlation among alleles at different loci in a population (Bartley et al. 1992) to generate an estimate based upon a single sampling period (Peel et al. 2004).

To test for recent bottlenecks I used the heterozygous excess method (Piry et al. 1999) in the program BOTTLENECK (version 1.2.0, Cornuet and Luikart 1996) and applied the single step (SMM) and two phase (TPM; 90% single step mutations, 12% variance among steps) mutation models (Piry et al. 1999) and a one tailed Wilcoxon sign-rank test. I used a mode shift in allele frequency distribution test that is available in BOTTLENECK, as an additional test for genetic bottlenecks per population (Cornuet and Luikart 1996). I tested for the presence of an isolation-by-distance pattern (Wright 1943) for brown trout and for rainbow trout within the stream network using the ISOLDE option in Genepop (Version 3.4). I measured geographic distance as waterway distance in meters in ArcMap10 (ESRI) using the midpoints of the sampling section sections and $F_{ST}/(1-F_{ST})$ as a measure of genetic distance for the isolation-by-distance test.

RESULTS

Fish species assemblages.– I recorded eight native and four non-native fish species during electrofishing (Table 4) and gill net sampling (Table 5) in the Glen Alpine Creek watershed. Native fish species included Lahontan cutthroat trout, mountain whitefish *Prosopium williamsoni*, Lahontan redband *Richardsonius egregius*, speckled dace *Rhinichthys osculus*, Paiute sculpin *Cottus beldingii*, tui chub *Gila bicolor*, mountain sucker *Catostomus platyrhynchus*, and Tahoe sucker *Catostomus tahoensis*. Tahoe and mountain sucker were recorded as sucker spp. in order to prevent misidentification as these two species can hybridize (Decker 1989). All four non-native

fish species encountered in the upper watershed were in the family Salmonidae and included brook trout, lake trout, brown trout, and rainbow trout. The total number of fish species observed in stream sections varied from one to eight (Table 4). The total number of fish species observed in lakes varied from one to five (Table 5).

Visual Implant Alpha Tags.— I tagged a total of 60 brown trout and 33 rainbow trout with Visual Implant Alpha tags in the stream network in 2010. Six tagged brown trout and two tagged rainbow trout were recaptured in 2011. The six recaptured brown trout were relocated in stream section seven, the same stream section these individuals trout were sampled from in 2010 (Figure 4). The two recaptured rainbow trout were located in stream section 22, which was also the same stream section that they were originally sampled and tagged in 2010.

Brown trout distribution.— Brown trout were found in the same nine stream sampling sections in both years (sections; 2, 3, 5, 6, 7, 8, 9, 11, and 14; Figure 4). Relative abundance of brown trout in 2010 varied from 0.063 to 1.59 catches per minute ($\bar{X} = 0.652$, $SE \pm 0.223$) with the highest relative abundance in stream section seven. In 2011 catch per unit effort varied from 0.124 to 1.397 ($\bar{X} = 0.534$, $SE \pm 0.167$) with the highest relative abundance in stream section eight. Brown trout were also present in Heather, Lily, and Grass lakes where catch per unit effort varied from 0.155 ± 0.036 to 0.339 ± 0.062 fish per hour. The highest relative abundance of brown trout was in Lily Lake (Figure 3).

Rainbow trout distribution.— Rainbow trout were present in the same nine stream sampling sections in both survey years (sections 2, 3, 5, 6, 7, 8, 9, 11, and 22; Figure 4). In 2010 catch per unit effort varied from 0.021 to 1.107 fish per minute ($\bar{X} =$

0.235, SE \pm 0.114). In 2011 catch per unit effort varied from 0.056 to 2.36 fish per minute (\bar{X} = 0.405, SE \pm 0.232). The highest relative abundance of rainbow trout in the stream network was in section 22 in both years (2010, 1.107 fish per minute; 2011, 2.36 fish per minute). Rainbow trout were found in Gilmore, Grass, and Lily lakes and the catch per unit effort varied from 0.009 ± 0.009 to 0.131 ± 0.042 fish per hour. The highest relative abundance of rainbow trout was in Lily Lake.

Molecular markers.— Possible null alleles were found using Micro-checker, but no systematic patterns of HWE deviation were observed in that no sampling location was out of Hardy Weinberg equilibrium (HWE) at all loci, and no locus was out of HWE in all sampling locations (Brown trout Table 6, $P = 0.00038$, adjusted for multiple comparisons and based on 2600 randomizations; Rainbow trout Table 7, $P = 0.00032$, adjusted for multiple comparisons and based on 3200 randomizations).

Population genetic structure.— I sampled a total of 279 adult rainbow trout and 350 adult brown trout in the upper Glen Alpine Creek drainage. Bayesian genotype clustering patterns for adult fish were similar across years and therefore years were combined for analysis.

Brown trout – A model with five genotype clusters was the best fit of the data for brown trout (Figure 5). Individuals found in Heather Lake and its outlet (sampling sections 14 and 15, hereafter “Heather”) were assigned almost exclusively to one group (Figure 6). Individuals from Grass Lake and its inlet and outlet, (sampling sections 9, 10, and 11; hereafter “Grass”) grouped together and were located above a barrier separating sampling sections 8 and 9. There were two main genotype clusters located in sampling sections 6, 7, and 8 (hereafter “middle populations”) that were heavily admixed and are therefore

grouped together. Sampling sections 1, 2, 3, 4 and 5 (hereafter “lower”) grouped together for the fifth genotype cluster. Average levels of expected (H_E) and observed (H_O) heterozygosity were not statistically different the Bayesian genotype clusters (Heather, Grass, middle or lower; H_E , $F = 0.5047$, $df = 3$, $P = 0.680$; H_O , $F = 0.0921$, $df = 3$, $P = 0.960$). Allelic richness also did not differ among populations ($F = 0.7562$, $df = 3$, $P = 0.53$; Table 8). There were two loci that had significant positive F_{IS} values; Strutta58 in the lower population and SsaD157 in the middle population (Table 8, $P = 0.00125$, adjusted for multiple comparisons and based on 800 randomizations). All pairwise comparisons among brown trout populations were statistically significant (Table 9; Heather-Grass, $F_{ST} = 0.1209$; Heather-middle, $F_{ST} = 0.1395$; Heather-lower $F_{ST} = 0.1331$; Grass-middle, $F_{ST} = 0.0563$; Grass-lower, $F_{ST} = 0.0645$; and middle-lower $F_{ST} = 0.0425$; $P_{adj} = 0.00833$).

Three gradient barriers were coincident with the observed brown trout population structure. These barriers were located below sampling sections 6, 9, and 14 (Figure 6) respectively. The pattern of genetic population structure supports individual brown trout movement over these barriers and admixture largely follows an upstream to downstream movement pattern. N_e for the Heather, Grass, middle, and lower brown trout genotype clusters were; 11.2 (95% CI 7.7-18.3), 64.6 (95% CI 47-94.9), 84.4 (95% CI 73.3-98.0), and 108 (95% CI 92.6-127.8) respectively. The Grass Lake brown trout population had evidence for a genetic bottleneck under the TPM model ($P = 0.00684$). There was also evidence for an isolation-by-distance pattern (Mantel test, $P = 0.0084$; Figure 8).

Rainbow trout – A model with three genotype clusters was the best fit of the data for rainbow trout (Figure 5). An “upper” genotype cluster was identified at the outlet of

Gilmore Lake (section 22), a “middle” genotype cluster comprising fish from sections 3, 4, 5, 6, 7, 8, 9, 10, and a “lower” genotype cluster composed of fish from sections 1 and 2 (Figure 7). Although the average levels of heterozygosity were lower in the upper population (section 22; $H_E = 0.638$, $H_O = 0.639$; Table 10), they were not statistically significantly different than the middle or lower rainbow trout populations (H_E $F = 2.866$, 1.877 , $df = 2$, $P = 0.070$; H_O $F = 1.887$, $df = 2$, $P = 0.17$; Table 10). Average allelic richness was statistically significantly lower in the upper population compared to the other two rainbow trout populations (R_S $F = 7.263$, $df = 2$, $P = 0.0022$; Table 10). There were four loci with significant F_{IS} values, OMM1036, OMM1220, OMM1286 in the middle population and OMM1286 in the lower population (Table 10, $P = 0.00128$, adjusted for multiple comparisons and based on 780 randomizations). The upper, middle, and lower rainbow trout populations were also genetically differentiated from each other (upper- middle, $F_{ST} = 0.1398$; upper-lower, $F_{ST} = 0.1745$; and middle-lower, $F_{ST} = 0.0456$; $P = 0.0167$; Table 11).

Two barriers were coincident with the observed population structure pattern, Modjeska falls between sections 5 and 6 and Glen Alpine Falls between sections 2 and 3 (Figure 7). There was evidence supporting a pattern of individual movement and admixing of rainbow trout in an upstream to downstream direction. N_e for the upper, middle, and lower rainbow trout populations were; 16.7 (95% CI 15.5-18.0), 55.2 (95% CI 51.4-59.4), and 59.2 (95% CI 53.8-65.4) respectively. There was no evidence for a genetic bottleneck in any of the populations. There was evidence for a statistically significant pattern of isolation-by-distance (Mantel test, $P = 0.0574$; Figure 9).

Redd Spatial Analysis.— I located 14 salmonid redds in the stream section between Glen Alpine Falls and Fallen Leaf Lake 2011. Egg and alevin samples were genetically identified to species (Smith J. unpublished data). All 14 redds were rainbow trout redds. The redd locations were patchily distributed and generally clustered together with large sections of the stream reach lacking redds (Figure 10).

DISCUSSION

The objectives of this study were to characterize the distribution of brown trout and rainbow trout in the Glen Alpine Creek watershed, assess the connectivity of the populations in the upper watershed to populations in Fallen Leaf Lake, and additionally to examine the potential spawning effort of rainbow trout below Glen Alpine Falls in order to test whether the presence of these non-native fish species in the upper watershed, poses a threat to the success of the Lahontan cutthroat trout re-introduction by the USFWS in Fallen Leaf Lake, California. Although this study was conducted at a finer spatial scale than prior USFS and CDFW surveys documenting fish species distributions in this watershed, my findings were consistent with the earlier surveys of the drainage. There were only two sampling locations in the stream network in which brown and rainbow trout did not occur in sympatry in this study. These two areas were the outlet of Heather Lake which contained brown trout and the outlet of Gilmore Lake which contained rainbow trout (Figure 3). I also documented the presence of young-of-the-year rainbow and brown trout in multiple locations within the upper watershed, which confirmed naturalized reproduction of these species as no recent stockings have occurred. The presence and successful reproduction of these two fish species in the upper

watershed is a potential long term problem for the Lahontan cutthroat trout reintroduction program as the upper watershed may provide an ongoing source of immigrants into Fallen Leaf Lake.

