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Patterns of habitat use and movement of *Rana muscosa* in the northern Sierra Nevada with comparisons to populations in the southern Sierra Nevada, with additional information on the biogeography of the species.

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Abstract

Amphibian population declines and extinctions are occurring even in the world's least impacted areas. One of the best documented examples of a species decline in a protected area is that of the Mountain Yellow-legged Frog (*Rana muscosa*). This species occurs in mountain ranges in California and partially into Nevada and was once commonly seen in streams, lakes and ponds in montane habitats. Today, the species has declined dramatically despite the fact that it occurs almost entirely on protected public land. Several hypotheses have been proposed to explain the puzzling decline including introduced species, ultraviolet radiation, air pollution, climate change, and novel pathogens. This frog occurs in a biogeographically complex area and is an apparently ancient species comprising several genetically distant phylogeographic units, most of which are on the brink of extinction. Surprisingly, the two most deeply divergent forms can be found coming into close contact within the Sierran range. This study provides new detailed phylogeographic information and compares ecological data from populations within the Sierra Nevada. In particular, we present habitat associations for animals in the northern part of the Sierran range and compare them to those in the southern part of the range.

Introduction

Amphibian population declines are occurring worldwide, many in habitats regarded to be little impacted by human activities (Alford & Richards 1999; Houlahan et al. 2000; Wake 1991). In theory, because amphibians have small home ranges, their populations should be secure in large parks and other protected habitats (Blaustein & Wake 1990). Thus, the rapid decline and extinction of amphibian species from such areas is of great concern. Hypothesized mechanisms for declines in protected areas include emerging diseases (Berger et al. 1998; Carey 1992; Daszak et al. 1999), UV radiation and climate change (Blaustein et al. 1994; Kiesecker et al. 2001; Pounds et al. 1999), increased levels of air pollution and pesticide use (Davidson et al. 2001), introductions and spread of non-native predators (Adams 2000; Gillespie 2001; Kiesecker & Blaustein 1997; Knapp & Matthews 2000; Lawler et al. 1999; Vredenburg 2004), and synergistic interactions (Kiesecker & Blaustein 1995; Kiesecker et al. 2001; Relyea & Mills 2001). Many amphibian species that have declined or gone extinct are associated with montane aquatic habitats (Williams & Hero 1998). The best way to evaluate and reverse possible negative effects on populations of amphibians is to understand the stressors in the context of the biogeography and natural history of the species that are in peril.

The mountain yellow-legged frog, *Rana muscosa,* exists almost entirely on protected land in mountainous areas of California and part of Nevada (Figure 1) and yet has declined dramatically in the last several decades (Bradford 1991; Bradford et al. 1994; Drost & Fellers 1996; Fellers & Drost 1993). This species is endemic to two disjunct areas: 1) the Sierra Nevada mountains in California and Nevada, and 2) the San Gabriel, San Bernardino and San Jacinto Mountains in southern California. In the Sierran range, mountain yellow-legged frogs occur from near Antelope Lake (northern Plumas County) (Vredenburg et al. 2003 (in press)), south 490 km to Taylor Meadow in southern Tulare County; (Zweifel 1955) and range from 1,370 m to 3,660 m (Camp 1917; Grinnell & Storer 1924; Zweifel 1955). A few historic populations are known to have existed in the state of Nevada in the vicinity of Mt. Rose, near Lake Tahoe (Zweifel 1955). In southern California, the historic range included the San Gabriel, San Bernardino, and San

Jacinto Mountains with an isolated population at Mt. Palomar (in northern San Diego County; (Camp 1917) and ranged from 300 m to 2,300 m (Camp, 1917; Grinnell and Camp, 1917; Storer, 1925; Zweifel, 1955). This frog was once thought to have gone extinct in the San Bernardino Mountains (none were found between 1970-'93; Jennings and Hayes, 1994), but a small population was recently discovered (Jennings, personal communication).

Currently, mountain yellow-legged frogs are found scattered throughout nearly all of their historic range in Sierra Nevada, but the number of populations is greatly reduced (Jennings & Hayes 1994). This is most notable in the northern-most 125 km of the range (north of Lake Tahoe) and the southern-most 50 km, where only a few populations have been found in the last few years (Jennings & Hayes 1994). The current conservation situation in the Sierra Nevada is of great concern (Anonymous 2002; Drost & Fellers 1996; Knapp 1996; Knapp & Matthews 2000). In the southern California portion of their range, nearly all populations of mountain yellow-legged frogs have disappeared (Jennings and Hayes, 1994), and these disjunct populations are federally listed as endangered under the distinct population clause of the Endangered Species Act (Anonymous 2002). Meanwhile, the listing of the remaining Sierra Nevada populations as endangered was recently found to be "warranted" (Anonymous 2002), clearly, the remaining populations of mountain yellow-legged frogs are in grave danger. This species was originally described as two subspecies of the foothill yellow-legged frog, *Rana boylii,* with one subspecies (*Rana boylii muscosa)* occurring in the southern California mountain ranges and the second subspecies *R. boylii sierrae*; occurring in the Sierra Nevada(Camp 1917). On the basis of limited morphological data, the two subspecies were separated from *R. boylii*, joined and raised to the species level (Zweifel 1955). Recent molecular data (Macey et al. 2001) shows large differences between the frogs in these two disjunct areas, but most surprisingly, the deepest divergence in mitochondrial DNA was found within the Sierra Nevada and not between the two disjunct areas.

The geologic history of the Sierra Nevada is complex (House et al. 1998) and recent work on vertebrates in the area has shown that many species in the Sierran range show north to south phylogeographic breaks. For example this pattern is evident from genetic and morphologic work on the Yosemite Toad (*Bufo canorus*) (Shaffer et al. 2000)

and on several salamanders including in the family Salamandridae of the genus *Taricha* (Kuchta and Wake unpublished data), and in the family Plethodontidae in the genus *Batrachoseps* (Jockusch & Wake 2002; Wake et al. 2002), *Hydromantes* (Wake and Papenfuss unpublished data), and *Ensatina* (Moritz et al. 1992). A recent paper (Macey et al. 2001) showed that in *R. muscosa*, there was also a relatively large difference in mitochondrial DNA that similarly showed a north to south Sierran break in the species (see Appendix). Management agencies concerned with preserving evolutionary trajectories of species should be prepared to conserve the different evolutionary lineages discovered within the Sierra Nevada. While it is clear that proper management actions must be taken to conserve the species within the historical range, knowledge of the ecology and distribution of distinct evolutionary lineages is greatly needed for this effort.

Understanding the ecology and basic life history an organism is a key issue in managing for population persistence. Recent studies on habitat use and movement patterns of the mountain yellow-legged frog in the central and southern Sierra Nevada (John Muir Wilderness and Sequoia and Kings Canyon National Parks) have shown that deep bodies of fishless water are associated with most remaining populations (Knapp & Matthews 2000; Vredenburg 2002). In contrast, very little is known about the importance of deep fishless water bodies in the northern Sierra Nevada (from Yosemite National Park northward). A key life history component for the mountain yellow-legged frog that explains the tight association with deep fishless bodies of water is that the larval phase can take up to 3-4 years (Bradford 1983; Knapp & Matthews 2000; Vredenburg et al. 2003 (in press)). Since *R. muscosa* tadpoles are highly susceptible to trout predation (Vredenburg 2002, 2004), fishless areas are a key habitat characteristic of remaining populations. Therefore, understanding the breeding ecology of a threatened species is clearly a priority and while there is some information for mountain yellow-legged frog breeding in the southern Sierra Kings Canyon area (Vredenburg 2002), virtually nothing is know from the rest of the range. In this study we gathered information on the habitat associations of adult frogs, and we also conducted a study on the factors that influence the choice of breeding sites by *R. muscosa* and compared them to the more lake dominated study area in the southern Sierra Nevada (Vredenburg 2002). We studied

breeding site selection by taking habitat measurements at egg-laying sites as well as taking direct measurements of the egg masses without disturbing them.

Factors affecting reproductive success are thought to be strong selective forces affecting an animal's reproductive behavior and likely include environmental conditions, and presence of predators (especially exotic predators) and competitors. Because an animal's choice of breeding site is assumed to be based on maximizing reproductive success, an examination of breeding site choice should reveal much about the underlying factors affecting a species' reproductive success. In a heavily altered system, like much of the Sierra Nevada's aquatic habitat (Moyle & Randall 1998), it is important to know the factors that affect the reproductive behavior and hence reproductive success, without this, conservation efforts may be hampered. Only in this way can we build a good understanding of the requirements for increased reproductive success of this species. We explored the association between environmental and biological variables and the presence or absence of breeding, and infer which variables are most important in influencing the choice of breeding site by mountain yellow-legged frogs. We examined ponds, lakes and streams for evidence of breeding activity and measured a range of biotic and abiotic variables at study areas that included northern and southern clade mountain yellow-legged frogs (Figure 5) .

To study the habitat use and movements of post-metamorphic frogs, we used radio telemetry, and PIT tagging techniques to track frogs in the stream dominated northern Sierra Nevada habitats (Figure 5). We used compact radio-transmitters attached to adult frogs with beaded belts to be able to repeatedly locate animals. At each frog location, we recorded basic ecological data in order to compare habitat use within and between sites. We addressed habitat at two scales. At the larger scale, we characterized the general type of habitat used (i.e. ponds, lakes, low gradient streams, headwater streams). At the smaller scale, we characterized the microhabitat characteristics the frog selects for its various life-history needs (e.g., breeding, basking, cover, feeding, etc.).

Estimates of habitat use and dispersal distances have become increasingly important with the development of conservation techniques such as population viability analysis (PVA) and minimum viable population estimates. To document patterns of movement and habitat use for *R. muscosa* we used direct techniques including radio

telemetry tags and PIT tags (Heyer & et al. 1994). We also use molecular techniques (microsatellite DNA) to understand movement patterns over longer time scales. Using a combination of field techniques and lab techniques, we will be able to estimate habitat use and calculate movement between sub-populations. Understanding connectivity between populations is an important component in metapopulation persistence (Hanski & Gilpin 1991). The field movement data is used to tell us what habitat the frogs are using and where and when they are moving. The genetic techniques are used to give us a historical perspective of how the frog populations were once connected to each other.

The purpose of this study was to learn basic ecological aspects of the mountain yellow-legged frog in the Sierra Nevada and to make comparisons between stream-dwelling and lake-dwelling populations. We focused on the following questions:

What types of habitats does the mountain yellow-legged frog use in stream-dwelling populations in the northern part of the Sierran range and how does it compare to the information already gathered from mostly lake-dwelling populations southern part of the Sierran range (Kings Canyon)?

- What is occupancy rate of the frog in lakes vs streams?
- What is the occupancy rate of the frog in different types of streams (e.g., low gradient response, vs steeper transport, vs headwater source streams)?
- What microhabitat features are the frogs selecting?
- Are the frogs using a variety of habitat types?
- Are the frogs using different types of habitat for different portions of their life history (e.g., breeding vs adult)?

What are the movement patterns of the frogs in the northern part of its range?

- What distances are the frogs moving?
- Are the frogs using and moving among a variety of habitat types?
- How far from water (how far into the uplands) are frogs moving?
- If they are moving into uplands, what types of habitats are they using?
- Are frogs traveling overland to reach other aquatic sites?

METHODS

Breeding: Surveys were done by walking and snorkeling the shorelines of lakes and streams. Recorded variables include stream width, depth, substrate composition, lake or pond surface area, maximum depth, perimeter, near shore temperature, amount of terrestrial vegetation on shorelines, and presence or absence of aquatic predators (such as introduced trout) and potential competitors (such as other amphibian larvae or macro-invertebrates). The general type of habitat was characterized as either Spring, Creek, Lake, Marsh, or Pond/Marsh. A spring was defined as a very small creek where the max depth was <15 cm and the bank width was less than 75 cm. Springs and Creeks were characterized by containing flowing water. Moving water characterized as a "spring" did not necessarily originate from underground as in the classical sense of the word, but could have flowed out of a small pond for example. Creeks were similar to Springs but were larger. A Lake was defined as a standing body of water with a surface area greater than 400 m^2 whereas a pond had surface area less than 400 m^2. A marsh was defined as a shallow water area that contained emergent vegetation (such as sedges) throughout the entire wetted area. A marsh was basically a pond or lake that had filled silted in and had become overgrown with vegetation; a natural progressive state as ponds slowly transition towards meadows. A pond/marsh contained am mixture of the two habitat types. For each egg mass we measured the diameter of the egg mass, the substrate used for attachment, and the distance to the nearest conspecific egg mass. We also recorded the depth of the egg mass by recording the distance from the top of the egg mass to to surface of the water as well as the depth from the bottom of the egg mass to the bottom of the stream, lake or pond. When possible, we recorded the presence of garter snakes (*Thamnophis elegans*) and birds know to be predators on this frog (Clark's nutcrackers, Brewer's blackbirds), and potential competitors such as Pacific Tree Frogs (*Hyla regilla)* and Western Toad (*Bufo boreas*) and Yosemite Toads (*Bufo canorus*) (Vredenburg et al. 2003 (in press)). We relied on previous information for introduced trout presence when available. If this information was not known, we used gill nets (1-2) in ponds or lakes for

up to 12 hours to sample for fish presence (Knapp 1996). Presence of trout in streams was determined visually or by angling. We were only interested in noting the presence or absence of trout in the aquatic habitats and therefore did not allocate any effort to measuring trout densities.

Animal movements and habitat use: To address habitat use and movement we used radio telemetry, to track frogs in stream dominated northern Sierran habitats. We also used PIT tags (passive integrative transponders) to individually mark adults. Radios were affixed 2-6 weeks after breeding depending on our access to the site. Frogs were tracked weekly for the entire season which range from June to late October. Frogs were sighted approximately once a week and approximately once every two weeks they were captured, weighed, measured and released at site of capture. Radios were removed at the end of the season. To get movement data on more individuals, we inserted PIT tags into adult frogs (including those with transmitters) at all of the sites. A passive integrative transponder (PIT) is a radio-frequency identification tag that consists of an electromagnetic coil, tuning capacitor and microchip encased in glass. It is small (10 X 2.1mm, 0.05 g) and carries a 10 digit hexadecimal number that is read with a portable scanner. PIT tags are commonly used by herpetologists, and others to permanently mark free ranging animals (Heyer & et al. 1994); this method has proven to be very reliable with amphibians, including true frogs, and has had minimal impact on the survival of the study animals (Heyer & et al. 1994). After frogs were implanted with tags they were recaptured throughout the summer. In addition, frogs with PIT tags will be a valuable component of any future population monitoring studies.

Basic habitat variables were collected at each location where marked frogs were recaptured with PIT tags, or recaptured and / or re-sighted with radio-transmitters. Hand held GPS units were used to record locations and additional data were recorded directly onto PalmPilot handheld devices using a Palm version of Microsoft Excel. We also recorded distance to water, basic substrate characteristics, vegetation cover, air and water temperature, air speed, cloud cover and time of day. These variables have all been shown to be important in *Rana muscosa* in the southern Sierra Nevada. While data from PIT tagged frogs and radio belted frogs was collected at the same time, the data were

analyzed separately. In the future, the two data sets will yield results that can be compared and contrasted, giving us a better understanding of habitat use and how it compares to habitat use in the Southern Sierra Nevada.

When frogs moves to a new location along a stream (defined as >10 m from the previous sighting), we collected the following detailed information on habitat characteristics:

Channel Data

+ Water depth at thalweg
+ Wetted channel width:

Stream Data

+ Water temperatures were taken by placing a handheld thermometer in the water at 0.1 m from the water's edge and 2 cm under water.
+ to record stream habitat: we visualized a 1 m^2 plot extending from the water's edge into the water with the near shore edge centered on the frog location. In the $1m^2$ plot we:
    1. Recorded the dominant substrate.
    2. Recorded the percent cover by cover class for:
        - herbaceous submergent/emergent vegetation
        - woody submergent/emergent vegetation
        - woody debris
        - total cover, included any type of cover that a tadpole or frog could hide under including silt, wood, vegetation, vegetative material such as leaves.
+ Depth of detritus: we measured at 0.1 m from the water's edge using a measuring rod.

Shoreline Data

+ We recorded Shoreline Habitat for each frog: we visualized a 1 m^2 plot extending from the high water line onto the shore away from the lake with the high water line edge centered on the station. In the $1m^2$ plot we:
    1. Recorded the dominant substrate.
    2. Recorded the percent cover by cover class for:

- herbaceous vegetation less than 1 m high
- woody vegetation less than 1 m high
- woody debris less than 1 m high. Stumps that are rooted in the ground are not included.

- total cover, included any type of cover that a tadpole or frog could hide under including boulders, dead leaves, wood, and other vegetative material.

+ Bank angle: We laid the measuring rod perpendicular to the stream flow on the dominant angle of the bank between the base of the bank and bankfull. We placed a clinometer on the top of the depth rod and record the angle (degrees). For vertical or undercut banks, the gradient was recorded directly from the clinometer ($<90^o$). For banks that slope away from the water, the clinometer reading was subtracted from 180 to get the recorded gradient (between 90^o and 180^o).

+ Bank stability: We recorded bank stability as: 1 = stable, 2 = vulnerable, or 3 = unstable.
  1 = Stable: 75% or more cover of living plants, boulder/cobble aggregates, embedded logs,
  hardened conglomerate, or cohesive clay/silt banks.
  2 = Vunerable: 75% or more cover but one or more instability indicators ie: fracturing, blocking, or slumping or mass movement.
  3 = Unstable: less than 75% cover and had indicators of instability.
+ *Entrenchment ratio*:

We determined the location of bankfull on both sides of the channel and measured the distance between the two points (the bankfull width (Bw)). We measured the height of the tape at the thalweg to get the bankfull depth (Bd). We multiplied Bankfull Depth by 2 to get the Floodprone Depth (Fd). We extended the measuring tape across the channel so that it was level at the height of Fd. The distance between the two points where the tape met the channel banks was recorded as the floodprone width (Fw). Entrenchment ratio=Fw/Bw.

+ *Stream gradient*: We characterized the stream gradient (see below for details).

+ *Shade:* To measure shade in the middle of the wetted width of the stream, we held a densiometer approximately 0.5 m above the water's surface. Four readings were taken, facing the left bank, facing upstream, facing the right bank, and facing downstream. We held the densiometer level such that the bubble was in the circle and at elbow's length. We visualized four dots in each corner of each box. We counted the number of dots that had vegetation or other shade in them. We then recorded this number.

+ *Stream channel type:* Using the channel type key (see Appendix), we recorded the one-letter code (A, B, C, D, E, F, G) of the channel type that best represented the stream reach where each frog was located. We used the entrenchment ratio to assist in this determination.

+ *Valley Type*: When possible, we recorded the valley type that best represented the basin where the stream reach was contained using the two-letter codes below. We then determined the general description of the valley cross section with emphasis on the configuration of the valley floor. We divided this into types with a narrow valley floor (valley floor width (VFW) < 2.5 times stream active channel width (ACW) and types with a broad valley floor (VFW > 2.5 times ACW). Typical configurations may have had the active channel in several positions on the valley floor. See diagram below.

Narrow Valley Floor

SV = Steep V-Shaped valley or bedrock gorge (side slopes $>60^{o}$).

MV = Moderate V-Shaped valley (side slopes $> 30^{o}$, $<60^{o}$).

OV = Open V-Shaped valley (side slopes $<30^{o}$).

Broad Valley Floor
CT = Constraining Terraces. Terraces typically high and close to the active channel. Terrace surface is unlikely to receive flood flows and lacks water dependent (non-hydrophilic) vegetation.
MT = Multiple Terraces. Surfaces with varying height and distance from the channel. High terraces may be present but they are a sufficient distance from the channel that they have little impact.
WF = Wide-Active Flood plain. Significant portion of valley floor influenced by annual floods, and has water dependent vegetation (mesic meadow). Any terraces present do not impinge on the lateral movement and expansion of the channel. Please see Appendix B with a diagram reproduced from Rosgen (Rosgen 1996).

+ We classified the character of the stream channel as:
  1 = *low gradient* - Fine-grained streambanks dominated by clay, silt, sand or gravel particles. These reaches were usually 0.1-2%, though gradients ranged up to 3%. These streams are typically unconfined, but may be confined when downcutting has occurred.
  2 = *high gradient* - Includes all other streams.
  3 = *undeveloped channel* - drainages without a developed channel

We radio-tracked frogs throughout the entire active season (June-October) in 2003 and compare the results to those collected previously (1999; Vredenburg unpublished). In addition to the habitat data collected for each frog, we also calculated movement patterns using Arview (Version 3.0). Coordinates for each frog location collected throughout the field season were imported into ArcView and projected such that the distances between the locations could be measured. To understand how far frogs moved each time they changed location, we measured the distances between capture locations and then averaged those measurements for each frog. We present these data as the average

successive distance (SD). To understand how far frogs moved throughout the entire study period, we measured both the linear distance and the stream distance between the earliest location and the last (by date) location for each frog. We present these data as the net stream distance moved (NSD) and the net linear distance moved (NLD). We also calculated the gross distance moved for each frog. This calculation is a sum of all movements made for each frog, including movements back and forth for example along a stream. The sum for each frog was then averaged with all the frogs from each site. We averaged the movements of frogs from each site in order to make the data more easily viewable. We do not present statistical analyses for the movement patterns at this time, however, we will complete these analyses for the final report.

Population characteristics: We conducted basic surveys in order to get baseline information on population sizes and structure. Surveys were timed to target each of the life history stages. Surveys were conducted at snowmelt to count egg masses, and twice in mid to late-summer to count adults, metamorphs, and tadpoles. At snowmelt, multiple visits were required to determine the onset of breeding. Egg mass surveys occurred toward the end of breeding. Population surveys occurred 1 and 2 months after breeding.

Visual encounter surveys (VES) were used to search for egg masses, tadpoles, and frogs for relative abundance estimates. During surveys, the number of animals per life history stage was recorded. Egg masses were counted and their general stage recorded (i.e., not close to hatching, close to hatching, newly hatched). Tadpoles were visually counted by the size classes: a) first year ((<20 mm total length (TL), no legs)), b) second year (11- 39 mm TL, no legs), c) third year or more (>40 mm TL with rear legs and or front legs). Other life history stages include adult (≥46 mm> SVL), subadult (31-45 mm SVL), and metamorph (<30 mm SVL). When possible, all animals were counted individually. When this was not possible, adults were rounded to the nearest 10 and other life history stages will be rounded to the nearest 10 for numbers up to 100, rounded to the nearest 100 for numbers up to 1000, and rounded to the nearest 1000 for numbers greater than 1000. Up to 50 adults, subadults and metamorphs were captured, measured (SVL,

mm), and weighed (grams). Additional information on adult sizes will be obtained through the mark-recapture effort.

Biogeography and Gene Flow and the Species Question: In this study, we collected tissues from adult and larval mountain yellow-legged frogs to augment ongoing studies on biogeography and gene flow in this species. We then used genetic tools (microsatellites and mtDNA) already developed in previous studies to investigate the potential contact zone between the northern and southern clades in the phylogeny of what is currently recognized as *Rana muscosa*. We used the DNA to augment two ongoing studies, 1) a study using mitochondrial DNA to understand the historical biogeography of the species, and 2) a study using nuclear DNA (microsatellites) to understand gene flow between populations on a smaller geographic scale (within basin). Tissues were collected by preserving toe clips in 95% EtOH (Heyer et al. 1996). Funds from this study were used to process and analyze tissues we collect for this study, as well as tissues that have been collected by collaborators throughout the range of the species. In the lab, we also collected morphological data on adult specimens that were previously collected throughout the range of the species. These data will be used in future studies to compare morphological metrics between the clades identified using genetic techniques (Figure 8-9). All whole animal specimens examined are provided by the Museum of Vertebrate Zoology.

Phylogeography (MtDNA): To understand the evolution of the mountain yellow-legged frog throughout its extensive range we conducted phylogeographic analyses of 96 different localities spanning the known distribution. Genomic DNA was extracted from toe, thigh, and tail tissue using a Qiagen tissue extraction kit. Amplification of DNA through polymerase chain reaction (PCR) was performed with denaturation at 95 degrees Celsius for 30 seconds, annealing at 50 degrees Celsius for 30 seconds, and extension at 72 degrees Celsius for 90 seconds for 30 cycles. Amplified products were purified with ExoSAP-IT and then used in cycle-sequencing reactions with denaturation at 95 degrees Celsius for 15 seconds, annealing at 50 degrees Celsius for 15 seconds, and extension at 60 degrees Celsius for 4 minutes for 25 cycles. Cycle-sequencing products were cleaned

using Sephadex columns and then sequenced using an ABI 3730 Capillary Sequencer. Sequences for each sample were then compiled and analyzed using the program Sequencher. Output files were then used in the programs PAUP* (Swofford 1996) and MrBayes (Huelsenbeck & Ronquist 2001) to estimate phylogenetic relationships.

Initially, sequencing was targeted toward 2,000 bases of the mitochondrial genome. By reducing the length of amplified DNA to 500 bases, we were able to cut cost and time without significantly reducing the number of phylogenetically informative characters. The resultant target DNA sequence was a segment encoding the ND2 gene for which primers were designed (forward primer: 5' CCC CAA TAA CAC TGC TTC TCC AA 3'; reverse primer: 5' GAG GGT TAT GGT AAT AAT GTA TGT 3').

The 96 localities sequenced for the ND2 mitochondrial gene were analyzed using several different methods. The program Modeltest was used to first choose the appropriate model of DNA substitution for our dataset. With the chosen parameters, we used the program PAUP* (Swofford 1996) to perform a parsimony bootstrap analysis with 100 replicates and 100 maxtrees. In this analysis, the frequency that a given branch is found is recorded as the bootstrap proportion. These proportions are used as a measure of the reliability of individual branches in the optimal tree. Next we used the program MrBayes to perform Bayesian estimation of phylogeny (Huelsenbeck & Ronquist 2001). Bayesian inference relies on the posterior probability distribution of trees, which is the probability of a tree given a certain dataset. Through Markov chain Monte Carlo simulation, the program produced a large set of trees that were then compiled in PAUP* (Swofford 1996). A 50% majority rule consensus tree was produced which yields the frequency that each clade was found in all trees produced. The values of the branches are the probability of the true existence of that clade. From the Bayesian run, we also created a consensus phylogram tree which represents the relationships between the taxa and yields information on how much evolutionary change has occurred between them (the horizontal length of each branch) (Huelsenbeck & Ronquist 2001). The amount of change is also quantified in a distance matrix produced in PAUP* (Swofford 1996). This matrix gives the pairwise percent difference between all sequences used in the analysis.

Population Genetics- microsatellite analysis (nuclear DNA): Genetic analysis of population structure at different geographic scales throughout the range of the mountain yellow-legged frog is imperative. Microsatellites are useful genetic markers for analysis of closely related taxa due to the fast mutation rate. In our microsatellite analysis, each sample of DNA was extracted using the Qiagen tissue extraction kit and then amplified using PCR with denaturation at 94 degrees Celsius for 40 seconds, annealing at 40-56 degrees Celsius for 40 seconds, and extension at 72 degrees Celsius for 30 seconds for 35 cycles. Genetic Identification Services designed primers for 27 different microsatellite loci from CA and TAGA libraries, 12 of which amplify reliably and are potentially informative. The primers used in this PCR process have fluorescent labels that allow for fast and accurate processing in the ABI 3730 capillary sequencer. With four different fluorescent labels, each sample can be analyzed for four microsatellite loci at the same time, and 96 samples can be run per plate in the sequencing machine. The data produced from a sequencing run is evaluated in the ABI program GeneMapper, which determines the length of microsatellites and identifies different alleles present. This data is then analyzed in programs like Arlequin that can output statistics on population differentiation, genetic assignment of individuals to populations, and estimates of migration.

RESULTS AND DISCUSSION

Breeding:

During the 2003 mountain yellow-legged frog breeding season we searched for egg sites in localities spread over a large area of the Sierra Nevada (Figure 12-13). We recorded information on breeding site attributes and on egg masses at 6 locations within the northern clade of the frog (E1-E-6; Fig 13) and 7 sites in the southern clade (all within 60 Lake Basin; E7; Fig 13). We compared the generalized egg laying habitat at 312 egg masses in the southern clade to 248 egg masses in the northern clade for a total of 560 egg masses (Figure 14). The general type of habitat was either Spring, Creek, Lake, Marsh, or Pond/Marsh. The southern egg masses were predominantly laid in Spring habitat whereas the northern sites had a higher proportion of eggs laid in Marsh and Pond/Marsh habitat.

Anurans use a wide assortment of substrates for attachment of egg masses (Duellman & Trueb 1986). We recorded the attachment substrate for 560 egg masses, 312 in the southern clade and 248 in the northern clade (Fig 15). A higher proportion of the southern egg masses were attached to rock whereas in the northern egg masses they were more likely to be attached to vegetation. In addition, egg mass diameters were measured on 559 egg masses, 247 in the northern clade and 312 in the southern clade (Fig 16). The mean size for the northern clade was significantly larger than the mean size for the southern clade (North, mean =7.8 cm, SE = 0.29, SD = 4.57; South, mean = 5.11cm, SE = 0.1, SD = 1.87; t-test, $p < 0.0001$, df = 311, t = 8.68). We also measured linear distance from each egg mass to the nearest conspecific egg mass at 296 southern egg masses and 247 northern egg masses (Fig. 17). The mean distance between egg masses was larger in northern egg masses than in southern egg masses (North, mean = 30.46 cm, SE = 4.8, SD = 75.7; South, mean = 13.08; SE = 2.82; SD = 48.5; t-test, $p < 0.001$, df = 404, t= 3.11). The water flow rate, however, was not different between the two areas. Water flow was measured directly next to 296 and 248 sties in the southern and northern clade, respectively (Fig. 18). The mean flow rate was not significantly different between the two sites (North, mean = 0.05 ft / sec^2, SE = 0.006, SD = 0.11; South mean = 0.0567 ft / sec^2, SE = 0.006, SD = 0.09; t-test, p = 0.9, df = 541, t = -0.004). The depth of the egg masses also did not differ between the two clades. The mean depth from the top of the egg mass to the surface of the water was not significantly different between the northern and southern clades (North mean = 3.56 cm, SE = 0.47, SD = 7.37; South mean = 2.64 cm, SE = 0.22, SD = 3.8; two-tailed t-test, p = 0.078, df = 353, t= 1.76). The mean depth from the bottom of the egg mass to the creek bottom (or lake bottom, etc.) was not significantly different between the northern and southern clades (North mean = 2.35 cm, SE = 0.34, SD = 5.44; South mean = 2.45 cm, SE = 0.28, SD = 4.82; two-tailed t-test, p = 0.82, df = 496, t= -0.22).

Animal movements and habitat use:

In Table IV, we summarize the habitat variables measured at each frog location. We present mean values for the habitat variables organized by site. Across all sites, frogs were found close to shore in open canopy, low gradient sites (Table IV). In addition, in figures 42-45 we present additional data on habitat variables at radio-tracked frog capture locations. It is clear that mountain yellow-legged frogs were sighted most often in low water flow environments (Fig 45) such as pools and riffles in streams. Table VI shows the number of frogs that were followed with radio-transmitting belts at each site. We studied animal movement patterns of adult mountain yellow-legged frogs using Holohil Bd-2 transmitting radios at 4 northern clade locations in 2003 (Fig 12-13) using a total of 43 radios (Table VI). We also present the movement patterns of mountain yellow-legged frogs from individuals radio-tracked in 1999 from the southern clade-Kings Canyon (Fig 13, site E7, and Table VI). We present the average distances traveled by individual frogs by study area (Figures 37-41). On average, frogs at Cow Creek moved greater distances than the other sites (Fig 37-39). Most of the difference is seen to be coming from the males. For example, the mean successive distance moved (SD) for male frogs at Cow Creek was >280 m whereas the mean values calculated for male frogs in nearby Baker Creek was nearly half that distance (Figure 39). When males and females are combined at each site, Cow Creek animals are still moving further than the remaining 5 sites (data from Ebbetts Pass is not shown, animals at that site did not move more than 20 m from the pond). When comparing Baker Creek animals, they also moved farther, especially for the mean successive distance (SD) than Deadwood, Lake 30-31, or Lake 12. When we compared the other distance measures, the net stream distance moved (NSD) and the net linear distance moved (NLD), we found the same pattern where Baker and Cow Creeks have higher values than the other sites. Most sites appear to show little difference between the sexes in movement distances with the exception of Cow Creek where males appear to be moving much further than females.

Figure 39 and 40 show comparisons between sites where mountain yellow-legged frogs co-occur with introduced trout and places where they do not co-occur. Our project was not designed to test the effects of introduced trout on movement patterns of the mountain yellow-legged frog; however, because it is well known that introduced trout have

negative effects on mountain yellow-legged frogs (Bradford 1989; Knapp & Matthews 2000; Vredenburg 2002, 2004) we present data from several fishless habitats and compare them to sites with introduced trout. Two of our radio-tracking study areas contained trout in close proximity to mountain yellow-legged frogs, Baker Creek and Lake 30-31 (Sixty Lake Basin). In the Sixty Lake Basin site, we know from prior studies that the reproductive sites for mountain yellow-legged frogs are always separated from introduced trout (Vredenburg, 2002)(Vredenburg 2004). In the Lake 30-31 area, post-metamorphic frogs leave the egg laying sites (fishless) and move into ephemeral ponds during much of the summer (fishless). Towards the end of the summer, the frogs move away from the ephemeral ponds as they dry and move into the main stream containing introduced trout (see Fig 33-36). Unfortunately, we do not have information on the breeding site locations at the Baker Creek site, but we do present movement data on adults from later in the season. We were not able to reach the Baker Creek sites early enough in the season to search for egg laying sites. On average, frogs at Cow Creek (without trout) moved longer distances than frogs from nearby Baker Creek (with trout; Fig 39), but most of that difference comes from the high successive distances (SD) from male frogs at Cow Creek. We also compare the movement data from Deadwood Canyon (without trout) to the data from the Lake 30-31 frogs (with trout; Fig 40). Additional statistical analyses are necessary to compare the frog movement patterns at these sites. We provide sample movement data on individual frogs (Fig 24-36) with scale bars. Arrows are drawn between successive frog identification locations and distances are measured between the points. In Figure 19 we show all of the point data for the radio-tracked frogs in Deadwood Creek, a fishless stream site. In Figure 20, we highlight the area with all of the points with shading. Figure 21 depicts all of the locations of radio-tracked frogs in Baker Creek and Cow Creek. Figure 22 shows all of the locations of radio-tracked frogs in the Sixty Lake Basin, and in Figure 23 we highlight the main study areas. In figures 24-36 we provide spatial maps with scale bars that show the movement patterns of individual frogs (Fig 24-27, Deadwood Canyon; Fig 28-29, Cow Creek, Fig 30, Baker Creek, Fig 31-32, Lake 12 in Sixty Lake Basin; Fig 33-36, Lake 30-31 in Sixty Lake Basin). Another study on movement ecology of the mountain yellow-legged frog showed that adults moved hundreds of meters, sometimes over dry land (Pope &

Matthews 2001). Here we report similar results on adult frogs from widely separated study areas in the Sierra Nevada.

Pylogeography (mtDNA): The addition of new genetic samples (see Table I) has greatly increased the spread of samples included in the study on mountain yellow-legged frog phylogeography. Figure 1 shows the distribution of available DNA tissue sample for this study. Figure 2 shows the localities of all of the samples included in the analysis, the colors designate the two major clades. Figures 7-9 show the relationships between the samples as a result of phylogenetic analysis. All analyses show strong support for monophyly for all mountain yellow-legged frog samples when compared to outgroups. Within the species, the analyses support northern and southern haplotypes with the geographic break occurring between the headwaters for the middle and south fork of the Kings River at Mather Pass (Fig 4-5). Interestingly, one sample collected east of Kearsage Pass falls out as part of the northern clade even though it is far south of the contact zone (Figure 4-5). Figure 7 shows the parsimony analysis with bootstrap values. This analysis gave strong support for the northern clade (92). Figures 8-9 are results from a Bayesean analysis and numbers of the trees represent confidence intervals (not bootstraps) for each clade. Figure 9 is a phylogram that shows the amount of genetic change between groups. An attached document (matrix.xls) gives the % divergence between all of the samples. On average, the northern and southern clades differed between 4-6%, a similar finding to the previous study of ND2 mitochondrial DNA (Macey et al. 2001). This level of divergence is seen between some frogs that are considered to be different species(Macey et al. 2001).

Contact Zone: Figures 3-6 show details of the contact zone between the northern and southern clade. Figure 6 shows all of the available samples in the contact zone. As populations decline around the contact zone in the Sierra Nevada, it is essential to know which major evolutionary clade an animal or population belongs to. Using the restriction endonuclease MscI, a quick and inexpensive Restriction Fragment Length Polymorphism (RFLP) test can reliably identify individuals. The RFLP test we designed is able to

assign an individual mountain yellow-legged frog in the contact zone to the northern or southern clade (Figure 9a). It also can identify any populations that may have both mitochondrial haplotype groups present indicating mixing across the presumed biogeographic barrier, although we have not found an example of this in the samples we analyzed for this study. The way the RFLP works is that the enzyme cuts the DNA segment at a specified sequence site which is only present in the southern clade, such that DNA from a southern clade animal is sliced into two pieces while DNA from a northern clade animal remains at full length (Figure 9a). The initial steps of the process are the same for sequencing in that DNA is extracted and then amplified using normal PCR protocol. But the process is condensed at this point, and only involves digesting the DNA with a restriction enzyme for 3 hours at 37 degrees Celsius. DNA separation on an agarose gel then distinguishes mitochondrial haplotypes. In this study, we analyzed sixteen samples from four geographically northern populations and ten samples from two southern populations along the contact zone. All samples have fallen into the hypothesized evolutionary clade predicted by geographic location north or south of the contact zone (Mather Pass), and so far no populations have had both mitochondrial types present. While analysis of more samples in the contact zone is necessary, it appears that genetic exchange across this zone is minimal. The lack of movement seen in the contact zone reaffirms the notion that animals do not move between different regions at appreciable frequency.

Population Genetics; Microsatellite Analysis (nuclear DNA):

Microsatellites (nuclear DNA) are useful genetic markers for analysis of closely related taxa due to the fast mutation rate. Primers for 27 different microsatellite loci from CA and TAGA libraries, were previously developed Genetic Information Systems under contract (Vredenburg, Moritz and Wake USFWS contract). Of the 27 loci delivered in 2001, 12 amplify reliably and are potentially informative and have been implemented in this study. Identifying the amount of variation in allele size and frequency is central to an analysis of population structure. If there is homogeneity across many populations, then we can assume that the lack of variation is a result of a high degree of admixture. But if we find a diversity of alleles at different frequencies in many populations, then we

can infer isolation of populations with little gene flow. For the mountain yellow-legged frog, population structure and equilibrium (long-term) migration rates are obtained using traditional variance-based methods of analysis, especially those that incorporate mutational divergence. The R_{st} statistic is an important measure of population variation and will be estimated in R_{st}Calc. Current connectivity is estimated by predicting the source of individuals relative to their capture location using maximum likelihood assignment methods, but this will take place in future studies.

The 12 sets of microsatellite primers that were designed have been optimized and initial sequencing reactions have been run. Multiple samples (ranging from 12-15) have been analyzed from each of six lakes in Sixty Lake Basin that span the range of distance and isolation relevant to anuran movement in the complex habitat (Figure 10-11). Variation in allele frequency is present between sites, though detailed analyses are still being performed (Figure 11a). The variation in microsatellites at this small spatial scale represents some degree of isolation of breeding sites and provides and indication of the extent of dispersal and migration in this species within a basin. Thus we conclude that the mountain yellow-legged frog has limited dispersal based on microsatellite analysis.

Population characteristics: Results from VES (visual encounter surveys) are presented in Table V. Population sizes in the northern clade are much smaller than in the southern clade, with the exception of Cow Creek. Previous data from the southern clade (Sixty Lake Basin, 21 populations) show that those populations are both larger and at higher density that the populations in the north (Vredenburg 2002, 2004). Repeated surveys (>980) of 21 populations in Sixty Lake Basin from 1996-2003 show average density of populations around 4 adults per 10 m of shoreline (Vredenburg 2004) and this has also been shown in surveys of many other populations in the Kings Canyon area (Knapp unpublished data). In this document we do not report densities of stream populations due to the linear nature of the habitat. For example VES methodology does not account for lengths of stream that should be surveyed. In stream populations, it is not clear where populations begin and end, therefore identifying correct stream lengths to use is problematic. We suggest that in stream environments, mark and recapture methods with result in more accurate population estimates than VES methods.

Conclusion/Remarks on Egg Laying: Although there were significant differences between northern and southern populations in terms of size and numbers of egg masses laid at sites, the type of habitat selected was similar across the two regions. In both the north and south, frogs chose low flow, open canopy areas to deposit their eggs. The egg deposition sites were predictable depending on the local habitat available. Mountain yellow-legged frogs lay their eggs in a highly clustered fashion usually. Tadpoles from previous years are usually nearby.

Conclusions on Movement and habitat use: Frogs in the north utilized more stream than pond habitat. This may be in part due to lack of available pond habitat, but even in areas with available pond habitat such as Deadwood Canyon, frogs were still found predominantly in streams. In Deadwood Canyon, a stream site, that also contains numerous permanent and ephemeral ponds, adult frogs almost exclusively were found in stream habitat. Baker Creek also has some available pond habitat yet not tracked animals utilized them. In fact, we only saw one frog in using the ponds, but we also studied Baker Creek later in the season. Baker Creek is interesting because it contains introduced trout. We predict that there must be off stream habitat that frogs can use for breeding purposes (we missed breeding at that site). We hope to conduct future studies on the interactions between introduced trout and mountain yellow-legged frogs in these types of habitats. The frogs we studied at all sites were predominantly found in low gradient, open canopy environments. Within northern sites, frogs were more often found along steams with C and E channel types, or with high entrenchment ratios. In addition, we also found a positive correlation between frog occurrence and bank stability. Frogs were more often found along stream stretches with stable banks. While our methods may not be sensitive enough to fully determine the importance of stream channel type on the distribution of mountain yellow-legged frogs, it does suggest that remaining populations occur mostly in non-degraded stream channels. This deserves further study.