Bayesian clustering analysis revealed that a percentage of individuals of both species sampled below barriers can be assigned to populations located higher in the watershed, strongly suggesting that these individuals most likely came from source populations located above the barriers. Assignment tests thus support the connectivity of populations of brown trout and rainbow trout within the upper watershed with populations of these fish species in Fallen Leaf Lake. The data also show that immigrants from the upper watershed are reproducing as I sampled admixed individuals with proportional membership in both the lower and upper watershed genotype clusters. Although the inference of the genetic data supports movement of fish, it is not known if downstream movement of these fish from the upper watershed is age specific. There may be a combination of age classes of fish that are moving downstream, or it may be that primarily juvenile fish are washed downstream over barriers in high flow conditions. This dynamic has management implications and deserves further study. For example, management activities such as the mechanical removal of fish often require age class specific techniques (Reynolds 1983, McInerny and Cross 2006) and it is difficult to ensure that all juvenile fish have been removed from an area without extensive monitoring (Thompson and Rahel 1996). I did not find direct evidence of downstream movement of adult fish using mark-recapture techniques, although this part of the study was conducted for only one year, and I recaptured a very low percentage of the tagged individuals for each species. Nonetheless, the recapture information suggests relatively

limited movement which is consistent with the overall patterns of distinct genetic subunits observed.

I found support for genetic population structure for both rainbow and brown trout populations in the Glen Alpine Creek watershed. All pairwise comparisons among populations identified by Bayesian genotype clustering analyses for both species were genetically differentiated and were also associated with geographic barriers. Additionally, there was some support for an isolation-by-distance pattern in both species using a Mantel test. Therefore, the genetic population structure of brown and rainbow trout in the Glen Alpine Creek drainage appears to be shaped by a combination of isolation-by-distance and geographic barriers.

The population of rainbow trout located at the outlet of Gilmore Lake contained the highest relative abundance of rainbow trout in the stream network and also appeared to be the most isolated rainbow trout population as evidenced by the high and statistically significant pairwise F_{ST} estimates with the other two genotype clusters (see Table 11). Average allelic richness was also significantly lower in this population than in the other two rainbow trout populations.

In order for a genetic inference of effective population size to be accurate the samples analyzed need to be representative of the population they are collected from (Bartley 1992, Waples 1990). The effective population size estimates from NeEstimator for the brown trout genotype clusters were very close to the actual sample sizes (Table 8). This may indicate that the samples were not large enough or that the number of loci used in the analysis was insufficient to accurately estimate effective population sizes using samples from a single sampling period (Bartley et al. 1992). The effective population size

estimates for rainbow trout appear to be less problematic. The use of 13 microsatellite loci and the number of individuals sampled in the upper and middle populations (in excess of 90 individuals) indicate that effective population sizes are likely to be accurate (Bartley et al. 1992). However, the relatively small effective population sizes and significant pairwise F_{ST} estimates among the genotype clusters for brown and rainbow trout suggest limited gene flow and small and fairly isolated populations.

Hybridization between rainbow trout and LCT in this system, if not managed, could lead to a hybrid swarm and genetic assimilation leading to the genetic extinction of any Lahontan cutthroat trout population established in Fallen Leaf Lake. The patchy spatial distribution of redds in the stream reach below Glen Alpine Falls suggests that the available spawning habitat may be very limited. This result is not overly surprising considering that the substrate is largely composed of cobble and bedrock which is considered to be suboptimal spawning substrate (Bjornn and Reiser 1991, Knapp and Vredenburg 1996). However, because lower Glen Alpine Creek is currently the only available spawning habitat, hybridization between rainbow trout and Lahontan cutthroat trout is likely to occur.

Rainbow trout and brown trout are not the only non-native species threats to the successful reintroduction of Lahontan cutthroat trout in Fallen Leaf Lake, but mitigation of the threats posed by these species will be necessary. Additional recovery threats in this system include introduced brook trout, mysid shrimp, and lake trout. Although brook trout have been shown to be an impediment to cutthroat trout recovery and are naturalized in this system's upper watershed, they are not established in Fallen Leaf Lake, and their reproduction has not been documented in the reach below Glen Alpine

Falls. Introduced mysid shrimp may have altered the food web in Fallen Leaf Lake by reducing the larger native cladoceran zooplankton species which are prey items for juvenile cutthroat trout. Lake trout predation represents a significant threat to establishment of a Lahontan cutthroat trout population in Fallen Leaf Lake and removal strategies are currently being explored.

The challenges presented by introductions of non-native fish species are not unique to native trout recovery, or limited to this geographic location. As a result many management agencies have implemented non-native fish removal as part of their species management and recovery plans. Widespread introduction of fish into historically fishless high alpine lakes throughout the Sierra Nevada range have reduced native frog distribution and abundance (Knapp 2005). It has been shown that mechanical removal of introduced fishes in these lakes can in some cases, lead to re-colonization by nearby source populations of frogs (Knapp et al. 2007). In the Colorado River system non-native fish species are implicated in the decline of the humpback chub *Gila cypha* a listed native cyprinid species. Recovery approaches for humpback chub by federal and state agencies include mechanical removal of sympatric non-native fish species and translocations of humpback chub (Coggins et al. 2011, Yard et al. 2011). Non-native salmonid introductions have especially impacted native trout recovery and solutions including barrier construction and non-native salmonid removal have been undertaken. Some examples of these include Yellowstone cutthroat trout *O. c. bouvieri* in Yellowstone Lake, Apache trout *O. apache* in Arizona, and brook trout recovery in the Appalachian Mountains. In Yellowstone Lake illegally introduced lake trout are a major threat to continued persistence of native Yellowstone cutthroat trout in the lake and large scale

lake trout removal activities are ongoing (Syslo et al. 2011). Recovery of Apache trout in Arizona is known to be impeded by the presence of introduced brown and rainbow trout (Rinne and Minckley 1985, Avenetti et al. 2006), and management including non-native removals, barrier construction and stream dewatering has been used to create isolation of these species. While brook trout are known to hinder recovery of inland cutthroat trout subspecies, they themselves are threatened in the southern and central Appalachian Mountains due in large part to non-native salmonid introductions (Larson and Moore 1985, Fausch 2008), and chemical and mechanical removals of these introduced salmonids are part of many management plans.

MANAGEMENT IMPLICATIONS

Non-native salmonids are impeding the successful reintroduction and establishment of Lahontan cutthroat trout into the historically occupied lacustrine habitat of Fallen Leaf Lake (Al-Chokhachy et al. 2009, Meeuwig and Peacock 2012). A watershed level management approach needs to be undertaken if a Lahontan cutthroat trout population is to be successfully re-established in this lake habitat. The upper watershed should be incorporated into any management plan in order to mitigate the threats posed by movement of brown trout and rainbow trout from the upper drainage into the lake. Although this study establishes that there is movement from the upper watershed it also shows that there is population structure in both brown trout and rainbow trout in the upper drainage. This structure has been shown to be coincident with geographic barriers. Because of this it may be possible to design effective management solutions that take advantage of natural barriers. From a genetic perspective rainbow trout

present the most immediate threat to Lahontan cutthroat trout in this system because of the possibility of hybridization and, therefore, should be the focal species for management action in the upper watershed. Mechanical removal of rainbow trout may be successful in this system given that there are a large number of natural barriers to upstream movement and that there is evidence of separation of the genetic subpopulations. A top down approach in the watershed should be evaluated as the most isolated rainbow trout population is the highest elevation population and contains the highest relative abundance of rainbow trout in the stream network. Rainbow trout removal methods that take advantage of natural barriers can be assessed using genetic monitoring.

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Table Legends

- Table 1. Lake, sampling period, number of nets (N), net soak time in hours (mean \pm standard deviation), and gill net depth at the offshore ends of the gill nets (mean \pm standard deviation) for lakes sampled in the Glen Alpine Creek Watershed. Lakes sorted by elevation highest to lowest.
- Table 2. Primer information for 13 microsatellite loci used to determine the genetic connectivity of rainbow trout in the Glen Alpine Creek Watershed. Dye and multiplex, asterisk (*) denotes fluorescently labeled, Forward (F) and reverse (R) primer sequences, product size range in base pairs (bp), number of alleles, and GenBank Accession number.
- Table 3. Primer information for microsatellite loci used to determine the genetic connectivity of brown trout in the Glen Alpine Creek Watershed. Dye, asterisk (*) denotes fluorescently labeled, Forward (F) and reverse (R) primer sequences, product size range in base pairs (bp), number of alleles, and GenBank Accession number.
- Table 4. Sample size (N) and percent of sample made up of 9 species encountered among 18 electrofishing sample sections in the Glen Alpine Creek Watershed. Species codes are:(BN brown trout; RB rainbow trout; BK brook trout; SS sucker species; LR Lahontan redbside; SP speckled dace; LC Lahontan cutthroat trout; MWF mountain whitefish; PS Paiute sculpin).
- Table 5. Sample size (N) and percent of sample made up of 8 species encountered among 9 lakes sampled using gill nets in the Glen Alpine Creek Watershed. Lakes arranged by elevation highest to lowest. Species codes are:(BN brown trout; RB rainbow trout; BK brook trout; LK lake trout; TC Tui chub; SS sucker species; LR Lahontan redbside; SP speckled dace).
- Table 6. Brown trout F_{IS} values per locus per sampling site. Grouped by site and Bayesian cluster population (Heather, Grass, Middle, and Lower). Note the Middle population area contains two distinct breeding groups with heavy admixture. No F_{IS} values were significant at $P = 0.00038$ (adjusted for multiple comparisons after 2600 randomizations).
- Table 7. Rainbow trout F_{IS} values per locus per sampling site. Grouped by site and Bayesian cluster population (Upper, Middle, Lower). (NA) not available. Values in bold were significant at $P = 0.00032$ (adjusted for multiple comparisons after 3120 randomizations).

Table 8. Genetic diversity of brown trout in the Glen Alpine Creek watershed. Sample size (N), number of alleles (Na), observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness rarified to a sample size of 11 (R_S), inbreeding coefficient (F_{IS}) per population based on 800 randomizations indicative adjusted nominal level (5%) is 0.00125. Average H_O , H_E and R_S for Heather (0.654, 0.636, 4.000), Grass (0.682, 0.707, 4.861), Middle (0.655, 0.692, 4.762) and Lower (0.653, 0.680, 5.481).