CONSERVATION IMPLICATIONS:

Conservation of the mountain yellow-legged frog must become a central issue for land management agencies in the Sierra Nevada. A large number of Sierran populations have disappeared (Bradford 1991; Bradford et al. 1994; Vredenburg et al. 2003 (in press)), but the exact extent of decline is unclear due to the lack of systematic surveys (Jennings & Hayes 1994). Between 1989-'93, Bradford et al. (Bradford et al. 1994) resurveyed mountain yellow-legged frog 'historic sites' (documented between 1959-'79). In the western portion of Sequoia National Park (Kaweah River drainage) they resurveyed 27 historic sites and found no frogs at any of these locations (Bradford et al. 1994). Elsewhere in Sequoia and Kings Canyon National Parks (Kern, Kings, and San Joaquin River drainages), they resurveyed 22 historic sites and only 11 contained frogs (Bradford et al. 1994). Beginning just north of Kings Canyon National Park and running up into Yosemite National Park they resurveyed 24 historic sites and found frogs present at only 3 sites. In another study, Drost and Fellers (Drost & Fellers 1996) compared the presence of Sierran mountain yellow-legged frogs at historic sites (surveyed in 1915 by Grinnell and Storer) to distributions in 1995 and concluded that the species had generally "collapsed" compared to historical data. Grinnell and Storer (Grinnell & Storer 1924) stated that "the yellow-legged frog is the commonest amphibian in most parts of the Yosemite section." Drost and Fellers (1996) report finding frogs in only 2 of 14 historic sites (a single tadpole at one site, and an adult female at another). If we combine the data from the two resurvey studies in the Sierra Nevada (Bradford et al. 1994; Drost & Fellers 1996), there are 86 historic sites (data from 1915-'59), and only 16 contained frogs when they were revisited between 1989-'95. Therefore, only 18% of historic sites contained mountain yellow-legged frogs in recent surveys—that is a 82% decline. At the northern-most and southern-most part of the Sierran range (Butte and Plumas counties in the north, and Tulare County in the south), few populations have been seen since 1970 (Jennings & Hayes 1994). More recent surveys have reported very few mountain yellow-legged frog populations, but the authors were not able to compare to historic data in their survey areas due to the relatively low number of known historical sites(Knapp & Matthews 2000). The exact number of documented populations remaining in the Sierra Nevada is not available at this time, but several agencies (CDFG, USFS, NPS) and researchers (Knapp,

Davidson, Fellers, Vredenburg, Matthews and others) have discovered a large number of remaining populations. Unfortunately, most of the populations are very small. At this time, despite immense efforts by groups listed above, we are not aware of a single population north of Kings Canyon National Park (KCNP) that has more than 500 adults. Two hypotheses may be used to explain this pattern: 1) populations north of KCNP have suffered larger declines; or 2) populations north of KCNP may have always been smaller due to either habitat differences of phylogenetic differences. Given that Grinnell and Storer (1925) reported large numbers of mountain yellow-legged frogs at lakes in Yosemite, at this time, it seems likely that populations north of KCNP have suffered larger declines.

In southern California, mountain yellow-legged frog populations have declined nearly to extinction (Drost & Fellers 1996; Jennings & Hayes 1994; Stebbins & Cohen 1995). With only 6 - 8 extant populations, the largest having fewer than 100 adults (Mark Jennings, personal communication), the situation is tenuous at best for the mountain yellow-legged frog in southern California.

As one previous study showed, the results from our study clearly indicate that there are distinct evolutionary lineages of mountain yellow-legged frog within the Sierra Nevada and these should be conserved. While the Sierra Nevada contains the largest remaining populations (Anonymous 2002), all of the large populations (>500 adults) are contained within the southern clade (Fig 3). The goal of this study was to gain a better understanding of the northern populations, in particular to gain information of stream dwelling populations. After much difficulty, we were able to find several stream populations that were large enough to study, but there are few left. Most of our efforts on stream dwelling frogs were focused on populations in Deadwood Canyon, Cow Creek and Baker Creek (Figure 12-13), but we also were able to collect a significant amount of data at Rattlesnake Creek, Middle Creek and Summit Meadow. In addition, we collected information on northern lacustrine populations at Ebbetts Pass and Mono Pass.

Our work on the phylogeography of the species continues. Specimens from both clades are being examined morphologically and we are also attempting new molecular techniques. For example, nuclear markers can provide interesting comparisons to

mitochondrial datasets and highlight different evolutionary processes. We are beginning phylogenetic analysis of two nuclear genes (TROP and RP40) to assess how informative they may be as markers.

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Table Legends

Table I.
Microsatellites currently being used to test gene flow between *Rana muscosa* populations throughout the Sierra Nevada.

Table II.
Locality information for the samples included in the ND2 mitochondrial phylogeny for *Rana muscosa*.

Table III.
Site location information for the breeding study conducted in 2003.

Table IV.
Average values for habitat variables collected at individual radio-belted *Rana muscosa* at 4 sites in 2003.

Table V.
Visual encounter survey (VES) results from several sites in 2003.

Table VI.
Sample numbers of radio-tracked *Rana muscosa* at the different sites.

Figure Legends

Figure 1. A map of California with the known distribution of the Mountain Yellow-legged Frog (*Rana muscosa*). Points show available DNA tissue samples as of April 2004.

Figure 2.
A map of California showing all of the sample locations used in the ND2 mitochondrial phylogeny (green squares = northern clade; yellow squares = southern clade).

Figure 3.
A view of the two major mitochondrial clades for *Rana muscosa* within the Sierra Nevada (green squares = northern clade; yellow squares = southern clade).

Figure 4.
A view of the two major mitochondrial clades for *Rana muscosa* showing the major river drainages (note, some are missing) and the major roads.

Figure 5.
A view of the two major mitochondrial clades for *Rana muscosa* showing the topography of the area as well as the major roads. The sample east of Kearsarge Pass is part of the northern clade. The major break between the two lineages is along the Monarch Divide, between the middle and south fork of the Kings River.

Figure 6.
A view of the two major mitochondrial clades for *Rana muscosa* showing the available DNA samples in close proximity to the contact zone. For each local area, the numbers of sites and tissue per site are shown.

Figure 7.
Parsimony tree for the ND2 mitochondrial data set showing bootstrap values.

Figure 8.

This figure shows the 50% consensus tree of the ND2 mitochondrial data set for *Rana muscosa* from an analysis in MrBayes.

Figure 8a.

Parsimony and Bayesian phylogenies showing congruency in analysis. Both methods identified the Northern and Southern clades with similar confidence levels. Stars designate best supported branches.

Figure 9.

A phylogram using the consensus tree of the ND2 mitochondrial data set for *Rana muscosa* from an analysis in MrBayes. Confidence intervals are shows at the nodes.

Figure 9a.

Restriction fragment length polymorphism (RFLP) gel of six southern and six northern individuals, from mitochondrial DNA.

Figure 10.

Areas within the Sixty Lake Basin, Kings Canyon National Park selected for the first phase of the population genetics study on gene flow. Microsatellite analysis will be conducted on the 6 highlighted areas.

Figure 11.

A topographic map of the Sixty Lake Basin, Kings Canyon National Park showing the locations where genetic samples are available.

Figure 11a.

Allele frequencies per population for microsatellite locus D129. Frequency is equal to the percent of frogs with the allele out of the total number of frogs per population (~15 frogs).

Figure 12.
An overview showing the locations of the radio-tracking and egg-laying study sites.

Figure 13.
A closer view showing the locations of the radio-tracking and egg-laying study sites (note that site E-7 is shown in detail in Fig 10-11).

Figure 14.
A comparison of the generalized egg-laying habitats between northern and southern clade *Rana muscosa* (sample sizes are shown for each site on the x-axis).

Figure 15.
A comparison of the egg attachment substrate between northern and southern clade *Rana muscosa* (sample sizes are shown for each site on the x-axis).

Figure 16.
A comparison of the average egg mass size (diameter) between northern and southern clade *Rana muscosa* (sample sizes are shown for each site on the x-axis).

Figure 17.
A comparison of the distance to the nearest conspecific egg mass between northern and southern clade *Rana muscosa* (sample sizes are shown for each site on the x-axis).

Figure 18.
A comparison of the water velocity rate (feet/sec) averaged over a 20 second period measured at each egg mass between northern and southern clade *Rana muscosa* (sample sizes are shown for each site on the x-axis).

Figure 19.
A map showing all of the locations where radio-belted frogs were found in 2003 in the Deadwood Canyon site. The ecological data were recorded at each of these locations.

Figure 20.
A map showing all of the locations where radio-belted frogs were found in 2003 in the Deadwood Canyon site. The local areas where frogs were concentrated are shaded. The ecological data were recorded at each of these locations.

Figure 21.
A map showing all of the locations where radio-belted frogs were found in 2003 at the Cow Creek and Baker Creet sites. The ecological data were recorded at each of these locations.

Figure 22.
A map showing all of the locations where radio-belted frogs were found in 1999 in the Sixty Lake Basin, Kings Canyon National Park. The ecological data were recorded at each of these locations.

Figure 23.
A map showing all of the locations where radio-belted frogs were found in 1999 in the Sixty Lake Basin, Kings Canyon National Park. The local areas where frogs were concentrated are shaded.The ecological data were recorded at each of these locations.

Figure 24.
Movement patterns of radio-belted *Rana muscosa* in the Deadwood Canyon site in 2003. Distances between capture locations (shown as points) were calculated. Note the difference between stream distance and linear distance from point 1 to point 2.

Figure 25.
Movement patterns of two radio-belted *Rana muscosa* in the Deadwood Canyon site in 2003. Distances between capture locations (shown as points) were calculated.

Figure 26.

Movement patterns of one radio-belted *Rana muscosa* in the Deadwood Canyon site in 2003. Distances between capture locations (shown as points) were calculated.

Figure 27.
Movement patterns of one radio-belted *Rana muscosa* in the Deadwood Canyon site in 2003. Distances between capture locations (shown as points) were calculated.

Figure 28.
Movement patterns of one radio-belted *Rana muscosa* in the Cow Creek site in 2003. Distances between capture locations (shown as points) were calculated.

Figure 29.
Movement patterns of two radio-belted *Rana muscosa* in the Cow Creek site in 2003. Distances between capture locations (shown as points) were calculated.

Figure 30.
Movement patterns of three radio-belted *Rana muscosa* in the Baker Creek site in 2003. Distances between capture locations (shown as points) were calculated.

Figure 31.
Movement patterns of one radio-belted *Rana muscosa* in the Lake 12 site in the Sixty Lake Basin in 1999. Distances between capture locations (shown as points) were calculated.

Figure 32.
Movement patterns of one radio-belted *Rana muscosa* in the Lake 12 site in the Sixty Lake Basin in 1999. Distances between capture locations (shown as points) were calculated.

Figure 33.

Movement patterns of one radio-belted *Rana muscosa* in the Lake 30-31 site in the Sixty Lake Basin in 1999. Distances between capture locations (shown as points) were calculated. Introduced trout are present in the main stream seen flowing north on the west side of the map.

Figure 34.
Movement patterns of one radio-belted *Rana muscosa* in the Lake 30-31 site in the Sixty Lake Basin in 1999. Distances between capture locations (shown as points) were calculated. Introduced trout are present in the main stream seen flowing north on the west side of the map.

Figure 35.
Movement patterns of one radio-belted *Rana muscosa* in the Lake 30-31 site in the Sixty Lake Basin in 1999. Distances between capture locations (shown as points) were calculated. Introduced trout are present in the main stream seen flowing north on the west side of the map.

Figure 36.
Movement patterns of one radio-belted *Rana muscosa* in the Lake 30-31 site in the Sixty Lake Basin in 1999. Distances between capture locations (shown as points) were calculated. Introduced trout are present in the main stream seen flowing north on the west side of the map.

Figure 37.
Comparison of the mean movement patterns of all radio-belted *Rana muscosa* from 2003 and 1999. Baker Creek, Cow Creek, and Deadwood Canyon are in the northern mitochondrial clade while Lake 30-31 and Lake 12 are in the southern mitochondrial clade.

Figure 38.

Comparison of the mean movement patterns of all radio-belted *Rana muscosa* shown with the sexes separated from each other.

Figure 39.
Comparison of the mean movement patterns of all radio-belted *Rana muscosa* at Cow Creek and Baker Creek with the sexes separated from each other. Baker Creek contains introduced rainbow trout whereas Cow Creek is naturally fishless.

Figure 40.
Comparison of the mean movement patterns of all radio-belted *Rana muscosa* at Deadwood Canyon and Lake 30-31 in Sixty Lake Basin with the sexes separated from each other. The Lake 30-31 area contains introduced rainbow trout whereas Deadwood Canyon is naturally fishless.

Figure 41.
A comparison of the average gross distances moved by radio-belted *Rana muscosa* at all of the sites with the sexes separated from each other. This distance measure is calculated differently frm the previous graphs because it includes back and forth movements.

Figure 42.
A comparison of up-stream and down-stream stream gradients (shown as slope angle) at encounter sites for all 2003 frogs.

Figure 43.
A comparison of the channel type (Rosgen 1996; see appendix B) at each radio-belted frog capture location in 2003.

Figure 44.
A comparison of bank stability at each radio-belted frog capture location in 2003 (1 = stable, 2 = vulnerable, 3 = unstable; as in Rosgen 1996).

Figure 45.

A comparison of the local habitat at each radio-belted frog capture location in 2003.

Table I

Twelve microsatellite locus primer sets currently being analyzed for *Rana muscosa*.

Locus Name	Primer Sequence (5' to 3')	Microsatellite Motif	Product Length	Flourescent Color	Flourescent Label
D119_F	ATGCAGTTTACAGTTTCACACG	$(TAGA)_{21}$	133	green	HEX
D119_R	ATCCCCACACACGCTCTA				
A11_F	AACTGATACTTTTGGGTGTCTG	$(CA)_{16}$	148	green	HEX
A11_R	CGATTACCTGTCTTGGTGTC				
D14_F	TCCATGTCCATTTTGTGTTTG	$(TAGA)_{8}$	175	green	HEX
D14_R	GGTTACAACTGGGAGGTGTTG				
D209_F	GCACAGGGACACACACATC	$(TAGA)_{13}$	189	red	CyV
D209_R	GCTCGGAGATAGGTAGGGG				
D11_F	GCGATACACACCCCTGAG	$(AGAT)_{11}(ATAT)(AGAT)_{17}$	196	red	CyV
D11_R	GAAGCGACTGGATTTTCTTG				
D129_F	CCAAAGACAGAGGCACTTAG	$(TAGA)_{14}(TGGA)(TAGA)_{11}$	205	red	CyV
D129_R	TGCTCAGGACCTGTAGGTAG				
D114_F	CCTGGTGCCATTATTTTTTTAG	$(TAGA)_{21}$	236	yellow	TAMRA
D114_R	TTATCCCGGAGGAGTACAGTC				
D208_F	AGTCCTTCTCCACTTTTTTCTC	$(TAGA)_{12}$	240	yellow	TAMRA
D208_R	CAGCCTGTTCTGGGTTATT				
A19_F	TTATGTGGGCATGGTAAGTG	$(CA)_{17}$	247	yellow	TAMRA
A19_R	CAAAGCAAATGGGATTTAGC				
A104_F	CAACGGGGACATTCTAAAG	$(CA)_{12}$	253	blue	6-FAM
A104_R	CCCCTAGTCTGCAAATAAAAA				
D125_F	GGTGCTGCATCACTATAATTTC	$(TAGA)_{13}$	270	blue	6-FAM
D125_R	ATGTGGACATTGGCTTTATTC				
D131_F	CCTTTGGAGGACGATACAGG	$(TAGA)_{13}$	284	blue	6-FAM
D131_R	GCAGACAGTAGCACAGCACAC				

Table II.

Locality information for samples in mitochondrial phylogeny.

Specimen ID	Site Name	Major Clade	Latitude	Longitude
CAS 203394	Independence Lake	N	39.48775	-120.28421
CAS 206093	Rock Creek	N	39.86452	-121.00064
CAS 209370	Faggs Reservoir	N	39.84125	-121.18679
CAS 209386	Silver Lake. Plumas	N	39.95894	-121.13589
CAS 209404	Rock Lake	N	39.94134	-121.14254
CAS 209668	Pine Grove Cemetery, Plumas	N	39.71904	-120.89915
CAS 227639	Lone Rock Creek	N	40.20039	-120.64747
CAS 227640	Boulder Creek	N	40.25435	-120.60281
JAM 70	Rodgers Lake	N	37.73335	-119.22633
LJR 095	Little Indian Valley	N	38.59368	-119.88543
LJR 260	Mono Pass	N	37.85230	-119.21954
LJR 1048	Roosevelt Lake	N	37.96890	-119.34396
MVZ 149008	Levitt Lake	N	38.26977	-119.61717
MVZ 180163	Ebbetts Pass, Obel Lake	N	38.52834	-119.77573
MVZ 227662	Sonora Pass, 1.7 mi SW Sonora Pass	N	38.33104	-119.65471
RAK 100	N of Humphreys Basin	N	37.03225	-118.59565
RAK 123	Humphreys Basin	N	37.25831	-118.68290
RAK 1235	Gable Lakes #2	N	37.33035	-118.70003
RAK 2128	Bear Crk	N	37.33054	-118.80141
RAK 2378	North Palisade Peak	N	37.02406	-118.54264
RAK 2393	Mather Pass	N	37.03668	-118.47281
RAK 2638	Monarch Divide	N	36.86386	-118.59893
RAK 2695	Monarch Divide	N	36.94396	-118.57199

RAK 2727	Monarch Divide	N	36.88030	-118.59972
RAK 2737	Monarch Divide	N	36.88851	-118.72512
RAK 294	Summit Mdw	N	37.67930	-119.64765
RAK 3006	Amphitheater Lake	N	37.02305	-118.50293
RAK 3321	Near Slide Peak	N	36.89487	-118.68287
RAK 3489	Monarch Divide	N	36.88753	-118.66915
RAK 4066	Saddle Horse Lake	N	37.61139	-119.57666
RAK 4074	Merced Peak	N	37.68014	-119.39944
RAK lake 10090	Muir Pass, 1.5 km NW	N	37.11941	-118.69150
RAK lake 10100	E Wanda Lk	N	37.12197	-118.64196
RAK lake 10102	NW Wanda Lk	N	37.11428	-118.64374
RAK lake 10269	Amphitheatre Lk	N	37.01347	-118.49509
RAK lake 11517	Dusy Basin	N	37.09616	-118.55349
RAK lake 72996	Mt Conness (near Roosevelt Lk)	N	37.96890	-119.34396
VTV 060	Granite Lake, near Caples Lk	N	38.65190	-120.11030
VTV 061	4th of July Pk, SF American	N	38.67080	-120.03370
VTV 062	Mossy Pond, SF Yuba	N	39.37860	-120.46920
VTV 065	200m E Tamarack Lk	N	38.61230	-119.89130
VTV 075	Oliver Lake	N	39.98044	-121.32998
VTV 086	Haven Lake, MF Feather	N	39.67030	-120.63290
VTV 1022	Locality unspecified	N	38.18231	-119.74540
VTV 107	Lake Zitella	N	38.96050	-120.22540
VTV 110	Tragedy Creek	N	38.61930	-120.16740
VTV 1100	Gertrude Lk	N	37.61920	-119.14680

VTV 1101	Thousand Island Basin	N	37.73350	-119.19312
VTV 114	Deadwood Creek	N	38.60040	-119.99930
VTV 139	Lwr Pyramid Pk Lk	N	38.86190	-120.64140
VTV 152	Middle Creek	N	38.75810	-120.24960
VTV 1547	Locality unspecified	N	38.19589	-119.57981
VTV 1550	Above Peeler Lake	N	38.11730	-119.46135
VTV 1552	Thousand Island Lake	N	37.70423	-119.23950
VTV 1554	4th of July Lake, Kearsarge	N	36.76334	-118.35512
VTV 1555	Birch Creek	N	37.52787	-118.67653
VTV 1559	Horton Creek	N	37.31209	-118.67454
VTV 1560	Minaret Creek	N	37.64667	-119.14544
VTV 1575	Mills Creek	N	37.40222	-118.82347
VTV 371	Gable Lake #2	N	37.33110	-118.69050
VTV 372	Big Pine Lake #8	N	37.13790	-118.51720
VTV 979	Lake Camp Lake	N	37.25855	-119.05444
VTV 987	Cow Creek	N	37.17590	-118.43982
VTV 997	Baker Creek	N	37.16980	-118.46910
VTV 999	Rattlesnake Creek	N	39.33535	-120.47953
Y-258	Merced Pass, S Yosemite	N	37.62278	-119.41581
Y-358	NW Tilden Canyon Creek	N	38.06493	-119.61156
Y-638	Kuna Basin	N	37.79426	-119.22622
LJR 089	Laurel Basin	S	36.36813	-118.48107
MVZ 226112	Lake 1, 60 lk	S	36.81409	-118.42532
MVZ 230140	San Gabriel Mt	S	34.35140	-117.71010
MVZ 230141	Jan Bernardino Mt	S	33.77959	-116.77464
MVZ 230142	San Jacinto Mt	S	34.17718	-117.18184
RAK 1311	Upper Basin#2	S	37.01546	-118.47288
RAK 1727	Upper Basin #1	S	37.01420	-118.44000
RAK 1776	Headwaters Kern, W side	S	36.67590	-118.44282
RAK 2162	Near Muro Blanco	S	36.93756	-118.53603