Table 9. Pairwise F_{ST} values between brown trout genetic populations (Heather, Grass, Middle, and Lower) in the Glen Alpine Creek watershed. Pairwise values obtained after 120 permutations corrected $*P = 0.00833$.

Table 10. Genetic diversity of rainbow trout in the Glen Alpine Creek watershed. Sample size (N), number of alleles (Na), observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness rarified to a sample size of 64 (R_S), inbreeding coefficient (F_{IS}) per population based on 780 randomizations indicative adjusted nominal level (5%) is 0.00128.

Table 11. Pairwise F_{ST} values between rainbow trout genetic populations (Upper, Middle, and Lower) in the Glen Alpine Creek watershed, pairwise values obtained after 60 permutations corrected $*P = 0.0167$. Average H_O , H_E and R_S for Upper (0.639, 0.638, 6.325), Middle (0.739, 0.773, 10.500), and Lower (0.761, 0.773, 11.714).

Table 1.

| Lake | Sampling period | N | Soak time (h) | Depth (m) offshore end |
|-------------|-------------------------|---|---------------|---------------------------|
| Gilmore | July 29–August 12, 2011 | 9 | 12.8 ± 0.6 | 12.6 ± 8.0 |
| Alta Morris | September 9–11, 2011 | 6 | 12.2 ± 0.1 | 4.3 ± 0.5 |
| Half Moon | July 31–August 3, 2011 | 9 | 13.2 ± 0.7 | 3.2 ± 1.0 |
| Lost | August 14–16, 2011 | 6 | 12.4 ± 0.3 | 4.0 ± 1.8 |
| Triangle | August 14–16, 2011 | 6 | 13.0 ± 0.2 | 3.4 ± 1.7 |
| Heather | July 27–30, 2011 | 9 | 18.6 ± 4.0 | 9.0 ± 4.3 |
| Susie | July 27–August 11, 2011 | 6 | 12.2 ± 0.8 | 6.7 ± 3.9 |
| Grass | August 11–14, 2011 | 9 | 12.7 ± 0.7 | 4.0 ± 2.7 |
| Lily | August 7–17, 2011 | 9 | 12.2 ± 0.9 | 3.9 ± 2.3 |

Table 2.

| Locus | Dye / Multiplex | Repeat | Primer Sequence | Product Size (bp) | Allele # | GenBank Accession |
|---------|-----------------|-------------------------|--|-------------------|----------|-------------------|
| OCH10 | VIC / A | [ATC] [ATT] [ACT] | F:GGAGGTGATTCTATGGGTAAAT R:CAGATGGGCACTTAGATTGTT | 184-205 | 6 | AY374428 |
| OCH17 | *PET / A | [GACA] | F:TGCCGTCTCTGAACTTGTCAC R:GCTCAGGGGCAGAACGACAAATA | 200-288 | 20 | AY374434 |
| OCH20 | FAM / A | [TAGA] | F:CCCACGACAGAGCCCTACTAGAT R:CCTGGCCGTGCTTATGATAACTC | 226-318 | 20 | DQ979815 |
| OMM1315 | FAM / A | [CATC] | F: TACAGGGCTTGGCTCTATCTC R: GCCAAATACTTTCGCAAGG | 126-194 | 11 | G73554 |
| OMM1323 | FAM / A | [CATC] | F: TTTTGGCCAGCTCTGCTATGACA R:CATTACAGCACAACCTACGAAACCC | 202-246 | 6 | G73561 |
| OMM1325 | NED / A | [GATG] | F: TCTCTGCCAATGTGACATGCCT R: TAACTATCACTGCCACTCCTCGTG | 276-324 | 7 | G73562 |
| OCH9 | *PET / B | [TAA] | F:TTATGCAAGAAATGGCGTTGG R:TGGGGGTAGGCACGTCC | 177-231 | 15 | AY374427 |
| OMM1036 | VIC / B | [TATC] | F: TGTAGCAGGTGAGAATACCCA R: CACCATCTCCATCCTAGGC | 230-306 | 16 | AF346686 |
| OMM1220 | NED / B | [TCTA] | F: CTCTGGGACAGACTTATCAC R: CTATTGGACGATGCACAC | 146-222 | 16 | AF470002 |
| OMM1329 | VIC / B | [CATC] | F: GGGAAGTGTTACCATTACACAAG R: CATCCAGGAACGCACCTTTA | 175-211 | 9 | G73564 |
| OMM1302 | FAM / C | [CATC] [CT] | F: AGCCAGCCAATTAATACCCTG R: TTCTGTGTGGCCTAAACCTT | 217-297 | 16 | G73542 |
| OMM1037 | PET / C | [GAAA] | F: GCGACTGGATTTAATACTGC R: TCCTCTGACTGCCATTACATC | 164-248 | 19 | AF346687 |
| OMM1286 | FAM / D | [CCAT] | F: TCCCGAAGCCTCTGCATACTACGA R: ACGCGTCAGGCTAAGTGGCTATGT | 123-223 | 11 | AF470047 |

Table 3.

| Locus | Dye / Multiplex | Repeat | Primer Sequence | Product Size (bp) | Allele # | GenBank Accession |
|-----------|-----------------|--------|---|-------------------|----------|-------------------|
| Ssa85 | NED / A | [GT] | F: GGTGGGTCCTCCAAGCTAC R: ACCCGCTCCTCACTTAATC | 122-146 | 9 | U43692 |
| SsaD157 | VIC / A | [TAGA] | F: TCGAAATGGAACCTTTTGAATG R: TTAGGGCTGAGAGAGGAATAC | 274-350 | 26 | AF525204 |
| SsaD190 | FAM / A | [TAGA] | F: GGCATTGGAGGTAAGGACAC R: CCAGACCACTGAACTTCTCATC | 143-195 | 11 | AF525206 |
| Str60 | VIC / A | [CT] | F: CGGTGTGCTTGTCAGGTTTC R: GTCAAGTCAGCAAGCCTCAC | 121-137 | 7 | AB001057 |
| Ssa197 | FAM / B | [GTGA] | F: GGTTGAGTAGGGAGGCTTG R: TGGCAGGGATTTGACATAAC | 156-168 | 4 | U43694 |
| Str15 | VIC / B | [GT] | F: TGCAGGCAGACGGATCAGGC R: ATCCTCTACGTAAGGGATTTGC | 245-255 | 6 | AB001058 |
| Str73 | NED / B | [GT] | F: CCTGGAGATCCTCCAGCAGGA R: CTATTCTGCTTGTAAGTAGACCTA | 165-171 | 4 | AB001056 |
| BRUN13 | *FAM / C | [GT] | F: GAAACTAGGATGCCTGG R: TCTGACCCACACACAAGC | 185-248 | 17 | AF256667 |
| BRUN14 | *NED / C | [GT] | F: TCCTCTATCATACGGCTG R: ATCAGATGACCCAGTGCC | 119-127 | 5 | AF256661 |
| Strutta58 | *FAM / C | [GT] | F: AACAATGACTTTCTCTGAC R: AAGGACTTGAAGGACGAC | 227-306 | 20 | U60223 |

Table 5.

| Lake | N | BN | RB | BK | LK | TC | SS | LR | SP |
|-------------|------|-----|-----|------|-----|------|------|------|------|
| Gilmore | 1159 | 0 | 0.1 | 3.3 | 5.5 | 61.6 | 29.5 | 0 | 0 |
| Alta Morris | 101 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 |
| Half Moon | 369 | 0 | 0 | 94.9 | 0 | 0 | 0 | 0 | 5.1 |
| Lost | 95 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 |
| Triangle | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Heather | 958 | 2.2 | 0 | 18.6 | 0 | 0 | 0 | 65.4 | 13.8 |
| Susie | 344 | 0 | 0 | 42.2 | 0 | 0 | 0 | 46.8 | 11 |
| Grass | 926 | 1.9 | 0.2 | 9.4 | 0 | 0 | 0 | 78.2 | 10.3 |
| Lily | 670 | 5.5 | 2.1 | 0 | 0 | 0 | 43.9 | 38.9 | 9.6 |

Table 6.

| | Heather | | Grass | | | Middle | | | Lower | | | | |
|------|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | 15 | 14 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Ssa | | | | | | | | | | | | | |
| 190 | -0.067 | -0.333 | 0.25 | -0.25 | 0.137 | 0.133 | 0.11 | -0.058 | -0.04 | -0.031 | 0.052 | -0.176 | -0.057 |
| Ssa | | | | | | | | | | | | | |
| 85 | -0.053 | 0 | -0.286 | -0.081 | 0.17 | 0.17 | 0.13 | 0.247 | 0.232 | -0.105 | 0.061 | -0.29 | 0.212 |
| Str | | | | | | | | | | | | | |
| 60 | -0.231 | -1 | -0.2 | 0.333 | -0.07 | 0.251 | 0.11 | -0.055 | -0.18 | 0.06 | -0.299 | 0.063 | 0.129 |
| Ssa | | | | | | | | | | | | | |
| 197 | 0.149 | 0 | 0.143 | 0.394 | 0.345 | 0.169 | -0.047 | 0.121 | 0.464 | -0.109 | -0.214 | -0.111 | 0.022 |
| Str | | | | | | | | | | | | | |
| 15 | 0.245 | 0 | 0 | -0.111 | -0.248 | 0.1 | -0.142 | 0.405 | 0.038 | -0.057 | -0.084 | -0.22 | -0.055 |
| Str | | | | | | | | | | | | | |
| 73 | -0.111 | 0 | -0.5 | -0.19 | 0.036 | -0.057 | -0.042 | 0.083 | -0.18 | 0.086 | 0.08 | 0.063 | -0.093 |
| Ssa | | | | | | | | | | | | | |
| 157 | 0.009 | 0 | 0.478 | -0.154 | 0.062 | 0.167 | 0.043 | 0.146 | 0.147 | 0.146 | 0.026 | 0.231 | 0.144 |
| Stru | | | | | | | | | | | | | |
| 58 | 0.018 | 0 | 0.1 | -0.333 | 0.1 | 0.08 | 0.101 | 0.04 | 0.014 | 0.123 | -0.003 | 0.286 | 0.172 |
| Brun | | | | | | | | | | | | | |
| 14 | -0.011 | 0 | 0.294 | -0.087 | -0.083 | -0.077 | 0.005 | -0.319 | -0.056 | 0.015 | -0.126 | -0.081 | 0.095 |
| Brun | | | | | | | | | | | | | |
| 13 | -0.153 | -0.333 | -0.2 | -0.111 | -0.093 | 0.017 | -0.024 | -0.148 | 0.017 | -0.152 | -0.162 | 0.5 | -0.078 |

Table 7.

| | Upper | Middle | | | | | | | | Lower | |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------------|--------------|
| | 22 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| OCH9 | 0.032 | 0 | 0.118 | -0.079 | 0.158 | 0.333 | 0.082 | 0.029 | 0.009 | 0.071 | 0.021 |
| OCH10 | -0.011 | NA | -0.185 | -0.045 | -0.103 | 0 | -0.202 | 0 | 0 | 0.213 | 0.128 |
| OCH17 | -0.12 | -0.333 | 0.029 | -0.043 | -0.241 | 0.111 | 0.116 | -0.184 | -0.017 | -0.134 | 0.017 |
| OCH20 | -0.102 | 0 | 0.11 | -0.149 | 0.309 | -0.053 | -0.001 | -0.004 | -0.17 | -0.014 | -0.056 |
| OMM1315 | 0.082 | 0 | 0.254 | -0.025 | -0.108 | -0.176 | -0.079 | 0.015 | -0.201 | 0.004 | -0.067 |
| OMM1323 | 0.033 | -0.333 | -0.037 | -0.163 | -0.289 | -0.429 | -0.08 | -0.006 | -0.12 | -0.182 | 0.184 |
| OMM1325 | -0.065 | NA | -0.005 | -0.224 | -0.2 | 0.273 | -0.131 | -0.111 | -0.125 | -0.191 | -0.09 |
| OMM1036 | -0.034 | NA | 0.135 | -0.102 | 0.257 | 0.111 | -0.016 | -0.248 | 0.169 | -0.142 | -0.053 |
| OMM1220 | 0.065 | 0 | 0.054 | -0.063 | 0.052 | 0.273 | 0.09 | 0.227 | 0.172 | 0.06 | -0.044 |
| OMM1329 | -0.062 | -0.333 | -0.16 | -0.014 | -0.128 | -0.176 | -0.079 | 0.053 | -0.071 | -0.193 | -0.004 |
| OMM1037 | 0.023 | 0 | 0.117 | -0.107 | -0.095 | -0.2 | -0.053 | 0.043 | 0.191 | -0.074 | 0.041 |
| OMM1286 | 0.051 | 0 | 0.467 | 0.331 | -0.119 | 0 | 0.203 | 0.333 | 0.196 | 0.439 | 0.263 |
| OMM1302 | 0.068 | 0 | -0.141 | 0.069 | -0.134 | -0.6 | -0.03 | -0.192 | 0.243 | -0.1 | 0.065 |

Table 8.

| Locus | Heather | Grass | Middle | Lower | Locus | Heather | Grass | Middle | Lower | | |
|--------|-----------------------|--------|--------|--------|--------|-----------|-----------------------|--------|--------|---------------|---------------|
| | N | 11 | 49 | 164 | 126 | | N | 11 | 49 | 164 | 126 |
| SSa190 | Na | 5 | 7 | 6 | 11 | Str73 | Na | 3 | 4 | 4 | 4 |
| | H _O | 0.818 | 0.714 | 0.726 | 0.786 | | H _O | 0.545 | 0.694 | 0.669 | 0.603 |
| | H _E | 0.736 | 0.811 | 0.81 | 0.778 | | H _E | 0.481 | 0.682 | 0.658 | 0.587 |
| | R _S | 5 | 5.573 | 5.545 | 7.286 | | R _S | 3 | 3.831 | 3.292 | 2.964 |
| | <i>F_{IS}</i> | -0.118 | 0.121 | 0.104 | -0.01 | | <i>F_{IS}</i> | -0.143 | -0.017 | -0.029 | -0.028 |
| SSa85 | Na | 3 | 3 | 3 | 9 | SSaD157 | Na | 5 | 9 | 16 | 18 |
| | H _O | 0.545 | 0.571 | 0.555 | 0.532 | | H _O | 0.727 | 0.714 | 0.793 | 0.762 |
| | H _E | 0.506 | 0.633 | 0.665 | 0.609 | | H _E | 0.758 | 0.771 | 0.881 | 0.881 |
| | R _S | 3 | 2.998 | 2.999 | 3.819 | | R _S | 5 | 6.112 | 8.95 | 9.374 |
| | <i>F_{IS}</i> | -0.081 | 0.098 | 0.166 | 0.127 | | <i>F_{IS}</i> | 0.042 | 0.074 | 0.100* | 0.136 |
| Str60 | Na | 2 | 2 | 6 | 5 | Strutta58 | Na | 5 | 11 | 12 | 20 |
| | H _O | 0.545 | 0.49 | 0.409 | 0.516 | | H _O | 0.727 | 0.796 | 0.762 | 0.802 |
| | H _E | 0.416 | 0.488 | 0.473 | 0.527 | | H _E | 0.758 | 0.871 | 0.845 | 0.906 |
| | R _S | 2 | 2 | 2.452 | 2.636 | | R _S | 5 | 8.227 | 7.331 | 10.505 |
| | <i>F_{IS}</i> | -0.333 | -0.003 | 0.137 | 0.021 | | <i>F_{IS}</i> | 0.042 | 0.087 | 0.098 | 0.116* |
| SSa197 | Na | 3 | 3 | 4 | 4 | Brun14 | Na | 3 | 5 | 5 | 5 |
| | H _O | 0.545 | 0.388 | 0.445 | 0.413 | | H _O | 0.636 | 0.653 | 0.707 | 0.643 |
| | H _E | 0.636 | 0.608 | 0.471 | 0.443 | | H _E | 0.628 | 0.62 | 0.682 | 0.649 |
| | R _S | 3 | 2.979 | 2.973 | 2.896 | | R _S | 3 | 4.114 | 3.667 | 4.196 |
| | <i>F_{IS}</i> | 0.149 | 0.365 | 0.055 | 0.068 | | <i>F_{IS}</i> | -0.014 | -0.054 | -0.037 | 0.01 |
| Str15 | Na | 5 | 5 | 5 | 5 | Brun13 | Na | 6 | 13 | 13 | 14 |
| | H _O | 0.545 | 0.857 | 0.738 | 0.706 | | H _O | 0.909 | 0.939 | 0.744 | 0.77 |
| | H _E | 0.68 | 0.712 | 0.703 | 0.683 | | H _E | 0.762 | 0.872 | 0.728 | 0.734 |
| | R _S | 5 | 4.162 | 4.021 | 4.435 | | R _S | 6 | 8.614 | 6.391 | 6.694 |
| | <i>F_{IS}</i> | 0.205 | -0.206 | -0.023 | -0.034 | | <i>F_{IS}</i> | -0.205 | -0.078 | -0.022 | -0.049 |

Table 9.

| | Heather | Grass | Middle |
|--------|---------|---------|---------|
| Grass | 0.1209* | | |
| Middle | 0.1395* | 0.0563* | |
| Lower | 0.1331* | 0.0645* | 0.0425* |

Table 10.

| Locus | | Upper | Middle | Lower | Locus | | Upper | Middle | Lower |
|-------|----------------|--------|--------|--------|---------|----------------|--------|--------|--------|
| OCH9 | N | 92 | 113 | 67 | OMM1323 | N | 94 | 113 | 72 |
| | Na | 7 | 13 | 14 | | Na | 4 | 5 | 6 |
| | H _O | 0.576 | 0.779 | 0.851 | | H _O | 0.553 | 0.77 | 0.694 |
| | H _E | 0.59 | 0.864 | 0.881 | | H _E | 0.575 | 0.696 | 0.753 |
| | Rs | 6.3 | 11.91 | 13.91 | | Rs | 4 | 5 | 5.889 |
| | F_{IS} | 0.024 | 0.099 | 0.035 | | F_{IS} | 0.038 | -0.107 | 0.078 |
| OCH10 | N | 94 | 113 | 69 | OMM1325 | N | 94 | 111 | 71 |
| | Na | 3 | 5 | 5 | | Na | 4 | 6 | 6 |
| | H _O | 0.5 | 0.31 | 0.203 | | H _O | 0.479 | 0.658 | 0.69 |
| | H _E | 0.498 | 0.286 | 0.241 | | H _E | 0.451 | 0.612 | 0.614 |
| | Rs | 2.999 | 4.13 | 4.995 | | Rs | 3.681 | 5.969 | 5.901 |
| | F_{IS} | -0.005 | -0.085 | 0.158 | | F_{IS} | -0.061 | -0.075 | -0.125 |
| OCH17 | N | 94 | 113 | 69 | OMM1036 | N | 90 | 112 | 71 |
| | Na | 8 | 15 | 17 | | Na | 10 | 15 | 14 |
| | H _O | 0.809 | 0.858 | 0.899 | | H _O | 0.8 | 0.723 | 0.775 |
| | H _E | 0.724 | 0.885 | 0.877 | | H _E | 0.779 | 0.822 | 0.716 |
| | Rs | 7.649 | 13.999 | 16.763 | | Rs | 9.671 | 14.506 | 13.693 |
| | F_{IS} | -0.118 | 0.03 | -0.025 | | F_{IS} | 0.023 | 0.04 | 0.006 |
| OCH20 | N | 94 | 113 | 72 | OMM1220 | N | 93 | 106 | 64 |
| | Na | 8 | 18 | 16 | | Na | 10 | 16 | 15 |
| | H _O | 0.819 | 0.85 | 0.889 | | H _O | 0.742 | 0.745 | 0.906 |
| | H _E | 0.741 | 0.906 | 0.86 | | H _E | 0.791 | 0.868 | 0.898 |
| | Rs | 7.549 | 16.531 | 15.729 | | Rs | 9.563 | 15.38 | 15 |
| | F_{IS} | -0.106 | 0.063 | -0.033 | | F_{IS} | 0.062 | 0.142 | -0.009 |

Table 10 continued.