RAK 2962	Upper Basin	S	37.02271	-118.45886
RAK 2989	Upper Basin	S	37.02323	-118.45609
RAK 299	Hitchcock Lk	S	36.56159	-118.30698
RAK 3552	Tyndall Creek	S	36.61524	-118.43890
RAK 3584	Milestone Basin	S	36.64649	-118.45009
RAK 3713	Coyote Pass	S	36.35824	-118.47114
RAK 3924	Tyndall Creek	S	36.65296	-118.38343
RAK 559	Bighorn Plateau	S	36.62267	-118.34730
RAK 606	Vidette Creek	S	36.72440	-118.41607
RAK 671	Woods Lake	S	36.88670	-118.40018
RAK lake 10314	Woods Creek	S	36.95267	-118.41428
RAK lake 20226	Headwaters Kern	S	36.68959	-118.42365
S-376	Lake S. America	S	36.66033	-118.41967
S-387	Upper Kern (near Lk S America)	S	36.67802	-118.42436
S-508	Golden Bear Lake	S	36.72808	-118.35995
VTV 055	Bullfrog Lake	S	36.39820	-118.55360
VTV 1578	Mulkey Meadow	S	36.40240	-118.21144
VTV 874	Ansel Adams Lake	S	36.91430	-118.39640

Table III

SITE LOCATION INFORMATION

Site	Site ID	Northern / Southern	Lat	Lon	Dates Surveyed	Frogs Found	Egg Site	# Egg masses
Middle Creek	E-2	N	38.75882	120.24954	5/10, 5/27, 6/7-6/9	Yes	Yes	26
Rattlesnake Creek lake	E-1	N	39.33586	120.48079	6/14/2004, 7/15	Yes	Yes	22
Summit Meadow	E-6	N	37.6801	119.64908	6/23-6/24	Yes	Yes	38
Ebbetts Pass	E-4, R-2	N	38.55329	119.8225	6/25-	Yes	Yes	104
Mono Pass	E-5	N	37.85385	119.22022	26-Jun	Yes	Yes	300
Tragedy Creek	not shown	N	38.61233	120.16943	6/10,6/12	1 dead adult	No	
Emigrant Creek	not shown	N	38.64978	120.02421	6/11,7/17	No	No	
Cow Creek	R-3	N	37.18096	118.44459	8/11-	Yes	No	
Baker Creek	R-4	N	37.17007	118.46787	8/11-	Yes	No	

Deadwood Canyon	E-3, R-1	N	38.60176	119.99748	6/19-	Yes	Yes	32
4th of July Lake	not shown	N	38.6499	120.0246	17-Jul	No	No	
Sixty Lake Basin	E-7	S*	36.8179	118.4265	multiple dates and sites	Yes	Yes	310+

Table IV

MEAN HABITAT VARIABLES AT STUDY LOCATIONS

	Deadwood Canyon	**Baker Creek**	**Cow Creek**	**Ebbetts Pass**	**Total**
Canopy Cover					
Mean	11.48	3.59	0.00	23.68	10.53
Standard Error	1.14	2.30	0.00	5.23	1.14
Standard Deviation	11.74	11.25	0.00	684.15	26.16
Min	0.00	0.00	0.00	1.04	0.00
Max	56.16	40.00	0.00	100.00	100.00
Bank Angle					
Mean	134.47	141.46	110.07	139.61	132.28
Standard Error	3.39	7.33	6.81	8.64	2.76
Standard Deviation	34.88	41.42	36.02	35.89	37.10
Min	18.00	75.00	25.00	31.00	18.00
Max	180.00	180.00	175.00	180.00	180.00
% Aquatic Vegetation					
Mean	31.65	22.50	31.07	28.40	29.92
Standard Error	2.74	4.92	5.40	7.00	2.12
Standard Deviation	28.16	24.09	28.56	35.02	28.69
Min	0.00	0.00	5.00	0.00	0.00
Max	95.00	80.00	90.00	100.00	100.00
% Terrestrial Vegetation					

Mean	69.29	88.75	83.04	82.71	75.81
Standard Error	3.38	2.89	6.44	3.39	2.34
Standard Deviation	34.50	14.16	34.08	16.61	31.35
Min	0.00	50.00	0.00	30.00	0.00
Max	100.00	100.00	100.00	100.00	100.00
Wetted Width					
Mean	306.68	165.66	117.03	31.92	231.73
Standard Error	18.93	7.97	12.58	7.09	13.77
Standard Deviation	169.31	39.05	66.55	17.37	161.72
Min	35.00	86.00	35.56	15.00	15.00
Max	761.00	281.94	324.00	53.00	761.00
Thalweg Depth					
Mean	74.53	38.45	26.70	14.75	57.09
Standard Error	4.24	2.25	1.56	9.55	3.21
Standard Deviation	40.04	11.03	8.26	23.40	38.86
Min	1.00	21.00	12.50	4.00	1.00
Max	149.86	61.00	47.00	62.50	149.86
IR Temperature					
Mean	18.71	15.33	26.25	15.80	18.83
Standard Error	0.83	3.38	3.71	3.18	0.86
Standard Deviation	4.87	5.86	7.41	7.12	5.81
Min	10.00	11.00	18.00	6.00	6.00
Max	30.00	22.00	33.00	23.00	33.00
H20 Temperature					
Mean	16.35	14.17	14.13	16.17	15.75
Standard Error	0.46	0.80	1.01	1.33	0.39

Standard Deviation	4.57	3.09	5.24	6.49	4.95
Min	7.50	8.50	4.00	5.00	4.00
Max	29.00	18.00	21.50	28.00	29.00
Distance to H20					
Mean	24.04	22.92	2.33	58.50	26.02
Standard Error	7.15	12.74	0.33	33.14	6.54
Standard Deviation	35.77	22.06	0.58	66.27	38.69
Min	1.00	8.00	2.00	4.00	1.00
Max	150.00	48.26	3.00	140.00	150.00
Distance From Shore					
Mean	31.31	13.95	7.34	123.66	39.41
Standard Error	9.71	8.40	2.57	28.83	7.77
Standard Deviation	77.65	27.86	12.84	125.65	84.75
Min	0.00	0.00	0.00	0.00	0.00
Max	569.00	91.44	40.00	355.60	569.00
Submerged Depth					
Mean	17.19	13.14	15.46	6.88	14.84
Standard Error	2.91	3.42	2.92	2.53	1.77
Standard Deviation	22.35	11.34	14.29	10.42	18.62
Min	1.00	1.00	1.00	1.00	1.00
Max	102.00	32.50	59.00	40.00	102.00
Flow At Thalweg					
Mean	0.065555556	not available	not available	not available	
Standard Error	0.032365581	not available	not available	not available	
Standard Deviation	0.097096744	not	not	not	

		available	available	available	
Min	0.00	not available	not available	not available	
Max	0.25	not available	not available	not available	
Mean Flow near Frog					
Mean	0.033488372	not available	not available	not available	
Standard Error	0.016678458	not available	not available	not available	
Standard Deviation	0.109367962	not available	not available	not available	
Min	0.00	not available	not available	not available	
Max	0.70	not available	not available	not available	

Table V

VISUAL ENCOUNTER SURVEYS

Location and Dates Surveyed	**# adults**	**#juv**	**# tads**
Cow Creek			
11-Aug 2003	547	178	330
Deadwood Canyon			
20-Jun 2003	9	1	1
24-25 Jul 2003	34	26	56
10-Jul 2003	28	19	515
Rattlesnake Creek			
13-Jun 2003	6	0	0
15-Jul 2003	9	2	58
Middle Creek			
8-9 Jun 2003	12	10	3

Table VI

RADIO-TRACKED FROG SAMPLE NUMBERS

Location and Dates Surveyed	**Number of males**	**Number of females**	**year**	**Genetic Group**
Cow Creek	5	3	2003	North
Baker Creek	3	2	2003	North
Deadwood Canyon	10	12	2003	North
Ebbets Pass	1	5	2003	North
Sixty Lake Basin				
-Lake 30-31	8	6	1999	South
-Lake 12-13	6	6	1999	South
Total		68		

Figure 1.

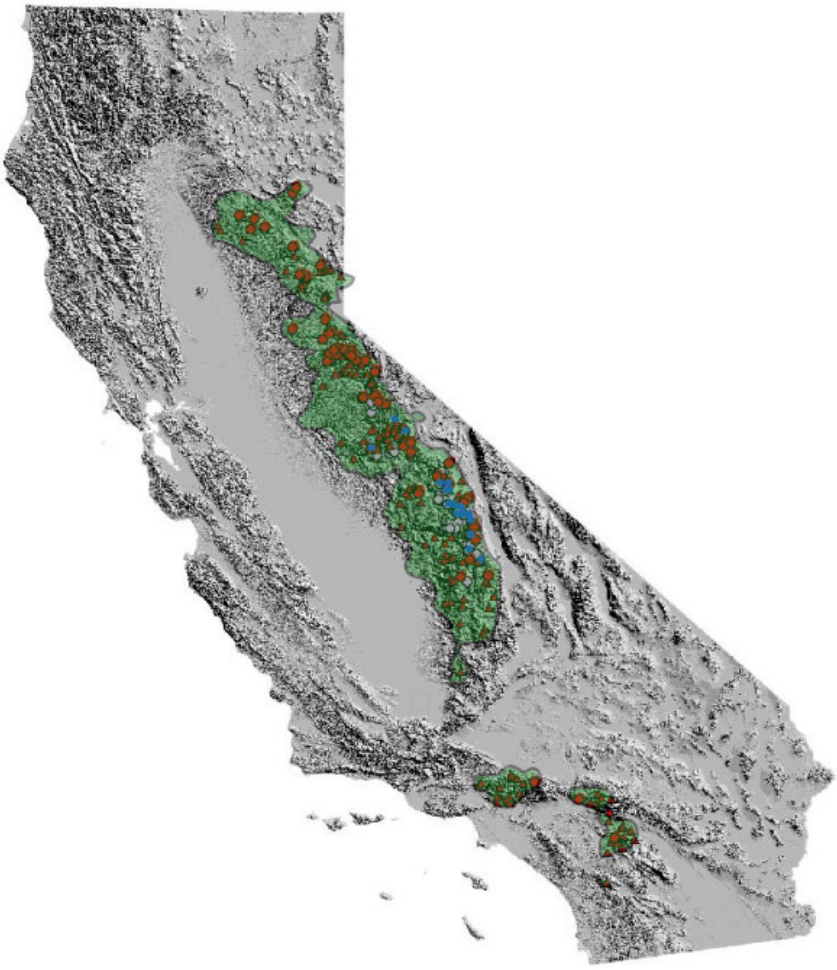


Figure 2.

Northern and Southern clades for the species.

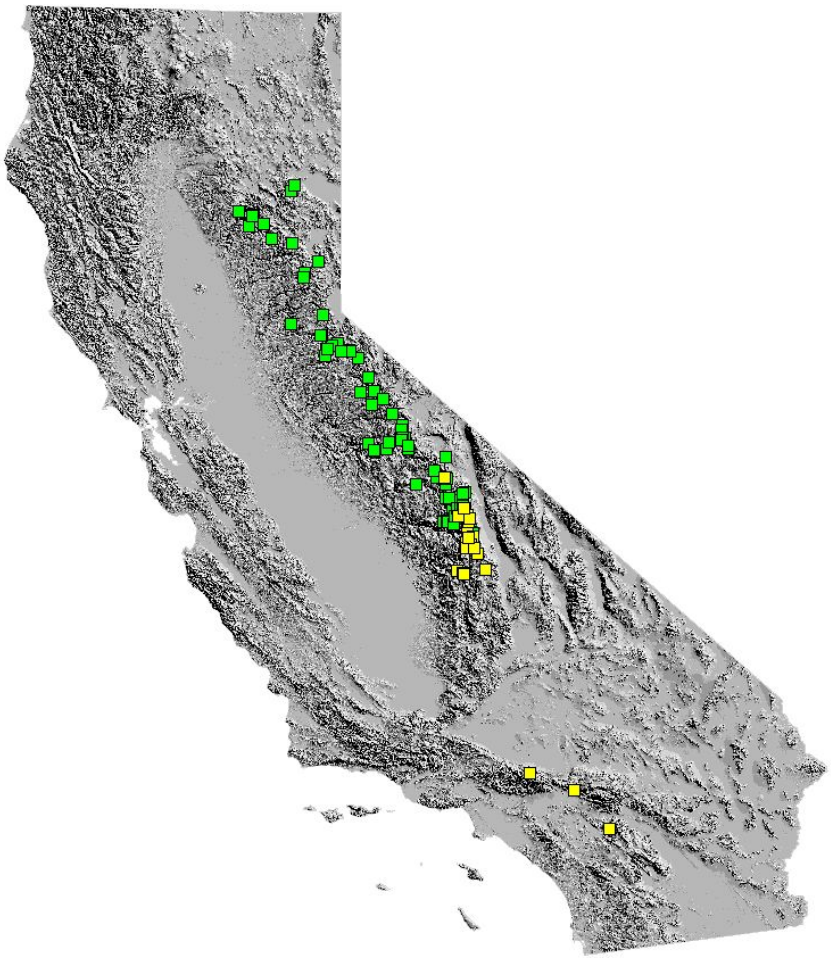


Figure 3.

The two clades in the Sierra Nevada.

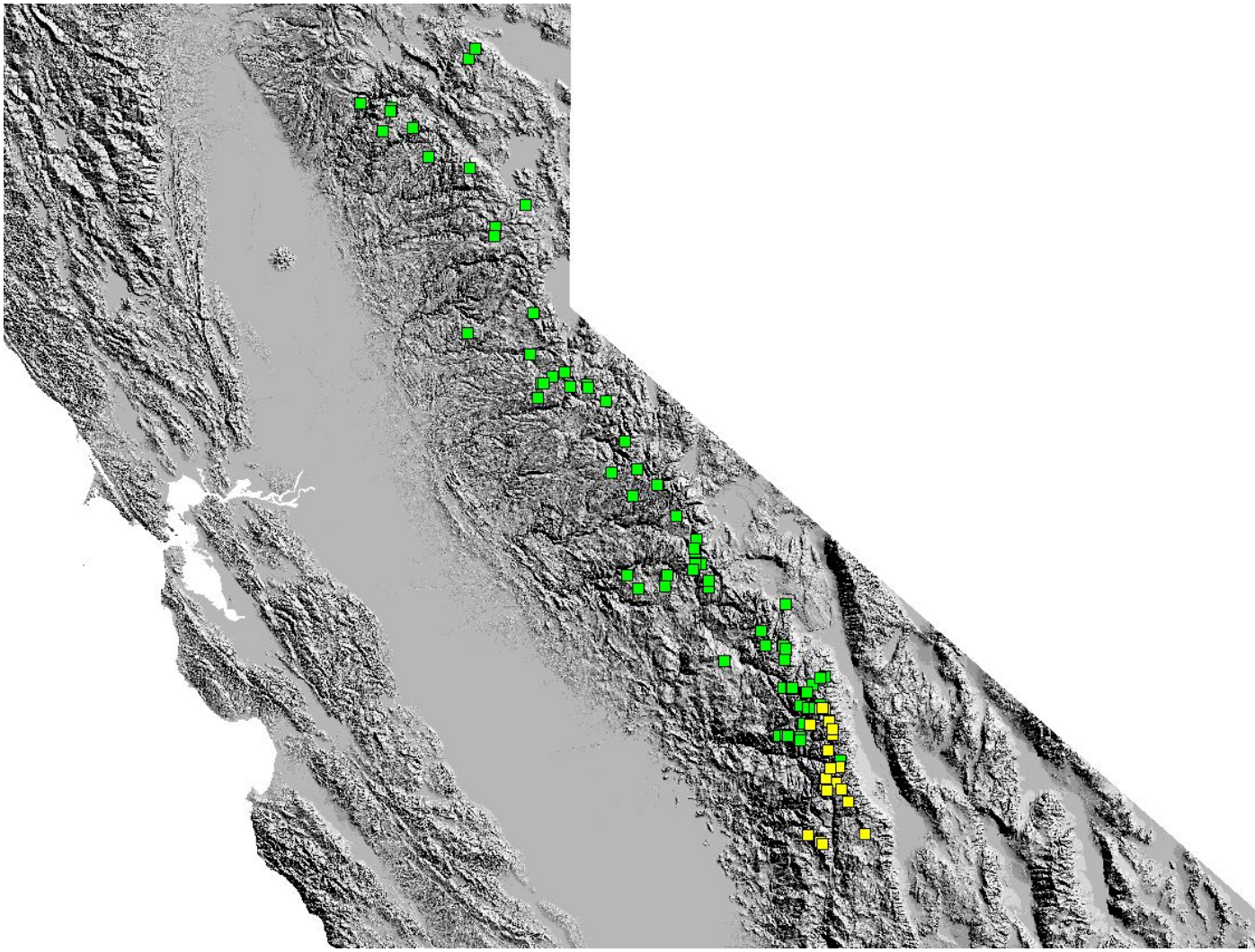


Figure 4

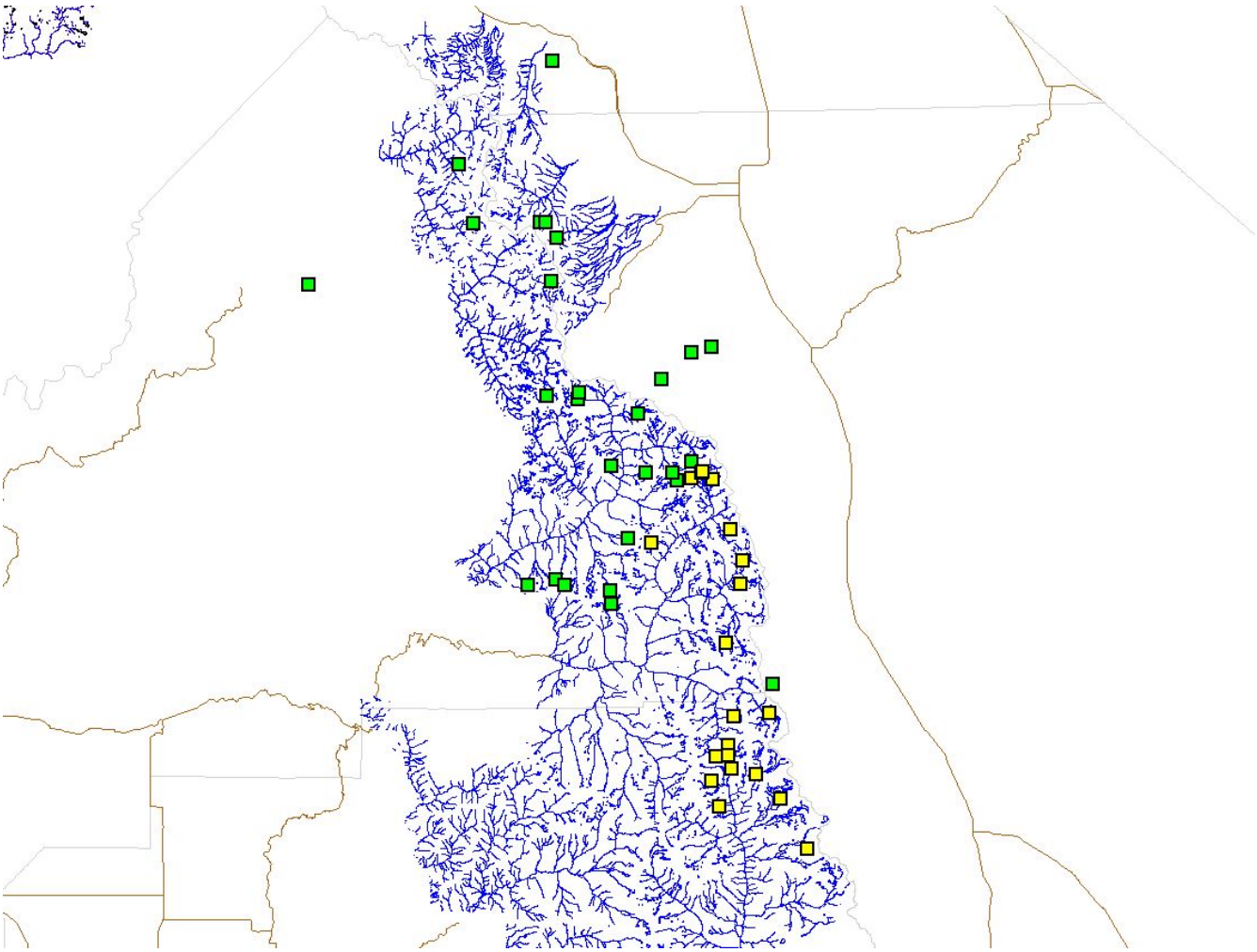
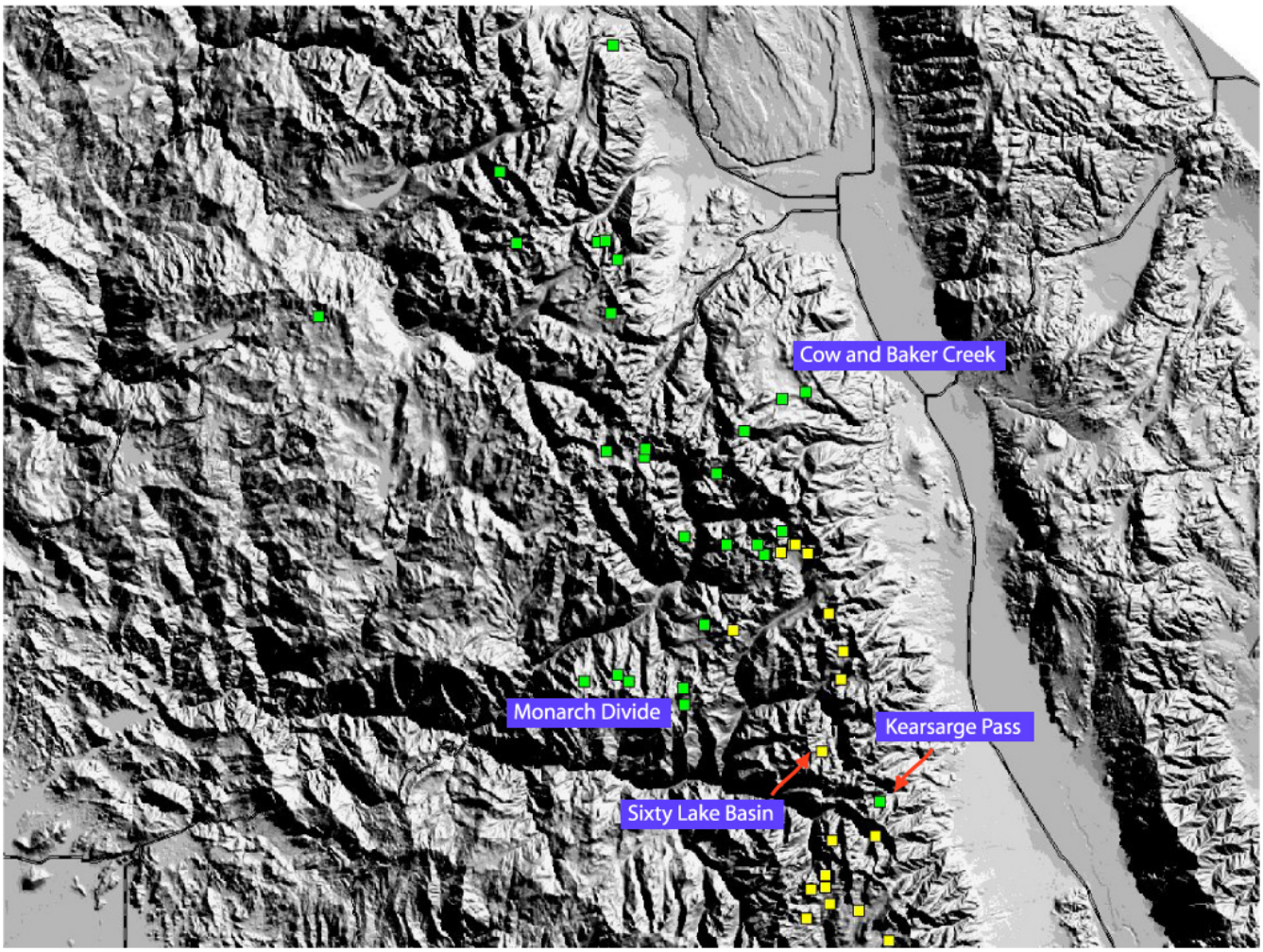


Figure 5.


Cow and Baker Creek
Monarch Divide
Kearsarge Pass
Sixty Lake Basin

Figures 6.

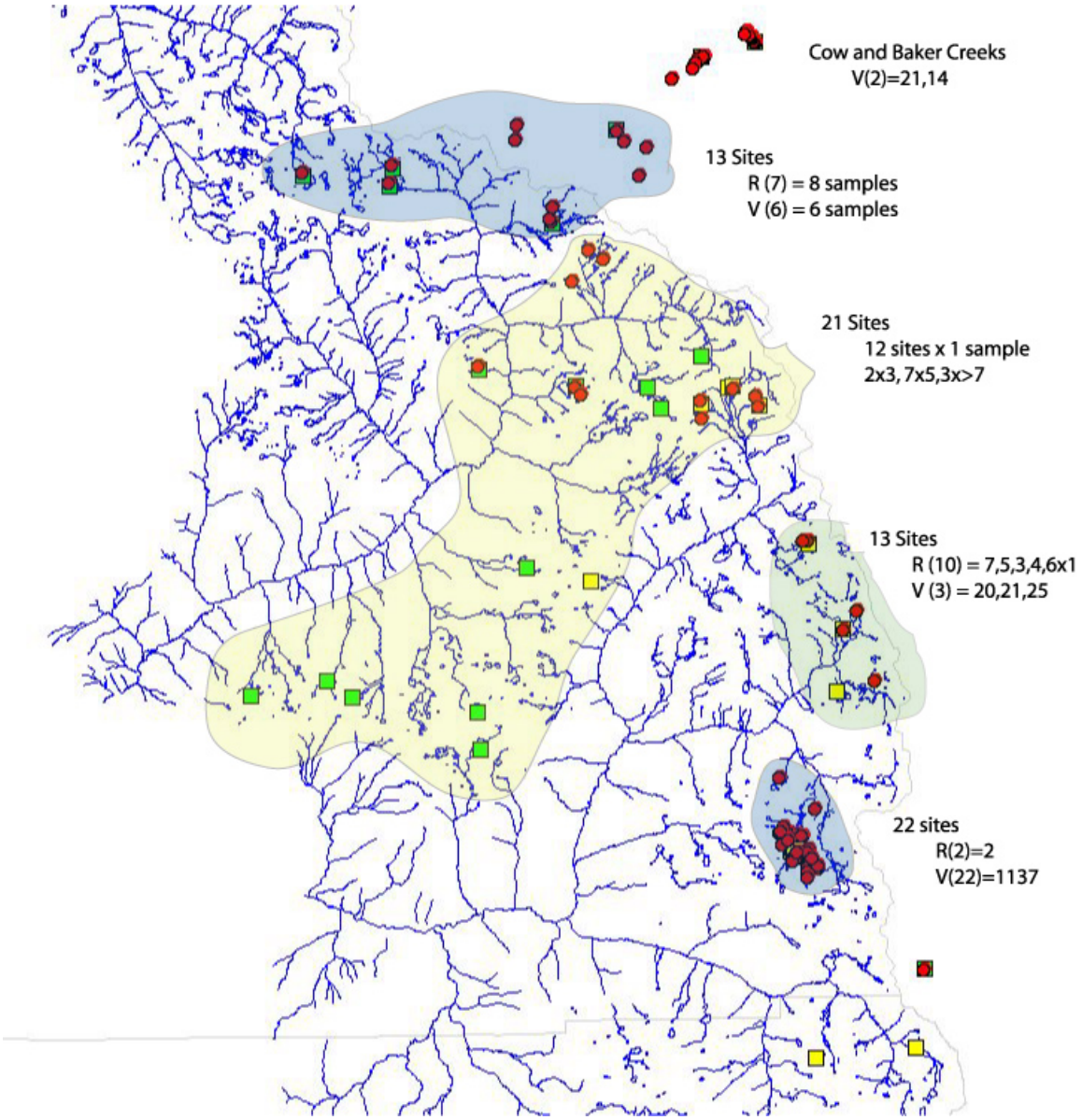

Cow and Baker Creeks
V(2)=21,14
13 Sites
R (7) = 8 samples
V (6) = 6 samples
21 Sites
12 sites x 1 sample
2x3, 7x5,3x>7
13 Sites
R (10) = 7,5,3,4,6x1
V (3) = 20,21,25
22 sites
R(2)=2
V(22)=1137

Figure 7.

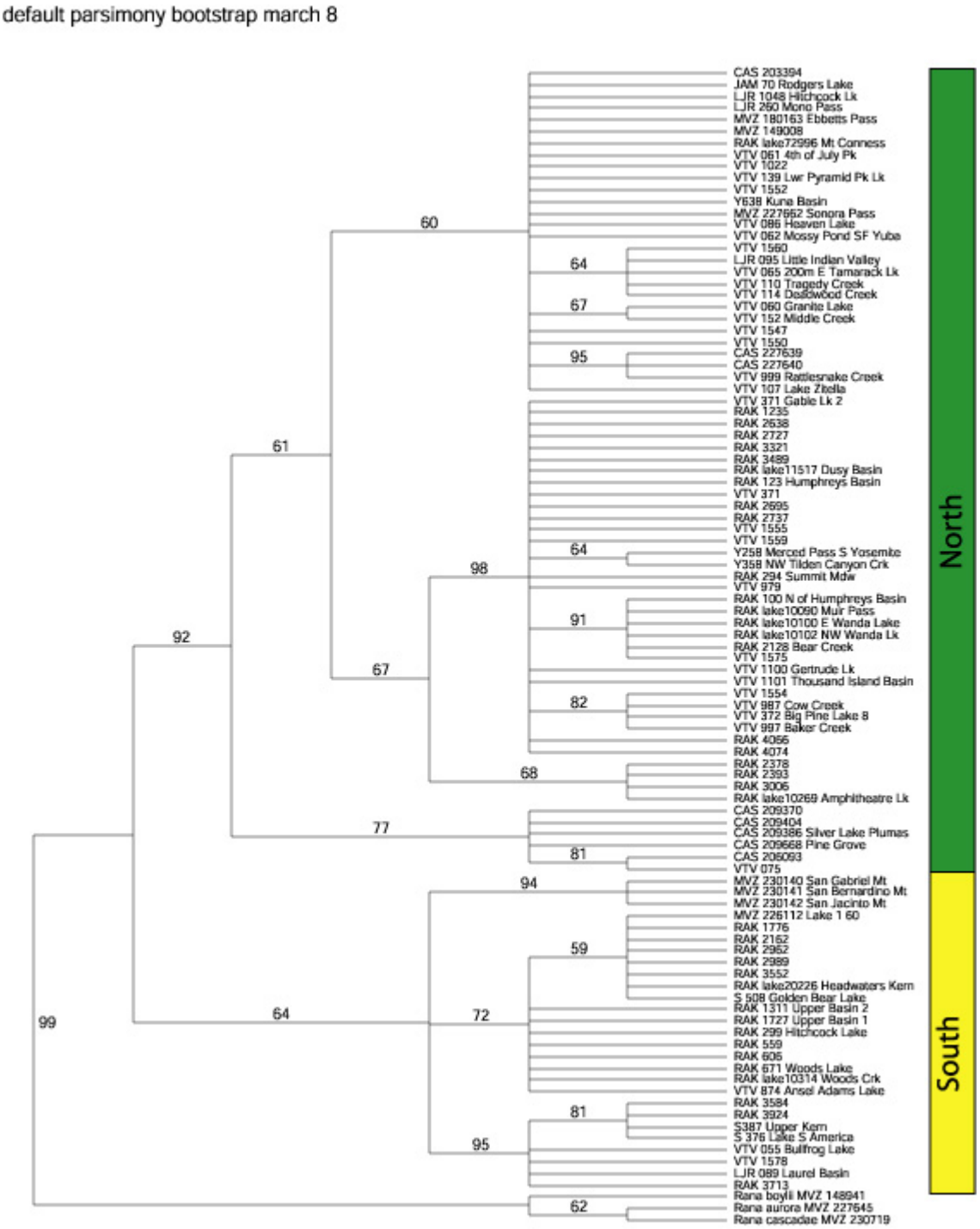

default parsimony bootstrap march 8
CAS 203394
JAM 70 Rodgers Lake
LJR 1048 Hitchcock Lk
LJR 260 Mono Pass
MVZ 180163 Ebbetts Pass
MVZ 149008
RAK lake72996 Mt Conness
VTV 061 4th of July Pk
VTV 1022
VTV 139 Lwr Pyramid Pk Lk
VTV 1552
Y638 Kuna Basin
MVZ 227662 Sonora Pass
VTV 086 Heaven Lake
VTV 062 Mossy Pond SF Yuba
VTV 1560
LJR 095 Little Indian Valley
VTV 065 200m E Tamarack Lk
VTV 110 Tragedy Creek
VTV 114 Deadwood Creek
VTV 060 Granite Lake
VTV 152 Middle Creek
VTV 1547
VTV 1550
CAS 227639
CAS 227640
VTV 999 Rattlesnake Creek
VTV 107 Lake Zitella
VTV 371 Gable Lk 2
RAK 1235
RAK 2638
RAK 2727
RAK 3321
RAK 3489
RAK lake11517 Dusy Basin
RAK 123 Humphreys Basin
VTV 371
RAK 2695
RAK 2737
VTV 1555
VTV 1559
Y258 Merced Pass S Yosemite
Y358 NW Tilden Canyon Crk
RAK 294 Summit Mdw
VTV 979
RAK 100 N of Humphreys Basin
RAK lake10090 Muir Pass
RAK lake10100 E Wanda Lake
RAK lake10102 NW Wanda Lk
RAK 2128 Bear Creek
VTV 1575
VTV 1100 Gertrude Lk
VTV 1101 Thousand Island Basin
VTV 1554
VTV 987 Cow Creek
VTV 372 Big Pine Lake 8
VTV 997 Baker Creek
RAK 4066
RAK 4074
RAK 2378
RAK 2393
RAK 3006
RAK lake10269 Amphitheatre Lk
CAS 209370
CAS 209404
CAS 209386 Silver Lake Plumas
CAS 209668 Pine Grove
CAS 206093
VTV 075
MVZ 230140 San Gabriel Mt
MVZ 230141 San Bernardino Mt
MVZ 230142 San Jacinto Mt
MVZ 226112 Lake 1 60
RAK 1776
RAK 2162
RAK 2962
RAK 2989
RAK 3552
RAK lake20226 Headwaters Kern
S 508 Golden Bear Lake
RAK 1311 Upper Basin 2
RAK 1727 Upper Basin 1
RAK 299 Hitchcock Lake
RAK 559
RAK 606
RAK 671 Woods Lake
RAK lake10314 Woods Crk
VTV 874 Ansel Adams Lake
RAK 3584
RAK 3924
S387 Upper Kern
S 376 Lake S America
VTV 055 Bullfrog Lake
VTV 1578
LJR 089 Laurel Basin
RAK 3713
Rana boylii MVZ 148941
Rana aurora MVZ 227645
Rana cascadae MVZ 230719
60
64
67
95
61
64
98
91
92
67
82
68
77
81
94
59
99
64
72
81
95
62
North
South

Figure 8.

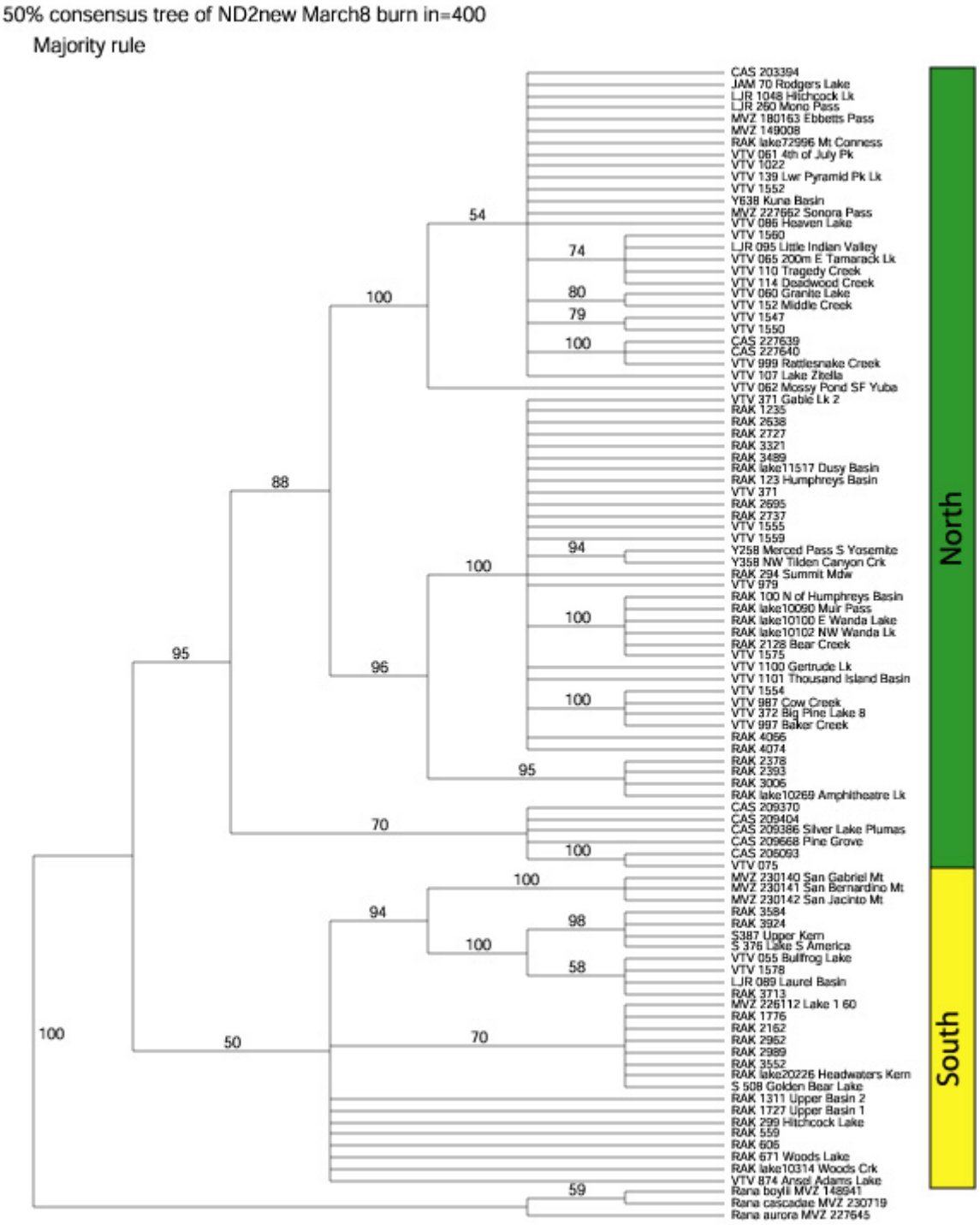

50% consensus tree of ND2new March8 burn in=400
Majority rule
CAS 203394
JAM 70 Rodgers Lake
LJR 1048 Hitchcock Lk
LJR 260 Mono Pass
MVZ 180163 Ebbetts Pass
MVZ 149008
RAK lake72996 Mt Conness
VTV 061 4th of July Pk
VTV 1022
VTV 139 Lwr Pyramid Pk Lk
VTV 1552
Y638 Kuna Basin
MVZ 227662 Sonora Pass
VTV 086 Heaven Lake
VTV 1560
LJR 095 Little Indian Valley
VTV 065 200m E Tamarack Lk
VTV 110 Tragedy Creek
VTV 114 Deadwood Creek
VTV 060 Granite Lake
VTV 152 Middle Creek
VTV 1547
VTV 1550
CAS 227639
CAS 227640
VTV 999 Rattlesnake Creek
VTV 107 Lake Zitella
VTV 062 Mossy Pond SF Yuba
VTV 371 Gable Lk 2
RAK 1235
RAK 2638
RAK 2727
RAK 3321
RAK 3489
RAK lake11517 Dusy Basin
RAK 123 Humphreys Basin
VTV 371
RAK 2695
RAK 2737
VTV 1555
VTV 1559
Y258 Merced Pass S Yosemite
Y358 NW Tilden Canyon Crk
RAK 294 Summit Mdw
VTV 979
RAK 100 N of Humphreys Basin
RAK lake10090 Muir Pass
RAK lake10100 E Wanda Lake
RAK lake10102 NW Wanda Lk
RAK 2128 Bear Creek
VTV 1575
VTV 1100 Gertrude Lk
VTV 1101 Thousand Island Basin
VTV 1554
VTV 987 Cow Creek
VTV 372 Big Pine Lake 8
VTV 997 Baker Creek
RAK 4056
RAK 4074
RAK 2378
RAK 2393
RAK 3006
RAK lake10269 Amphitheatre Lk
CAS 209370
CAS 209404
CAS 209386 Silver Lake Plumas
CAS 209668 Pine Grove
CAS 206093
VTV 075
MVZ 230140 San Gabriel Mt
MVZ 230141 San Bernardino Mt
MVZ 230142 San Jacinto Mt
RAK 3584
RAK 3924
S387 Upper Kern
S 376 Lake S America
VTV 055 Bullfrog Lake
VTV 1578
LJR 089 Laurel Basin
RAK 3713
MVZ 226112 Lake 1 60
RAK 1776
RAK 2162
RAK 2962
RAK 2989
RAK 3552
RAK lake20226 Headwaters Kern
S 508 Golden Bear Lake
RAK 1311 Upper Basin 2
RAK 1727 Upper Basin 1
RAK 299 Hitchcock Lake
RAK 559
RAK 605
RAK 671 Woods Lake
RAK lake10314 Woods Crk
VTV 874 Ansel Adams Lake
Rana boylii MVZ 148941
Rana cascadae MVZ 230719
Rana aurora MVZ 227645
54
74
100
80
79
100
88
94
100
100
95
96
100
95
70
100
100
94
98
100
58
100
50
70
59
North
South

Figure 8a.