| Locus | | Upper | Middle | Lower | Locus | | Upper | Middle | Lower |
|---------|-----------------------|-------|--------|--------|---------|-----------------------|--------|--------|--------|
| OMM1315 | N | 94 | 113 | 72 | OMM1329 | N | 94 | 113 | 71 |
| | Na | 6 | 7 | 11 | | Na | 5 | 7 | 9 |
| | H _O | 0.681 | 0.814 | 0.833 | | H _O | 0.649 | 0.858 | 0.845 |
| | H _E | 0.75 | 0.812 | 0.808 | | H _E | 0.615 | 0.837 | 0.804 |
| | Rs | 5.868 | 7 | 10.876 | | Rs | 4.68 | 7 | 8.998 |
| | <i>F_{IS}</i> | 0.092 | 0.003 | -0.032 | | <i>F_{IS}</i> | -0.055 | -0.026 | -0.051 |
| OMM1037 | N | 89 | 109 | 71 | OMM1302 | N | 94 | 110 | 67 |
| | Na | 10 | 14 | 16 | | Na | 7 | 13 | 16 |
| | H _O | 0.775 | 0.862 | 0.887 | | H _O | 0.309 | 0.809 | 0.881 |
| | H _E | 0.793 | 0.898 | 0.893 | | H _E | 0.331 | 0.816 | 0.902 |
| | Rs | 9.641 | 13.966 | 15.794 | | Rs | 6.618 | 12.189 | 15.819 |
| | <i>F_{IS}</i> | 0.023 | 0.04 | 0.006 | | <i>F_{IS}</i> | 0.069 | 0.008 | 0.024 |
| OMM1286 | N | 92 | 106 | 70 | | | | | |
| | Na | 4 | 9 | 9 | | | | | |
| | H _O | 0.62 | 0.575 | 0.543 | | | | | |
| | H _E | 0.657 | 0.748 | 0.796 | | | | | |
| | Rs | 4 | 8.911 | 8.914 | | | | | |
| | <i>F_{IS}</i> | 0.057 | 0.232 | 0.319 | | | | | |

Table 11.

| | Upper | Middle |
|--------|---------|---------|
| Middle | 0.1398* | |
| Lower | 0.1745* | 0.0456* |

Figure Legends

- Figure 1. The hydrographic Lahontan basin depicting the historical river and lake system distribution (shaded area) of Lahontan cutthroat trout *Oncorhynchus clarkii henshawi*.
- Figure 2. Map of the study area location showing the Glen Alpine Creek Watershed in grey. The inset map shows the geographical location of the study area in relation to California and Nevada.
- Figure 3. Study area lakes and major gradient barriers shown as black dots. These are barriers to upstream salmonid movement in all stream flow conditions.
- Figure 4. Sampling locations in the Glen Alpine Creek watershed. The 28 sampling locations are numbered. The named lakes; Le Conte, Jabu, Margery, and Lucille were not sampled in this study and are thought to be fishless.
- Figure 5. (A) The mean LnP(D) and SE of 10 runs conducted for each k for the multilocus genotype data generated for brown trout sampling sites in the Glen Alpine Creek watershed. (B) Delta k (Δk) plotted against number of genotype clusters for brown trout best fit of the data $k = 5$. (C) The mean LnP(D) and SE of 10 runs conducted for each k for the multilocus genotype data generated for rainbow trout sampling sites in the Glen Alpine Creek watershed. (D) Δk plotted against number of genotype clusters for rainbow trout best fit of the data $k = 3$.
- Figure 6. Study area map and Bayesian genotype clustering output showing the best fit of the data ($k = 5$) for brown trout coincident with barriers within the Glen Alpine Creek watershed. The structure output (bottom panel) has sampling location numbers that correspond to the numbers on the map along the bottom and the map colors correspond to breeding groups or genotype clusters depicted in the STRUCTURE graphic. Note the middle population is heavily admixed.
- Figure 7. Map and Bayesian genotype clustering output from program STRUCTURE showing the best fit of the data ($k=3$) for rainbow trout populations and the coincident barriers within the Glen Alpine Creek watershed. The structure output has sampling location numbers that correspond to the numbers on the map along the bottom and the map colors correspond to breeding groups or genotype clusters depicted in the STRUCTURE graphic.
- Figure 8. Isolation by distance analysis results for brown trout in the Glen Alpine Creek watershed using ISOLDE in Genepop showing a significant pattern ($P = 0.0084$, $R^2 = 0.331$, $Y = 2E-05X + 0.0298$).

Figure 9. Isolation by distance analysis results for rainbow trout in the Glen Alpine Creek watershed using ISOLDE in Genepop showing a significant pattern ($P = 0.0574$, $R^2 = 0.356$, $Y = 3E-05X + 0.356$).

Figure 10. Rainbow trout redd distribution in available spawning habitat within the stream reach between Fallen Leaf Lake and Glen Alpine Falls. The redd surveys were conducted in June through July 2011 and the black dots (N=14) represent redd locations.

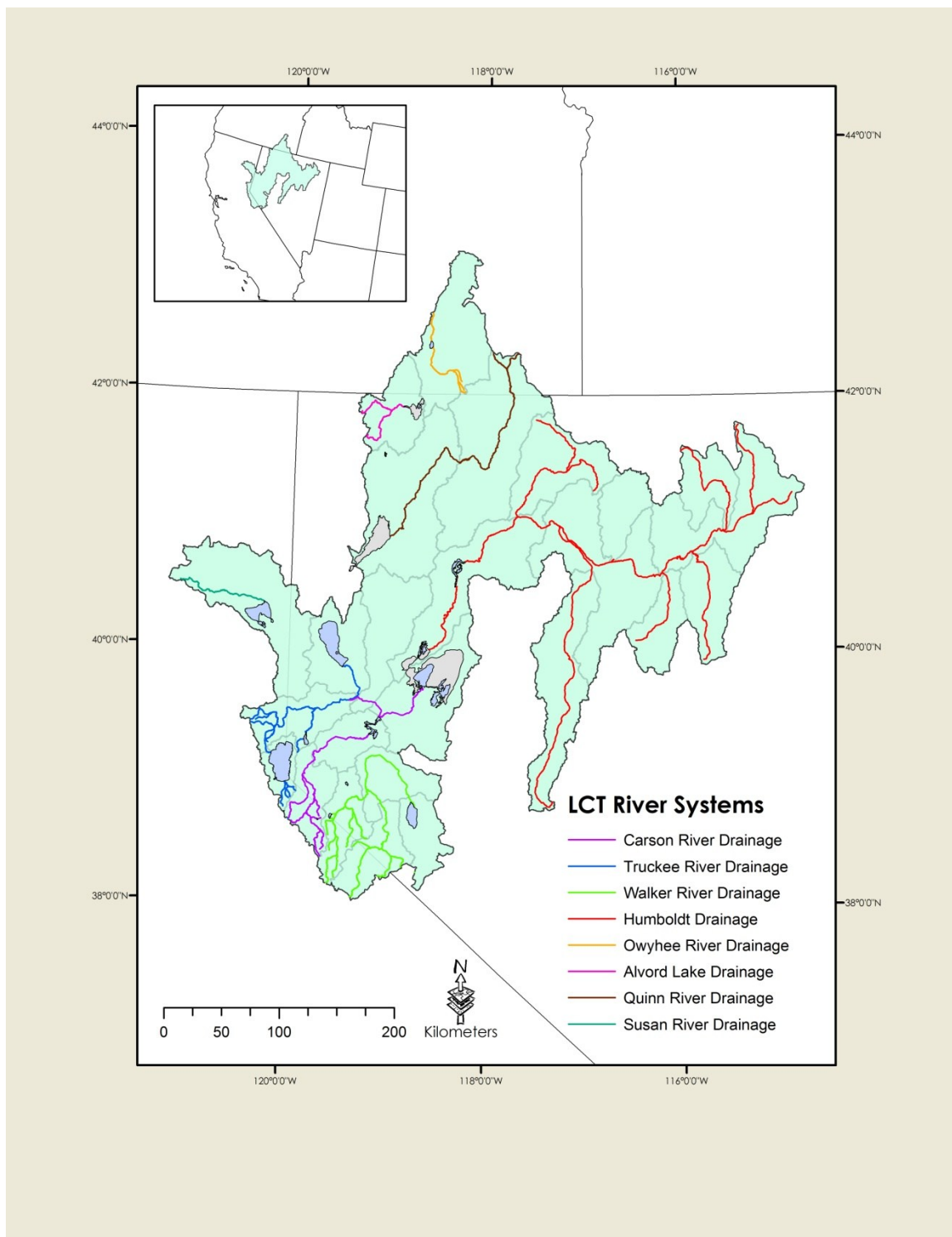


Figure 1.

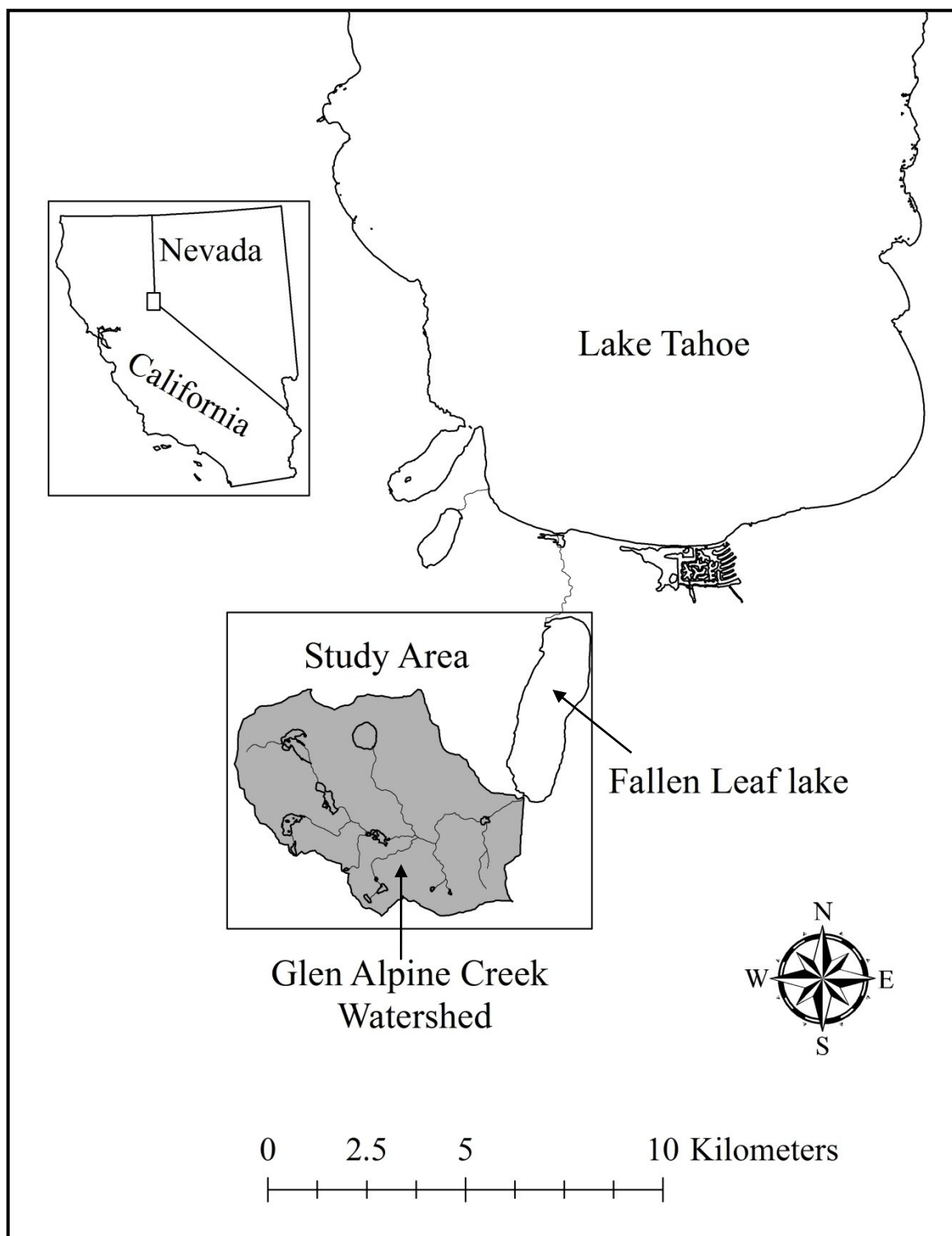


Figure 2.

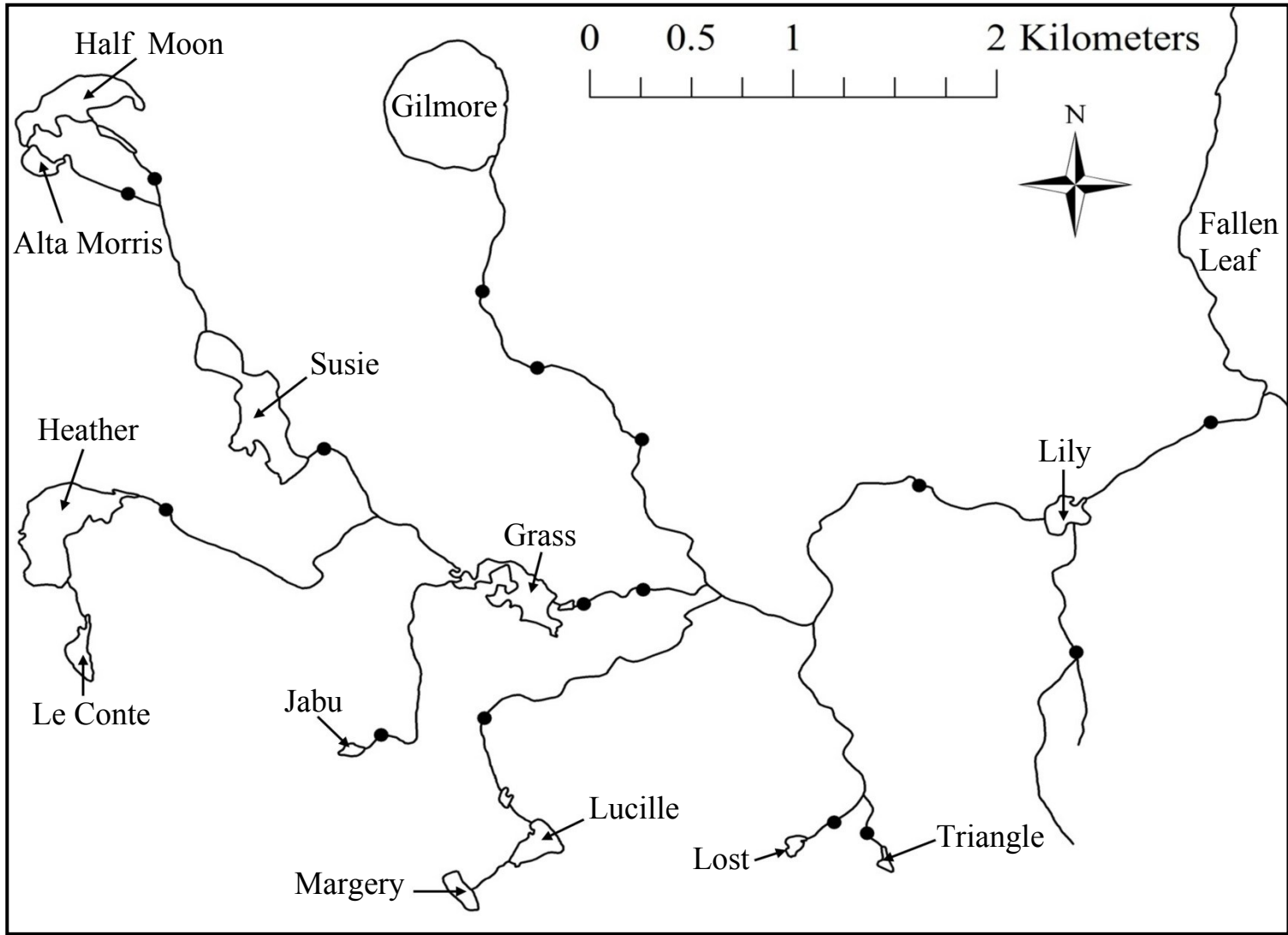


Figure 3.

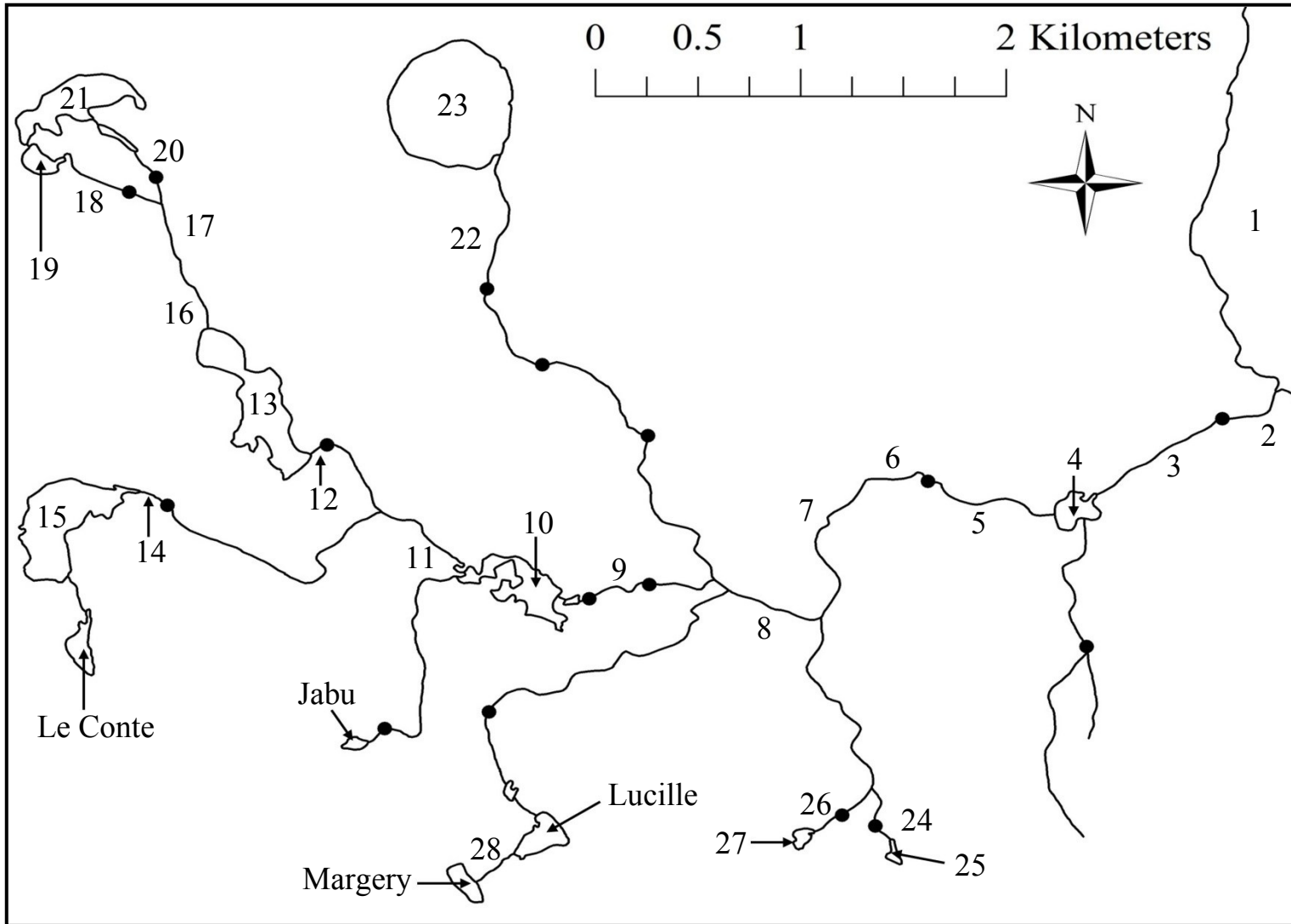


Figure 4.