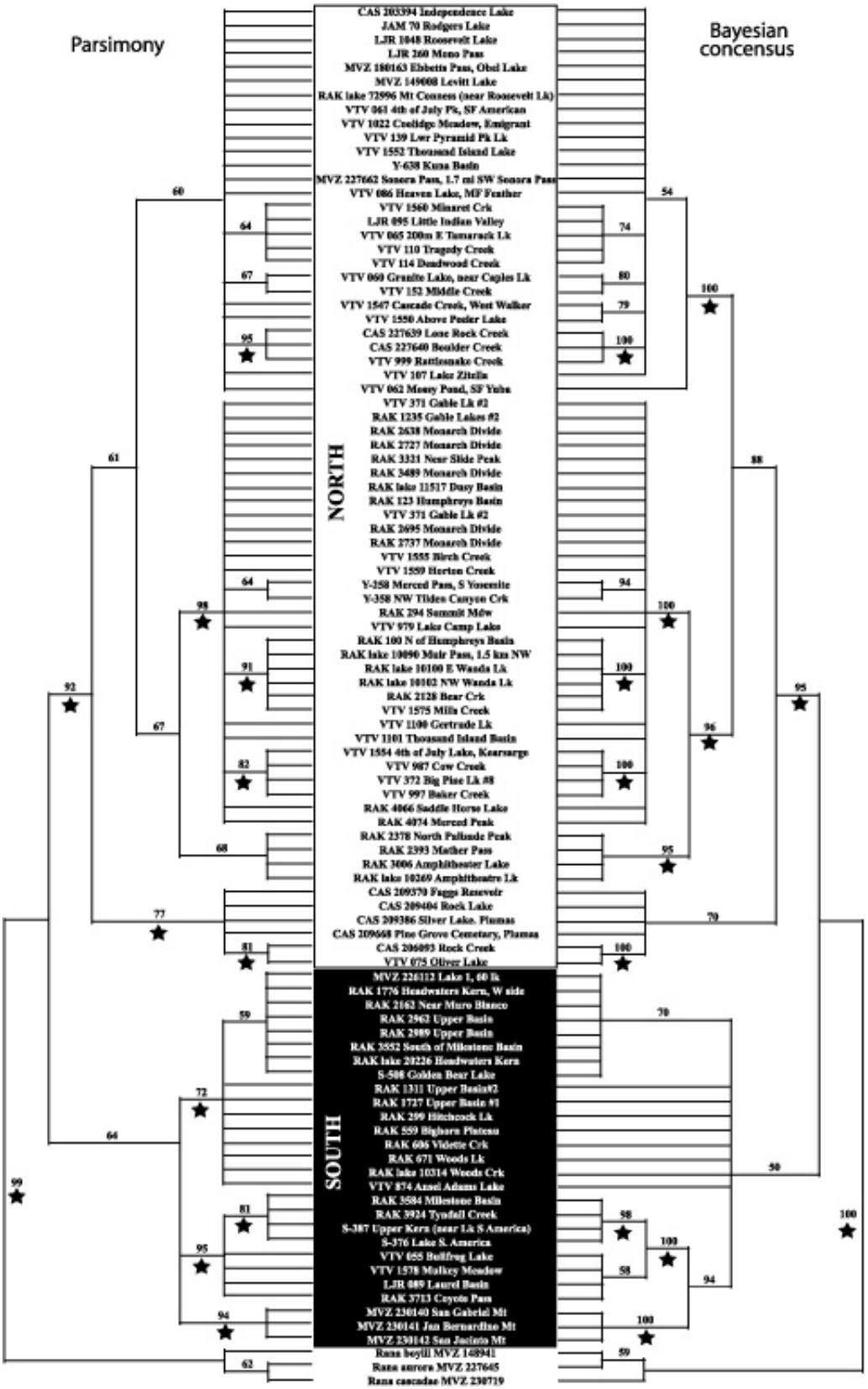

Parsimony
Bayesian concensus
CAS 203394 Independence Lake
JAM 70 Rodgers Lake
LJR 1048 Roosevelt Lake
LJR 260 Mono Pass
MVZ 180163 Ebbetts Pass, Obsi Lake
MVZ 149008 Levitt Lake
RAK lake 72596 Mt Conness (near Roosevelt Lk)
VTV 061 4th of July Pk, SF American
VTV 1022 Coolidge Meadow, Emigrant
VTV 139 Lwr Pyramid Pk Lk
VTV 1552 Thousand Island Lake
Y-638 Kuna Basin
MVZ 227662 Sonora Pass, 1.7 mi SW Sonora Pass
VTV 086 Heaven Lake, MF Feather
VTV 1560 Minaret Crk
LJR 095 Little Indian Valley
VTV 065 200m E Tamarack Lk
VTV 110 Tragedy Creek
VTV 114 Deadwood Creek
VTV 060 Granite Lake, near Cagles Lk
VTV 152 Middle Creek
VTV 1547 Cascade Creek, West Walker
VTV 1550 Above Peeler Lake
CAS 227639 Lone Rock Creek
CAS 227640 Boulder Creek
VTV 999 Rattlesnake Creek
VTV 107 Lake Zitella
VTV 062 Mossy Pond, SF Yuba
VTV 371 Gable Lk #2
RAK 1235 Gable Lakes #2
RAK 2638 Monarch Divide
RAK 2727 Monarch Divide
RAK 3321 Near Slide Peak
RAK 3489 Monarch Divide
RAK lake 11517 Dusy Basin
RAK 123 Humphreys Basin
VTV 371 Gable Lk #2
RAK 2695 Monarch Divide
RAK 2737 Monarch Divide
VTV 1555 Birch Creek
VTV 1559 Horton Creek
Y-358 Merced Pass, S Yosemite
Y-358 NW Tilden Canyon Crk
RAK 294 Summit Mdw
VTV 979 Lake Camp Lake
RAK 100 N of Humphreys Basin
RAK lake 10090 Muir Pass, 1.5 km NW
RAK lake 10100 E Wanda Lk
RAK lake 10102 NW Wanda Lk
RAK 2128 Bear Crk
VTV 1575 Mills Creek
VTV 1100 Gertrude Lk
VTV 1101 Thousand Island Basin
VTV 1554 4th of July Lake, Kearsarge
VTV 987 Cow Creek
VTV 372 Big Pine Lk #8
VTV 997 Baker Creek
RAK 4066 Saddle Horse Lake
RAK 4074 Merced Peak
RAK 2378 North Palisade Peak
RAK 2393 Mather Pass
RAK 3006 Amphitheater Lake
RAK lake 10269 Amphitheatre Lk
CAS 209370 Fuggs Reservir
CAS 209404 Rock Lake
CAS 209386 Silver Lake, Plumas
CAS 209668 Pine Grove Cemetary, Plumas
CAS 206093 Rock Creek
VTV 075 Oliver Lake
NORTH
MVZ 226112 Lake 1, 60 lk
RAK 1776 Headwaters Kern, W side
RAK 2162 Near Muro Blanco
RAK 2962 Upper Basin
RAK 2989 Upper Basin
RAK 3552 South of Milestone Basin
RAK lake 20226 Headwaters Kern
S-508 Golden Bear Lake
RAK 1311 Upper Basin#2
RAK 1727 Upper Basin #1
RAK 299 Hitchcock Lk
RAK 559 Bighorn Plateau
RAK 606 Vidette Crk
RAK 671 Woods Lk
RAK lake 10314 Woods Crk
VTV 874 Ansel Adams Lake
RAK 3584 Milestone Basin
RAK 3924 Tyndall Creek
S-387 Upper Kern (near Lk S America)
S-376 Lake S. America
VTV 055 Bullfrog Lake
VTV 1578 Mulkey Meadow
LJR 089 Laurel Basin
RAK 3713 Coyote Pass
MVZ 230140 San Gabriel Mt
MVZ 230141 San Bernardino Mt
MVZ 230142 San Jacinto Mt
SOUTH
Rana boylii MVZ 148941
Rana aurora MVZ 227645
Rana cascadae MVZ 230719

Figure 9.

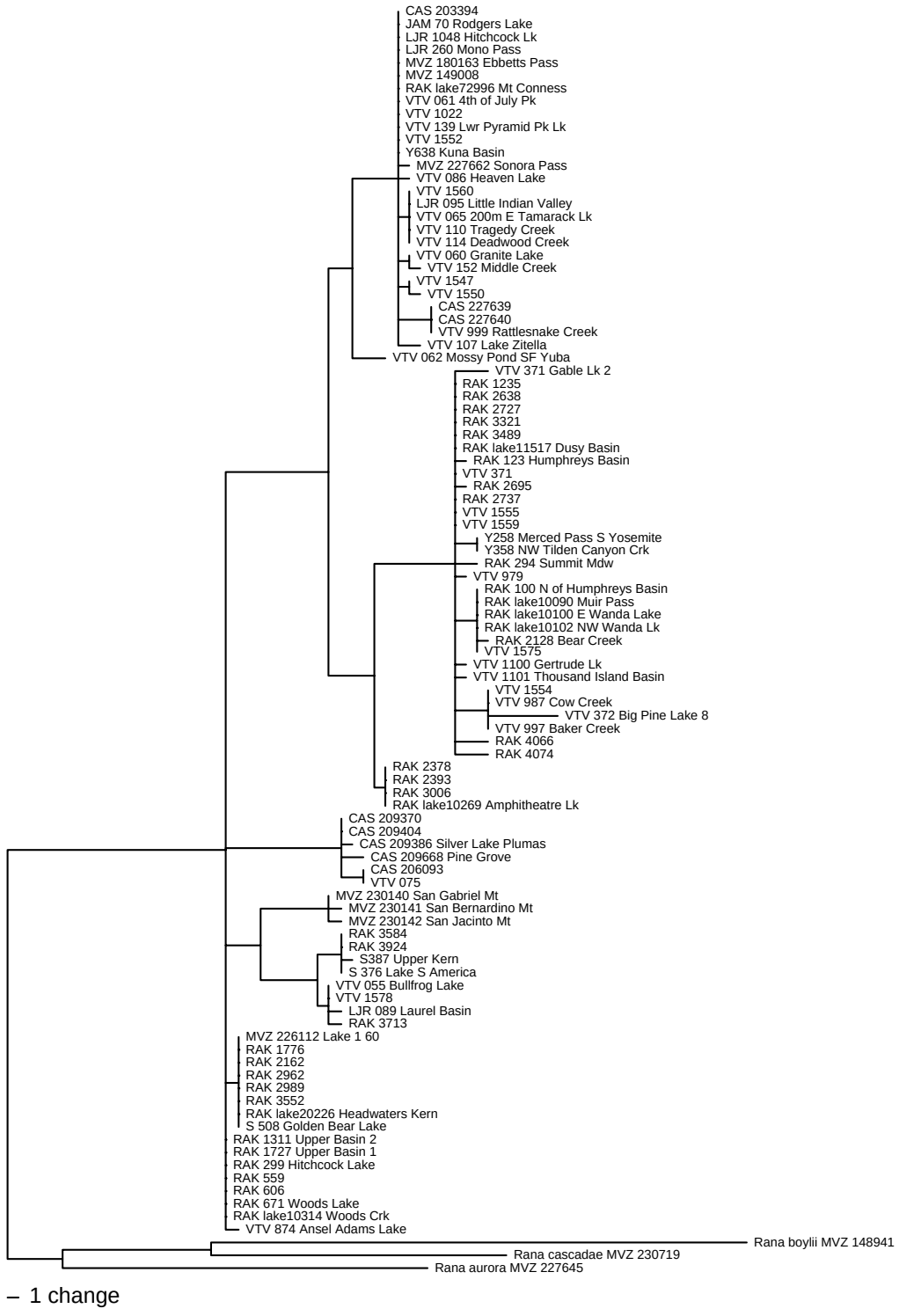

CAS 203394
JAM 70 Rodgers Lake
LJR 1048 Hitchcock Lk
LJR 260 Mono Pass
MVZ 180163 Ebbetts Pass
MVZ 149008
RAK lake72996 Mt Conness
VTV 061 4th of July Pk
VTV 1022
VTV 139 Lwr Pyramid Pk Lk
VTV 1552
Y638 Kuna Basin
MVZ 227662 Sonora Pass
VTV 086 Heaven Lake
VTV 1560
LJR 095 Little Indian Valley
VTV 065 200m E Tamarack Lk
VTV 110 Tragedy Creek
VTV 114 Deadwood Creek
VTV 060 Granite Lake
VTV 152 Middle Creek
VTV 1547
VTV 1550
CAS 227639
CAS 227640
VTV 999 Rattlesnake Creek
VTV 107 Lake Zitella
VTV 062 Mossy Pond SF Yuba
VTV 371 Gable Lk 2
RAK 1235
RAK 2638
RAK 2727
RAK 3321
RAK 3489
RAK lake11517 Dusy Basin
RAK 123 Humphreys Basin
VTV 371
RAK 2695
RAK 2737
VTV 1555
VTV 1559
Y258 Merced Pass S Yosemite
Y358 NW Tilden Canyon Crk
RAK 294 Summit Mdw
VTV 979
RAK 100 N of Humphreys Basin
RAK lake10090 Muir Pass
RAK lake10100 E Wanda Lake
RAK lake10102 NW Wanda Lk
RAK 2128 Bear Creek
VTV 1575
VTV 1100 Gertrude Lk
VTV 1101 Thousand Island Basin
VTV 1554
VTV 987 Cow Creek
VTV 372 Big Pine Lake 8
VTV 997 Baker Creek
RAK 4066
RAK 4074
RAK 2378
RAK 2393
RAK 3006
RAK lake10269 Amphitheatre Lk
CAS 209370
CAS 209404
CAS 209386 Silver Lake Plumas
CAS 209668 Pine Grove
CAS 206093
VTV 075
MVZ 230140 San Gabriel Mt
MVZ 230141 San Bernardino Mt
MVZ 230142 San Jacinto Mt
RAK 3584
RAK 3924
S387 Upper Kern
S 376 Lake S America
VTV 055 Bullfrog Lake
VTV 1578
LJR 089 Laurel Basin
RAK 3713
MVZ 226112 Lake 1 60
RAK 1776
RAK 2162
RAK 2962
RAK 2989
RAK 3552
RAK lake20226 Headwaters Kern
S 508 Golden Bear Lake
RAK 1311 Upper Basin 2
RAK 1727 Upper Basin 1
RAK 299 Hitchcock Lake
RAK 559
RAK 606
RAK 671 Woods Lake
RAK lake10314 Woods Crk
VTV 874 Ansel Adams Lake
Rana boylii MVZ 148941
Rana cascadae MVZ 230719
Rana aurora MVZ 227645
– 1 change

Figure 9a.

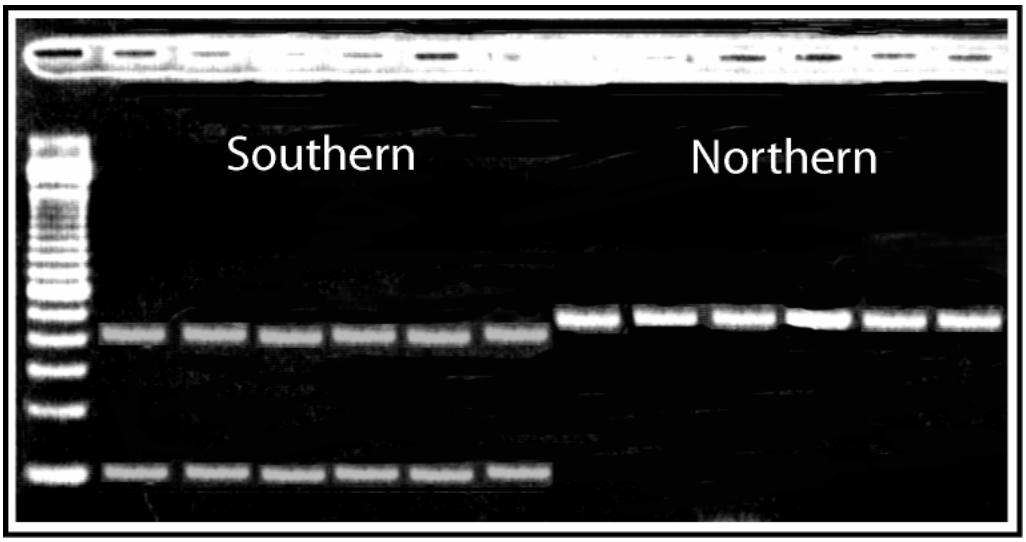


Figure 10.

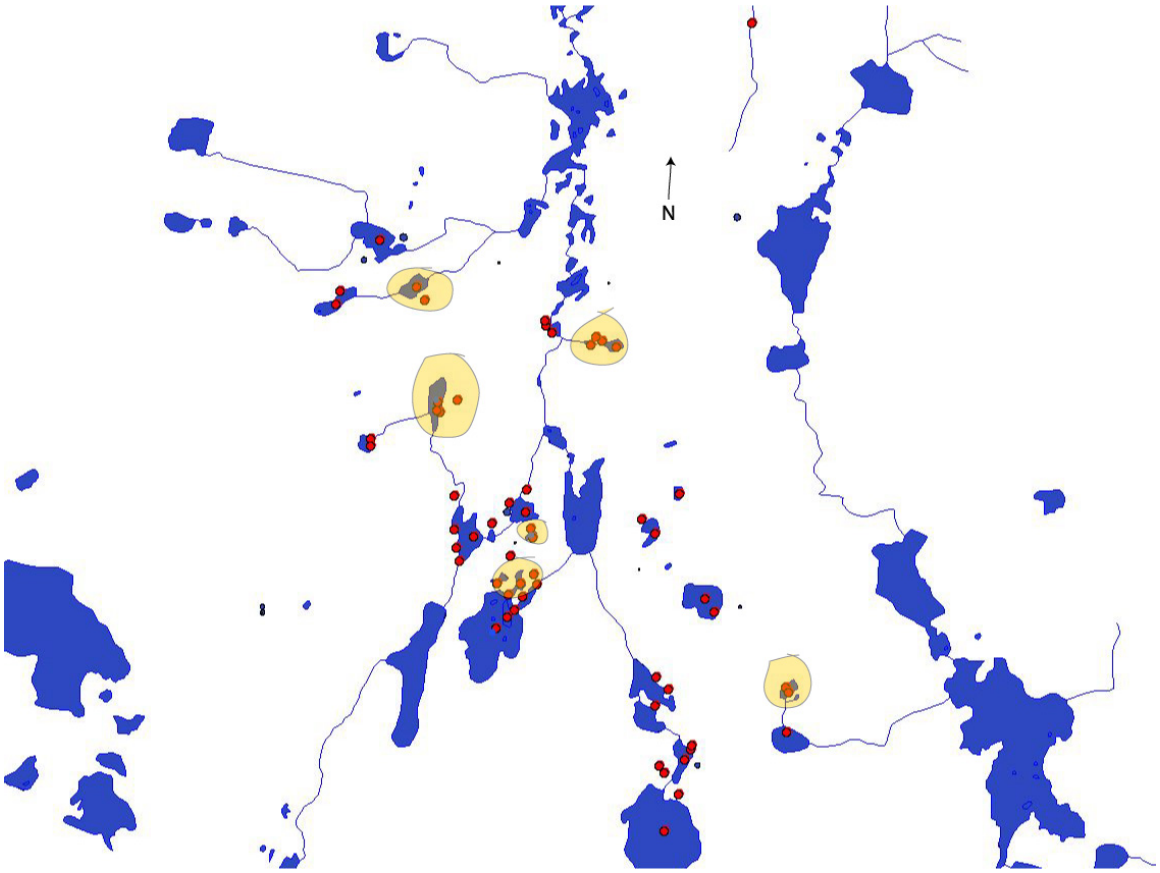


Figure 11.

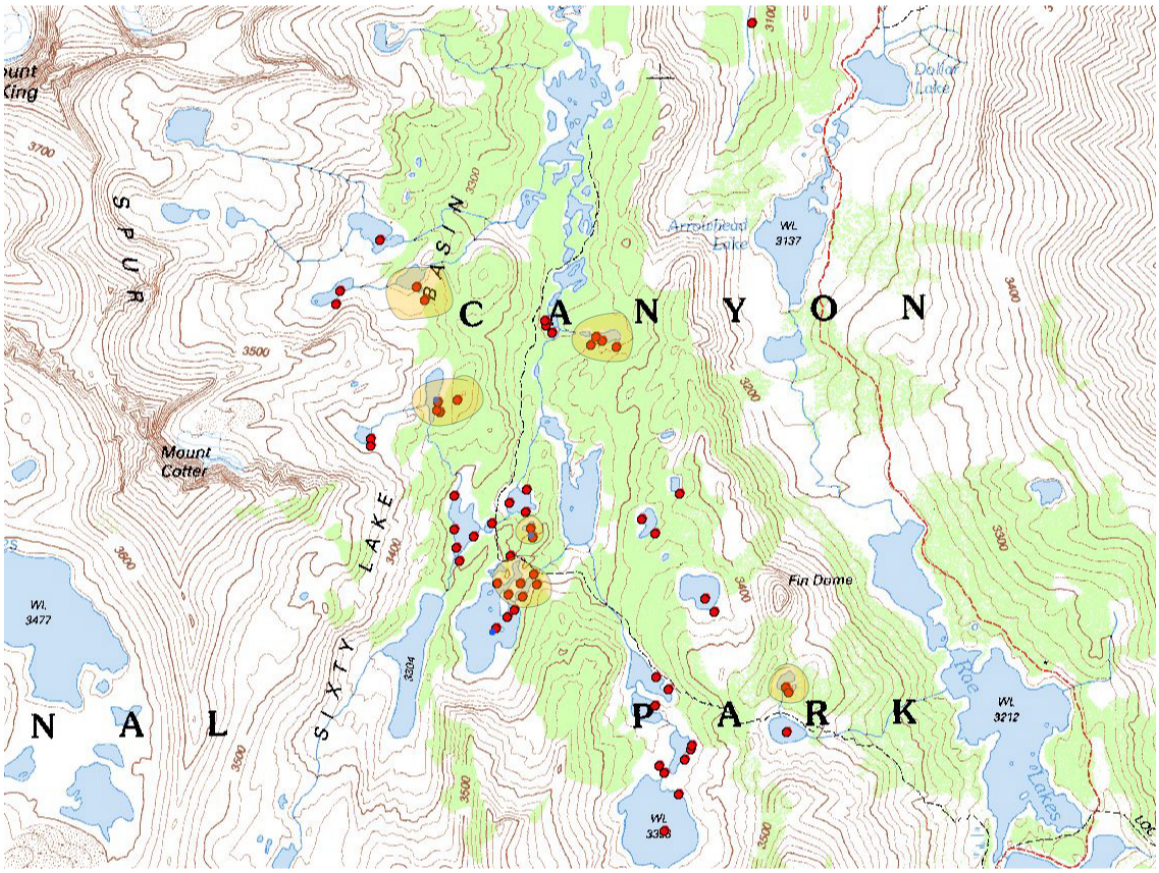

SPUR
BASIN
CANYON
Mount Cotter
SIXTY LAKE
Fin Dome
NAL
PARK
Dollar Lake
Arrowhead Lake
WL 3137
WL 3477
WL 3212
WL 3356
Rae Lakes
3700
3600
3500
3400
3300
3200
3100

Figure 11a.

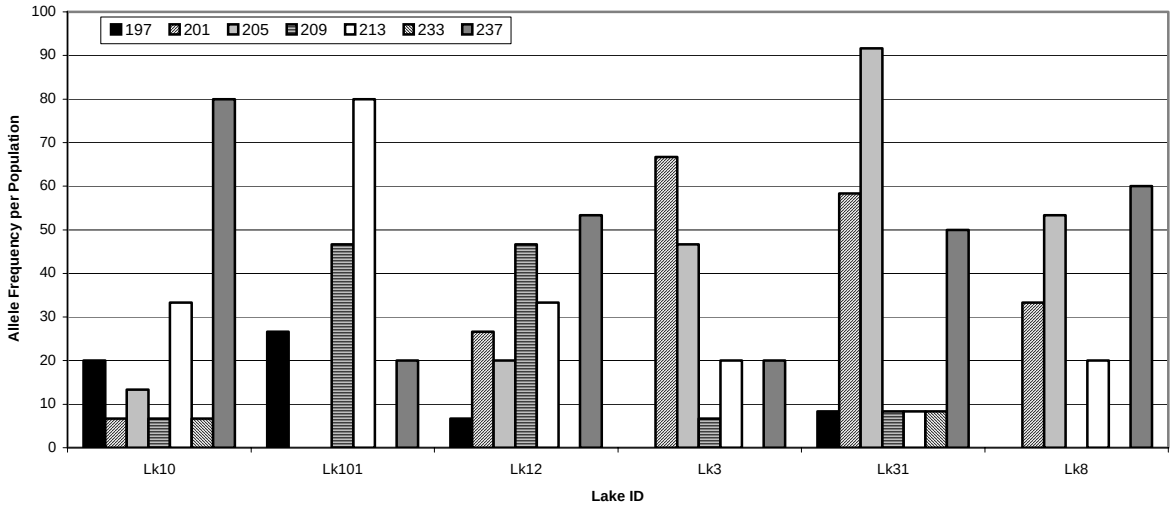

197
201
205
209
213
233
237
100
90
80
70
60
50
40
30
20
10
0
Allele Frequency per Population
Lk10
Lk101
Lk12
Lk3
Lk31
Lk8
Lake ID

Figure 12.

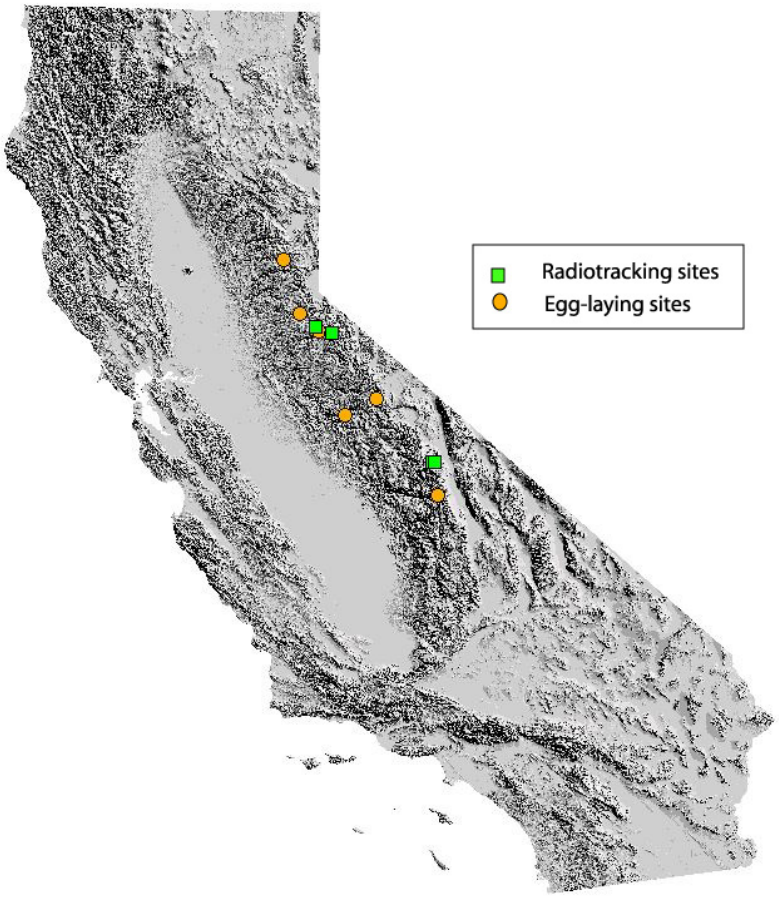


Figure 13.

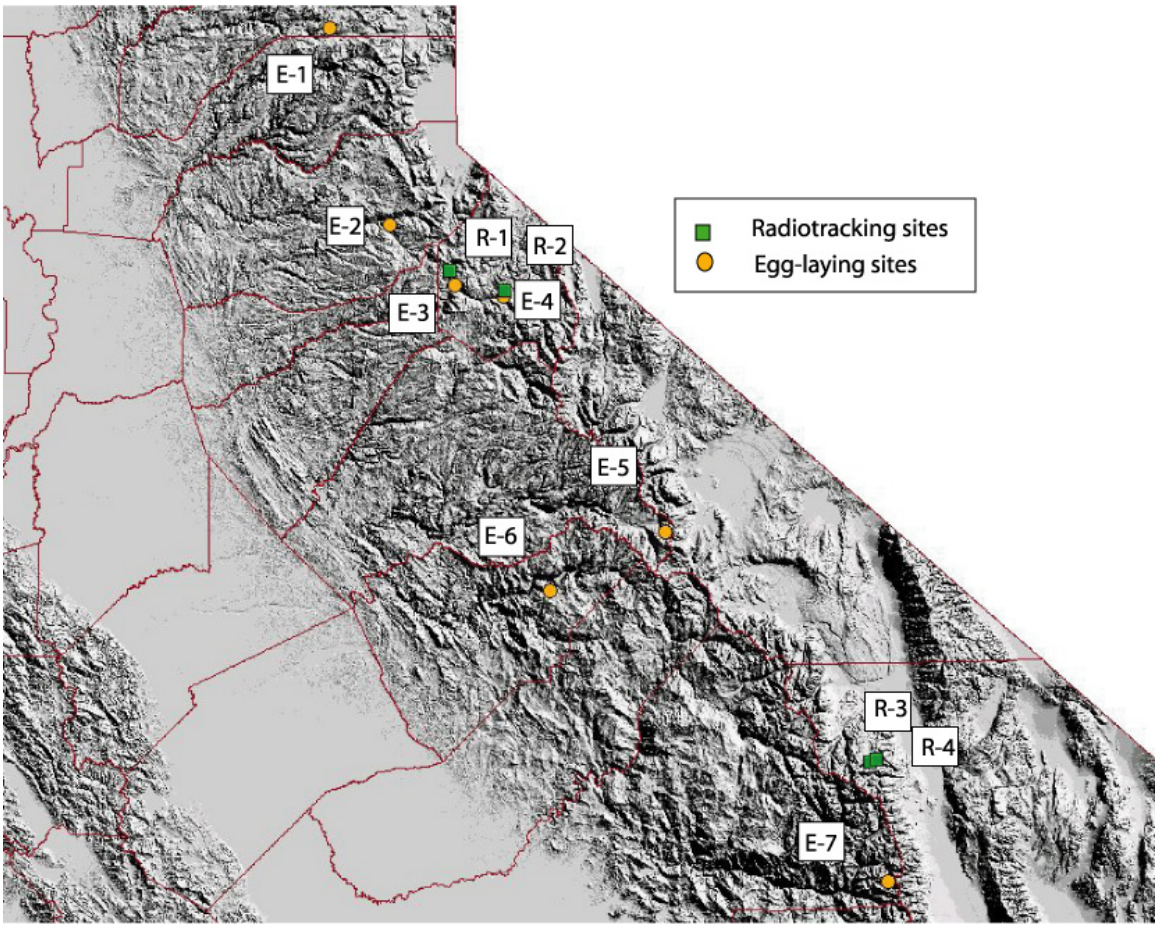

E-1
E-2
R-1
R-2
E-3
E-4
E-5
E-6
R-3
R-4
E-7
Radiotracking sites
Egg-laying sites

Figure 14.

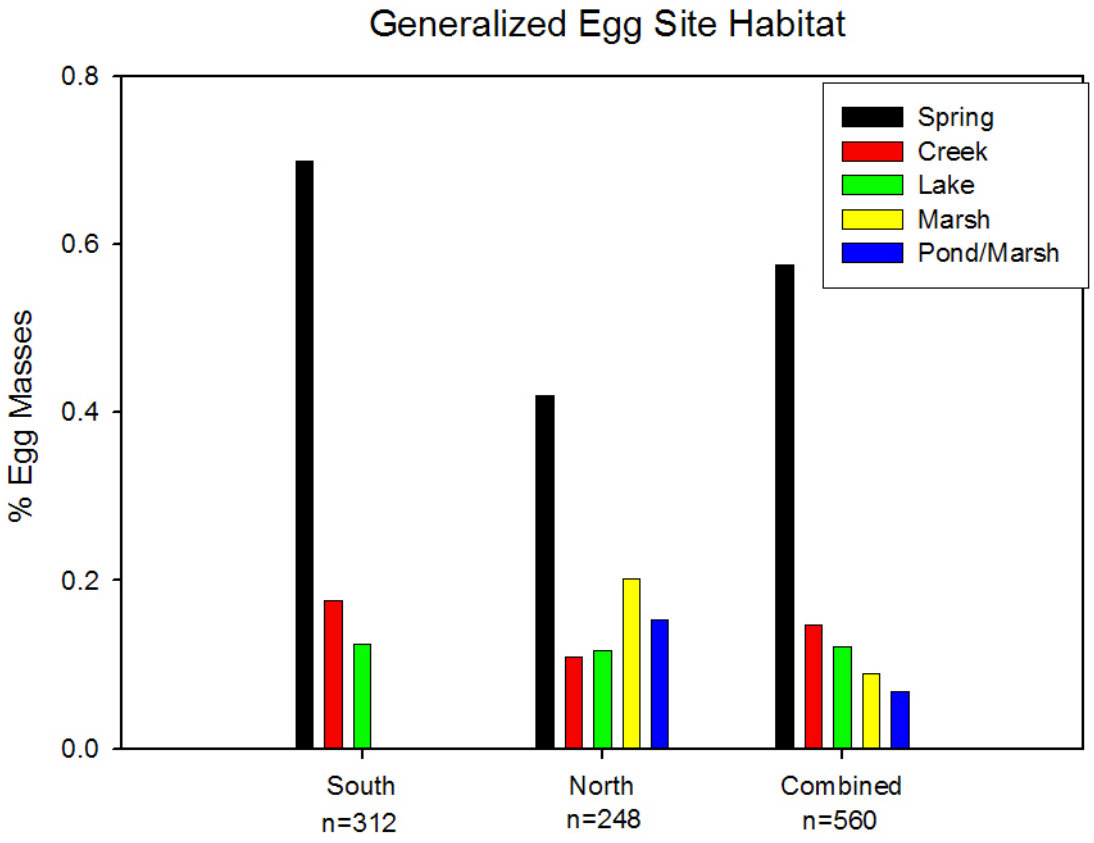

Generalized Egg Site Habitat
% Egg Masses
0.0
0.2
0.4
0.6
0.8
Spring
Creek
Lake
Marsh
Pond/Marsh
South
n=312
North
n=248
Combined
n=560

Figure 15.

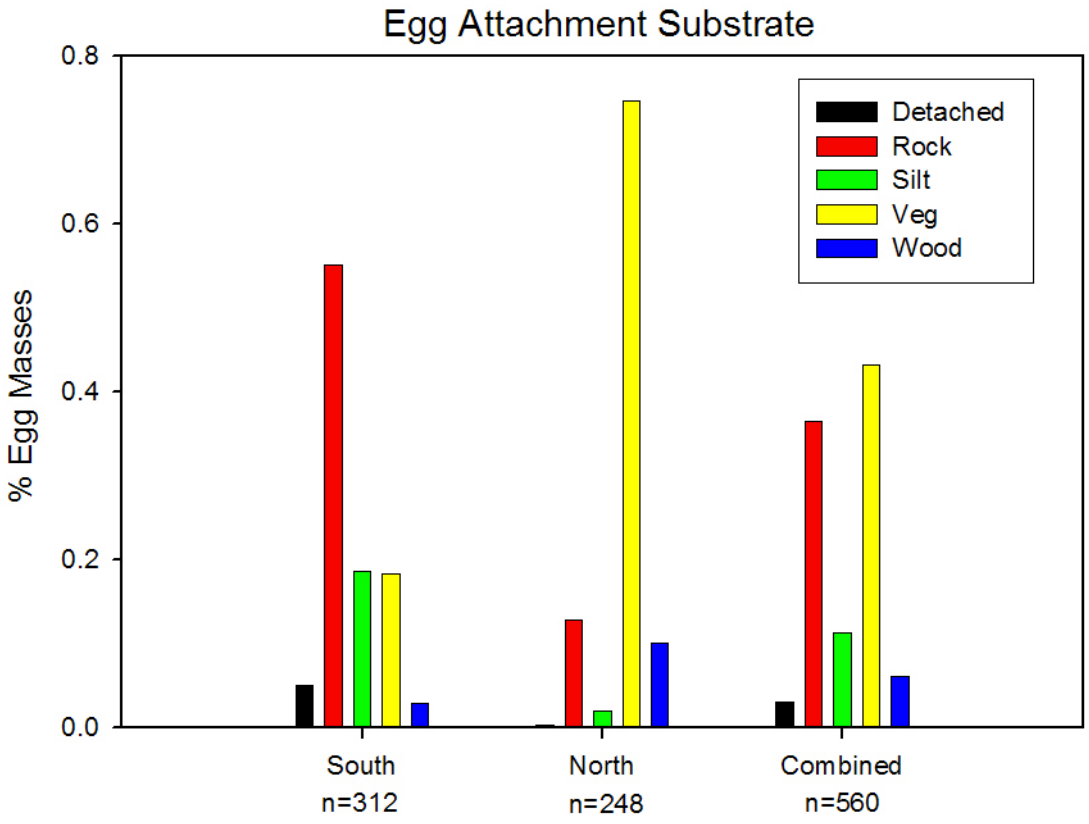

Egg Attachment Substrate
% Egg Masses
0.8
0.6
0.4
0.2
0.0
Detached
Rock
Silt
Veg
Wood
South
n=312
North
n=248
Combined
n=560

Figure 16.

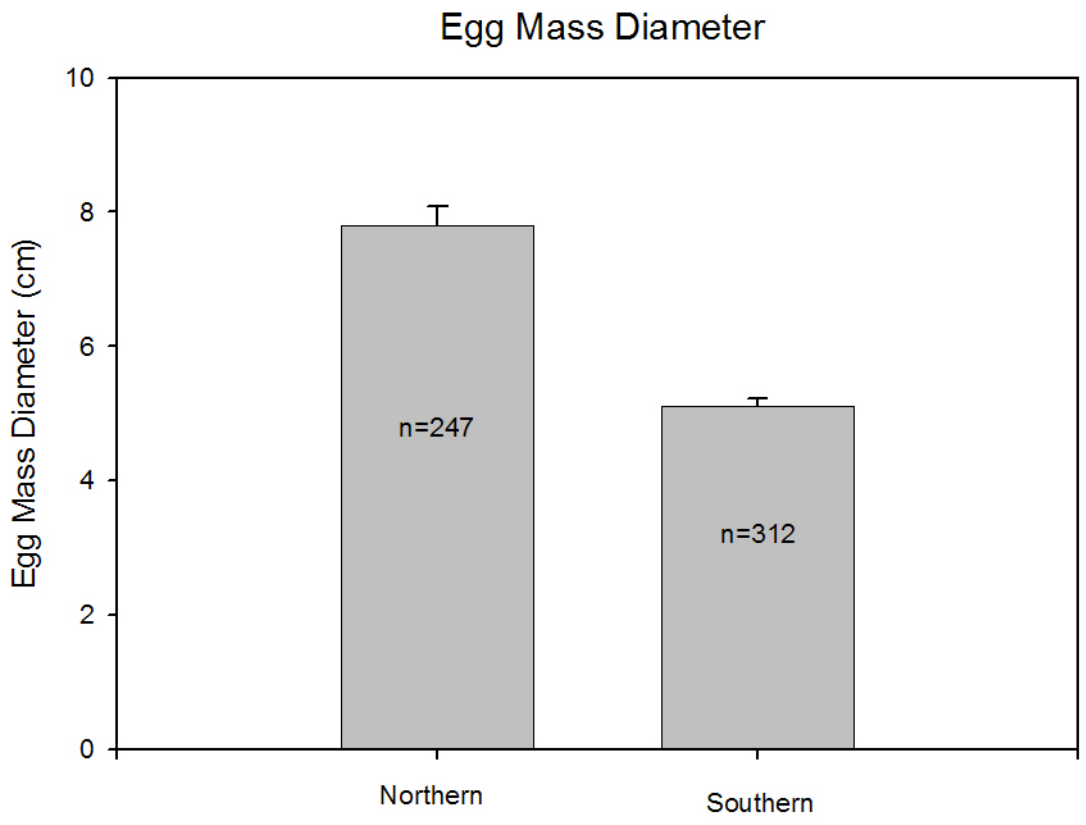

Egg Mass Diameter
Egg Mass Diameter (cm)
10
8
6
4
2
0
n=247
n=312
Northern
Southern

Figure 17.

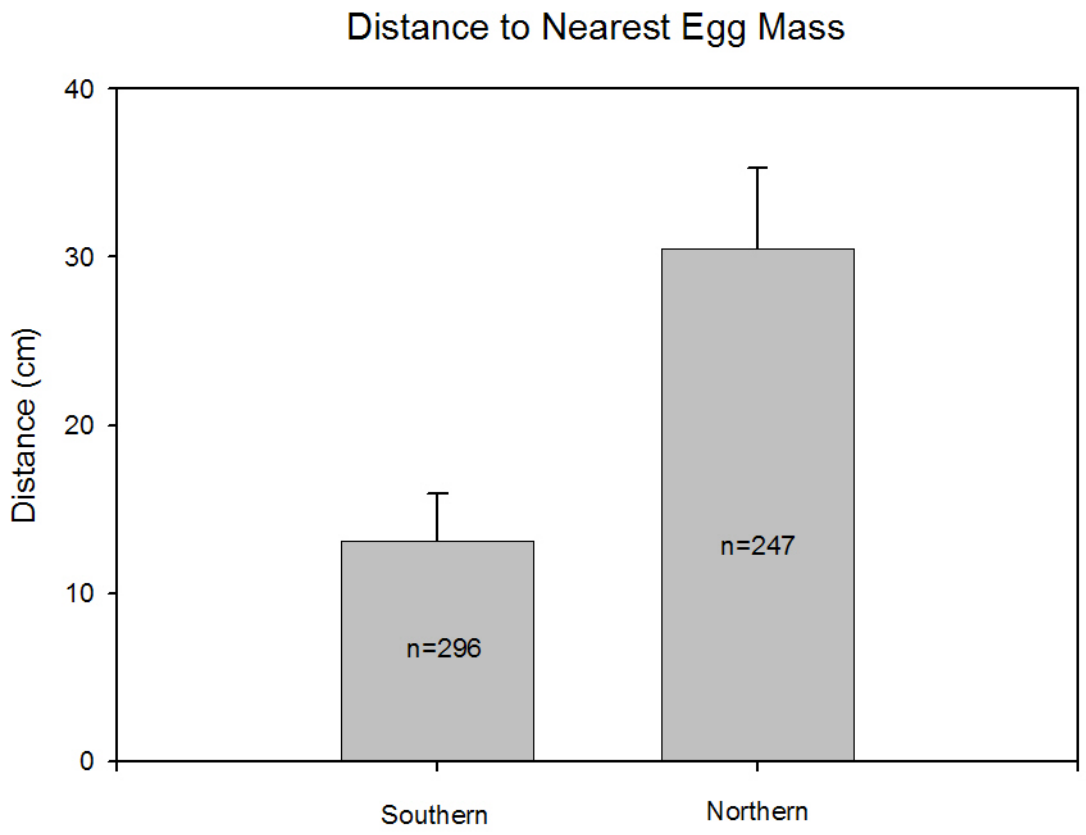

Distance to Nearest Egg Mass
Distance (cm)
40
30
20
10
0
n=296
n=247
Southern
Northern

Figure 18.