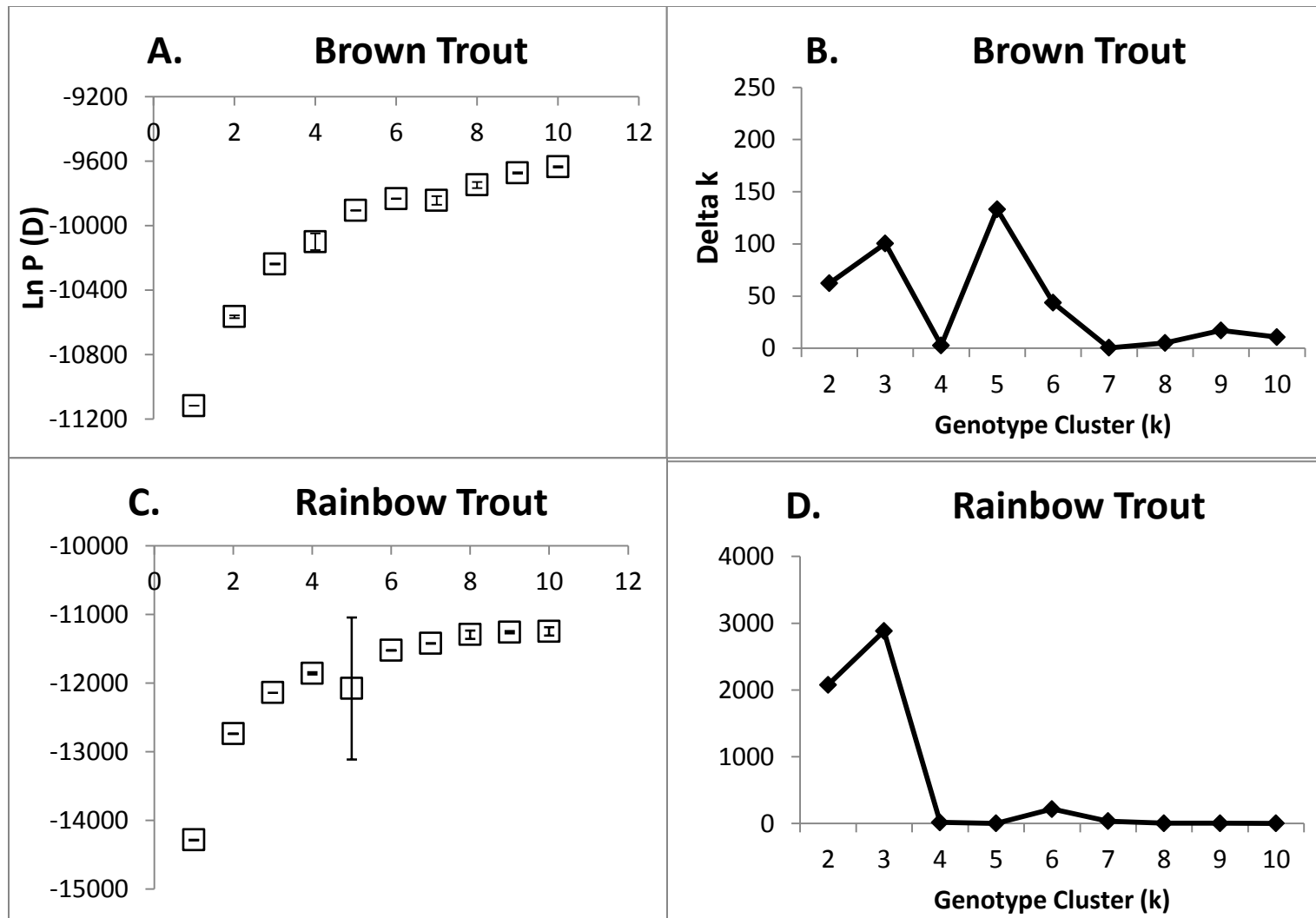


Figure 5.

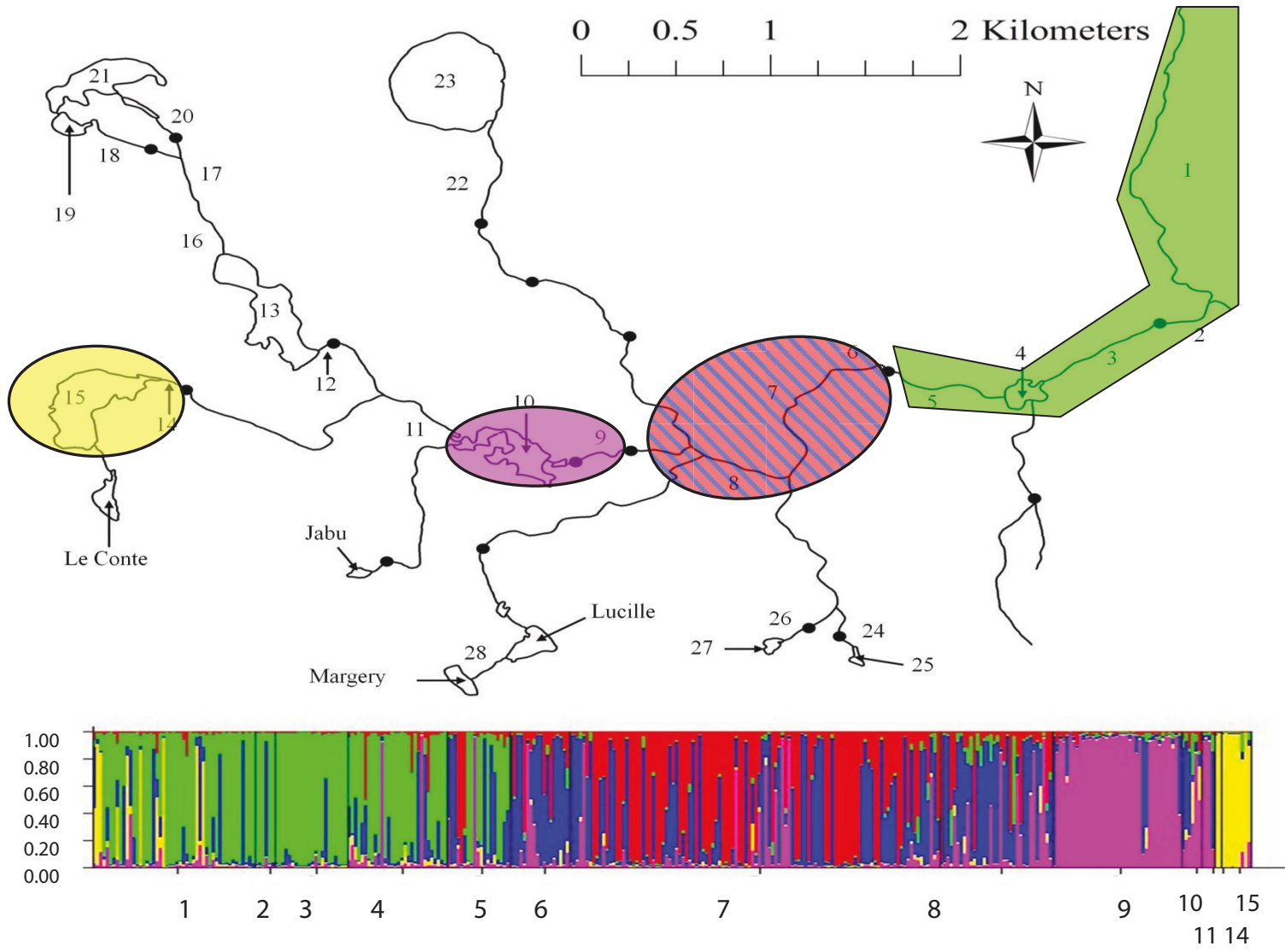


Figure 6.

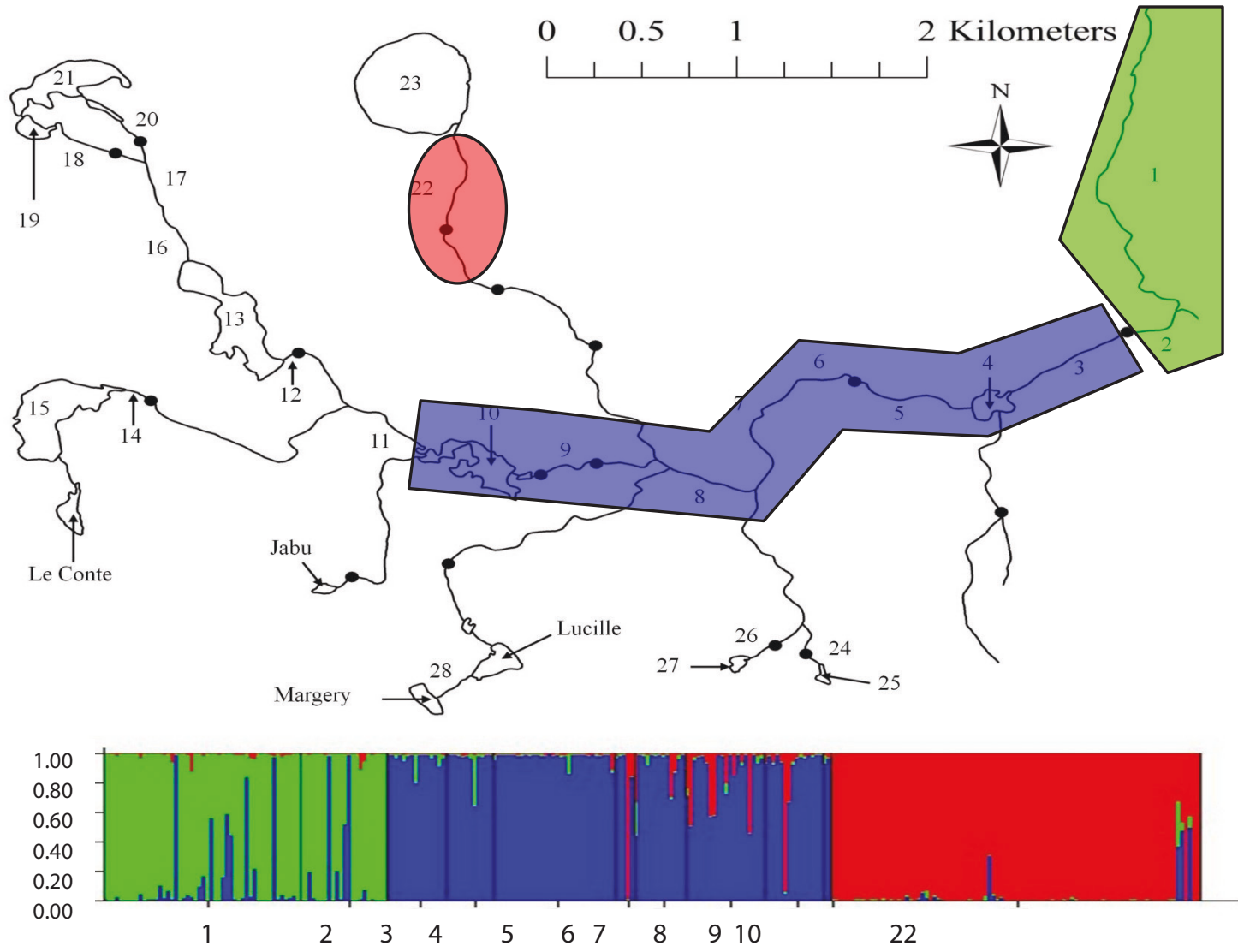


Figure 7.

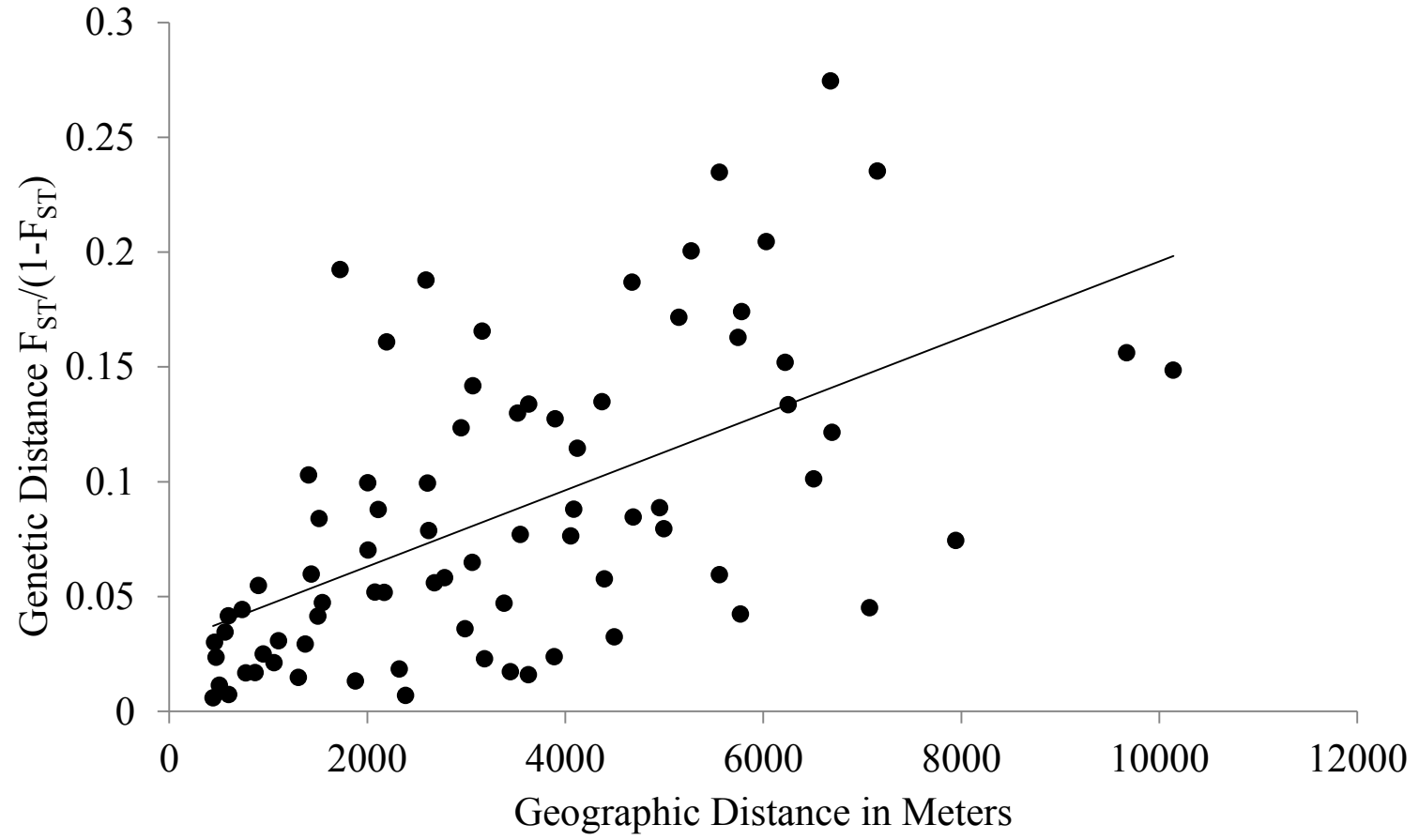


Figure 8.

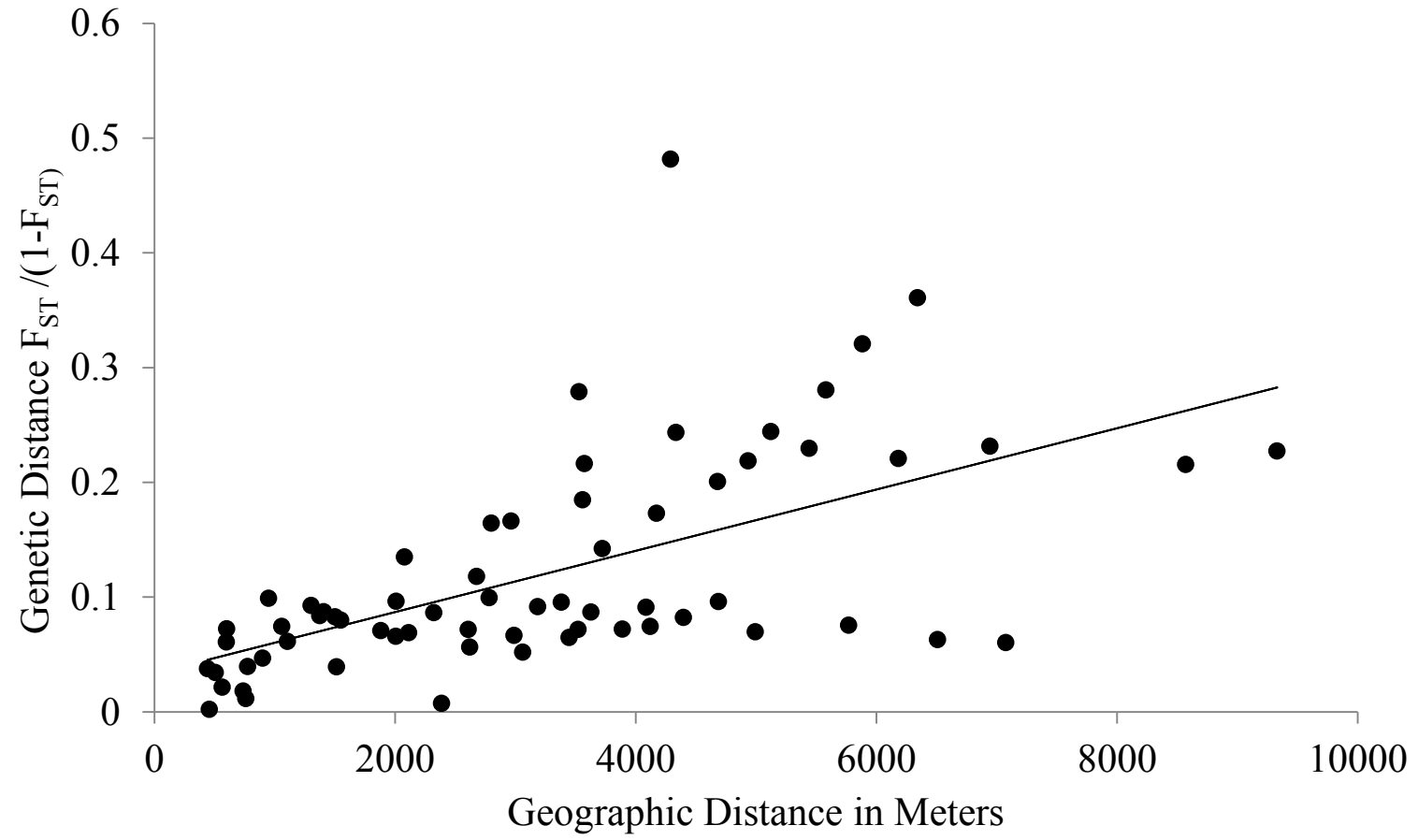


Figure 9.

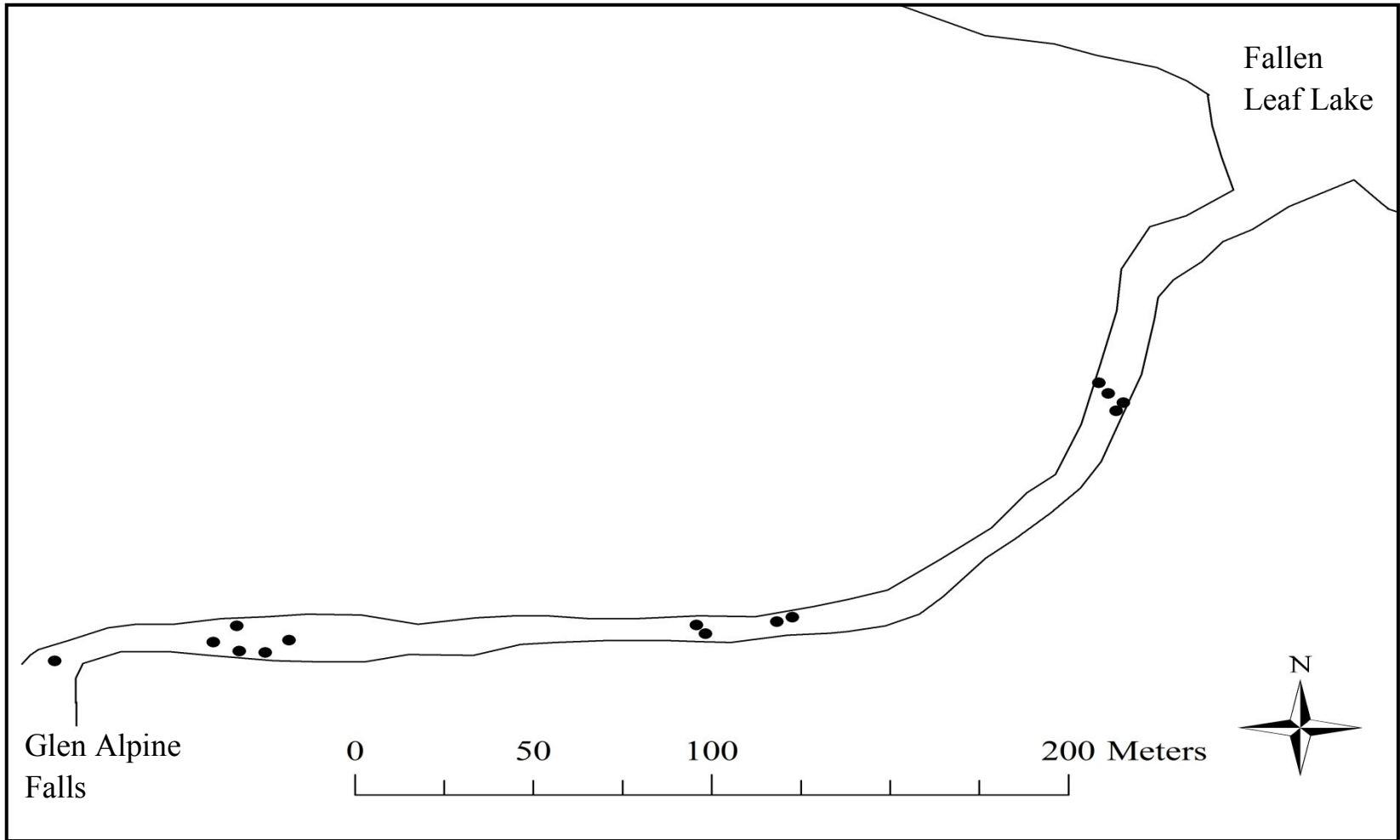


Figure 10.