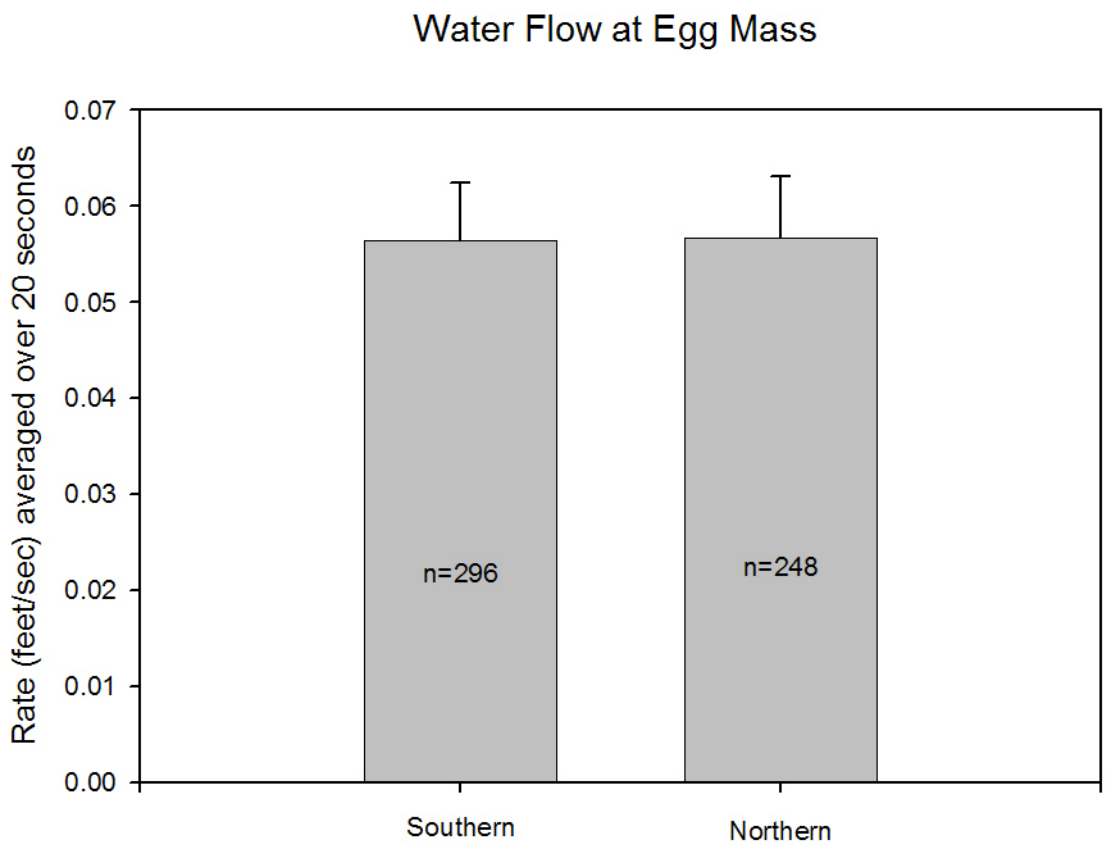

Water Flow at Egg Mass
Rate (feet/sec) averaged over 20 seconds
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
n=296
n=248
Southern
Northern

Figure 19.

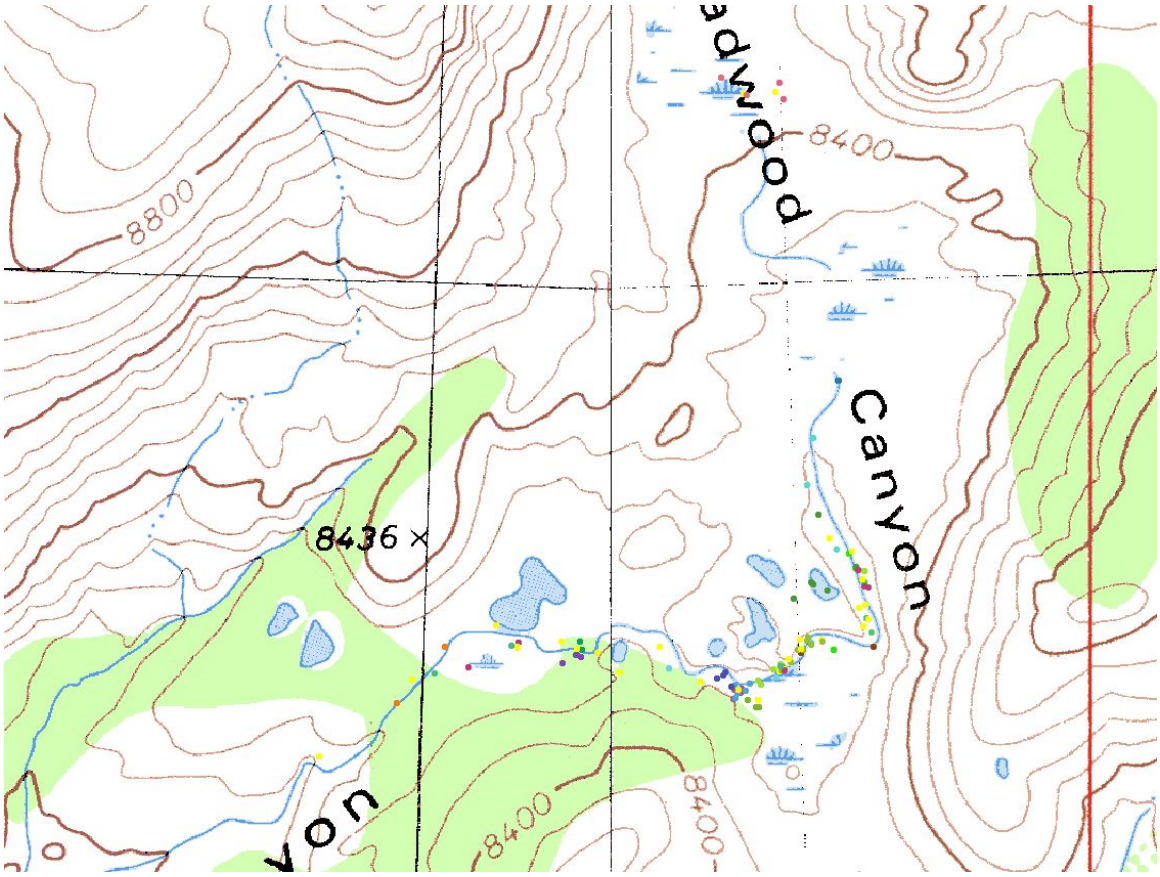


Figure 20.

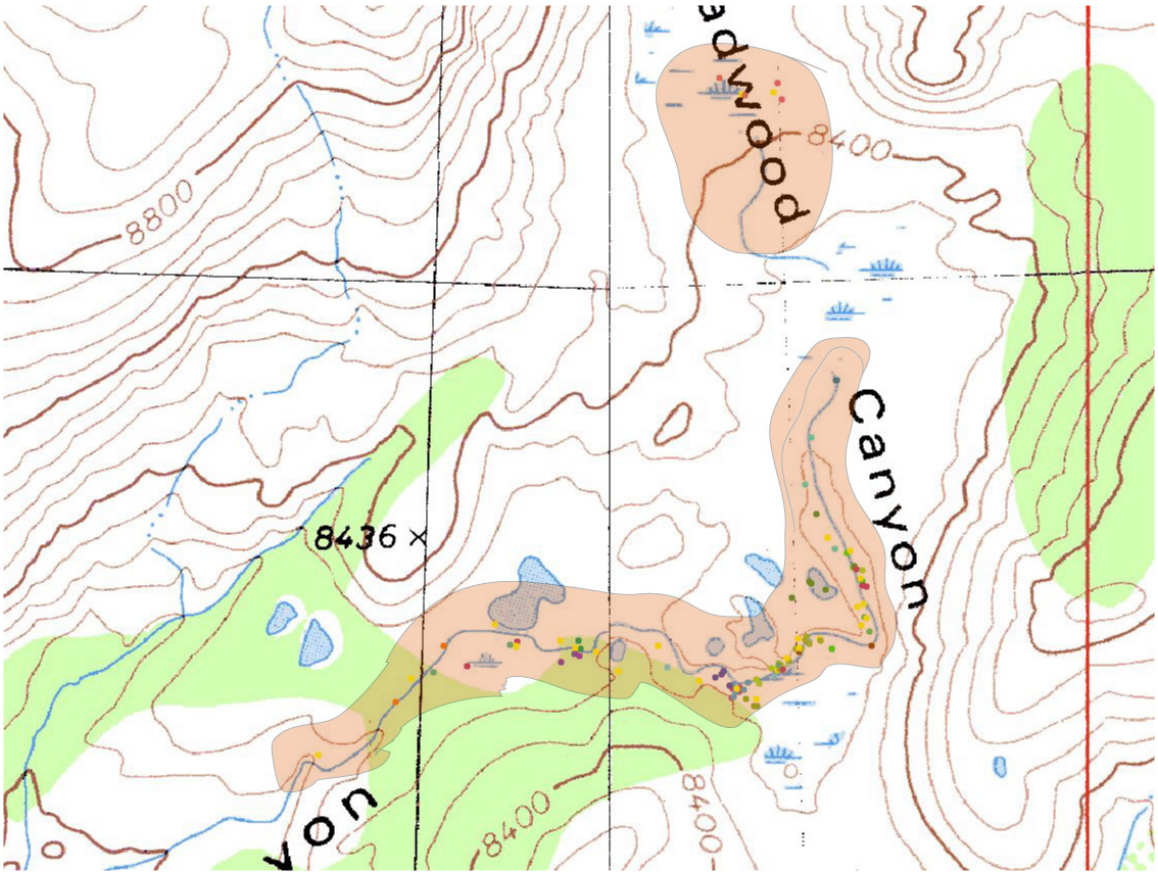


Figure 21.

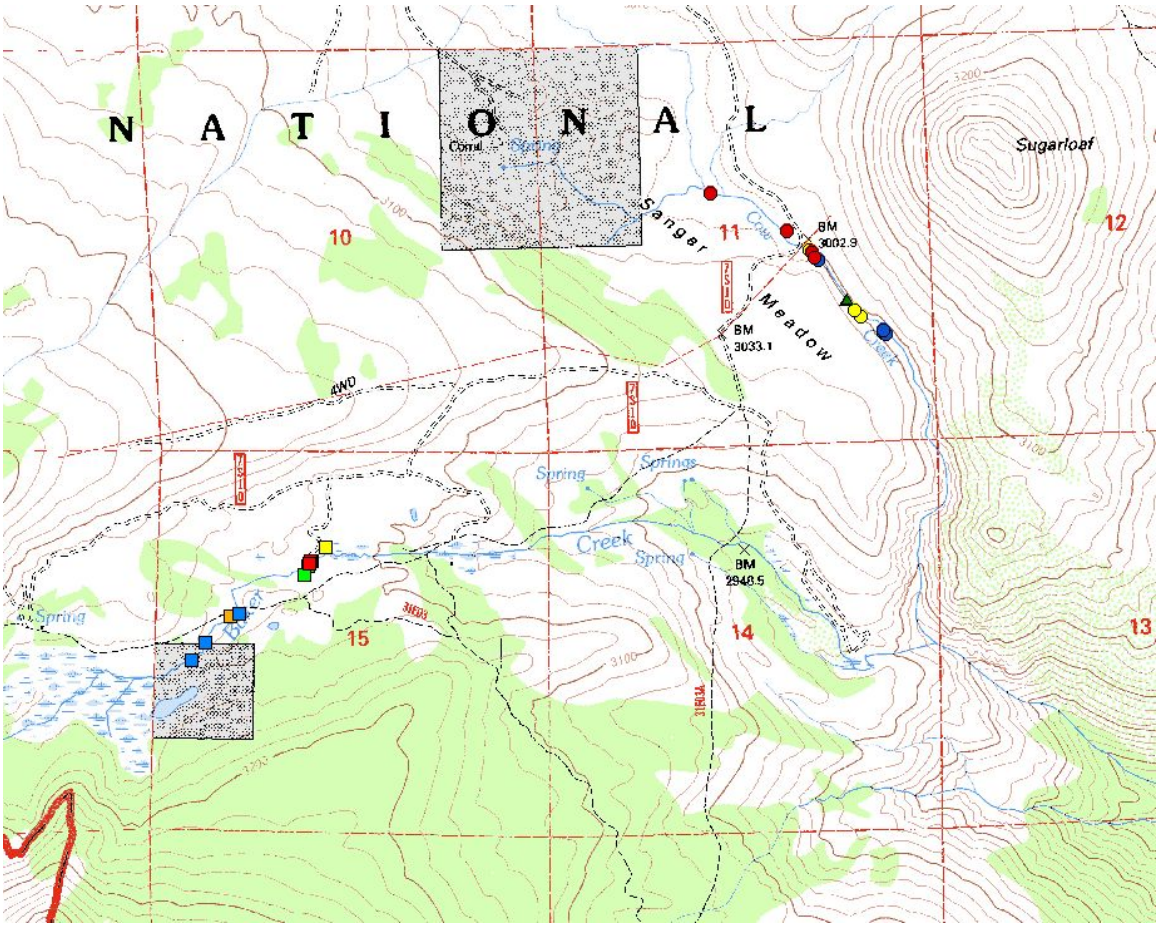

N A T I O N A L
Corral
Spring
Sanger
Creek
10
11
12
13
14
15
BM
3002.9
BM
3033.1
Meadow
Creek
4WD
Spring
Springs
Spring
Creek
Spring
BM
2948.5
Sugarloaf
3100
3200

Figure 22.

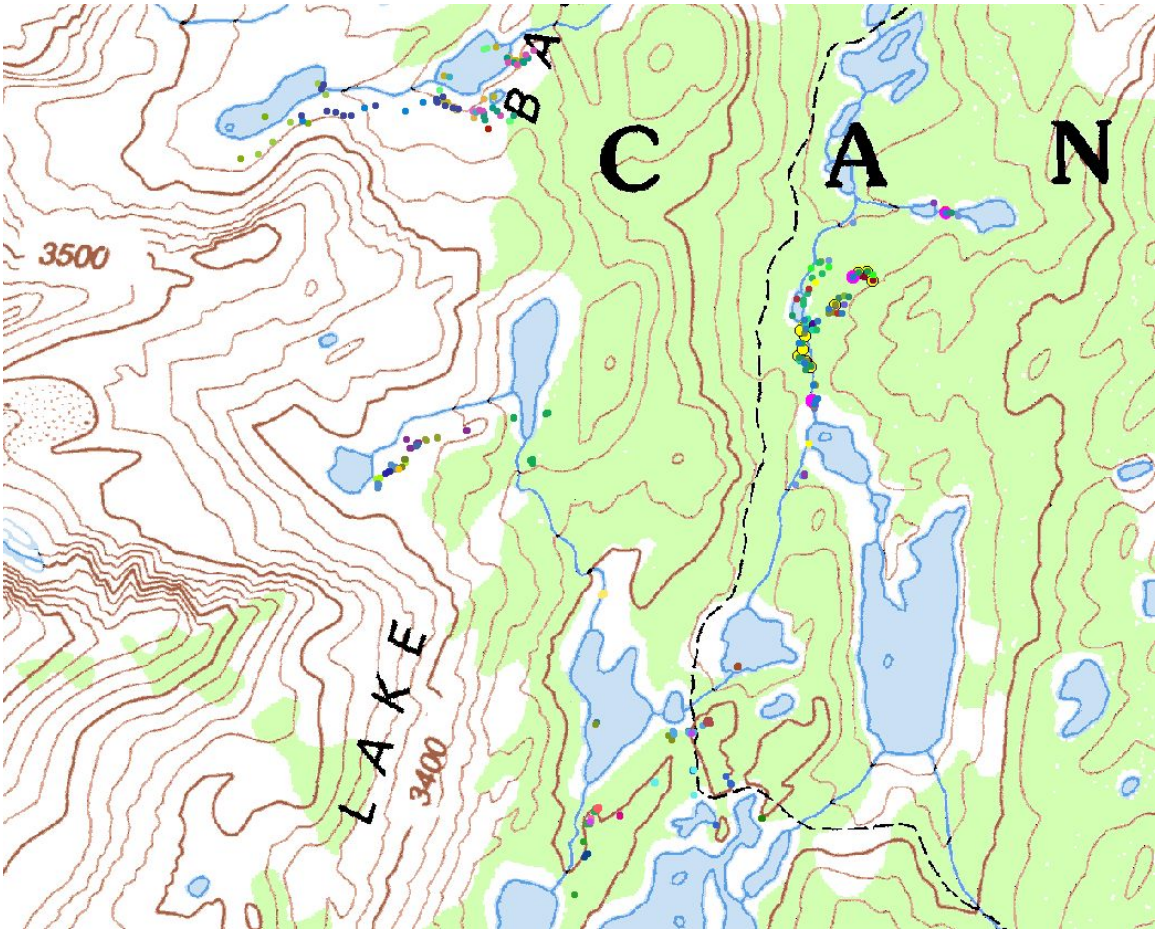


Figure 23.

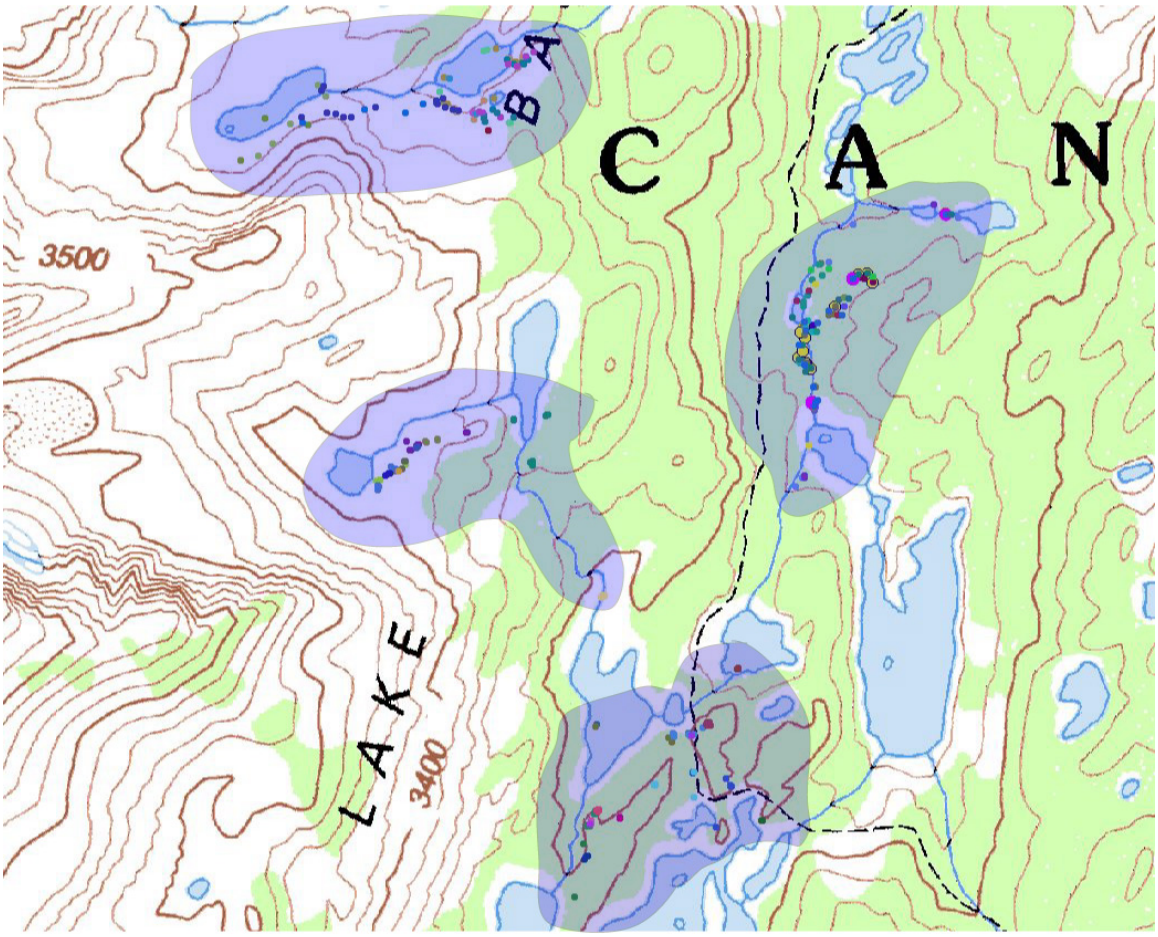


Figure 24.

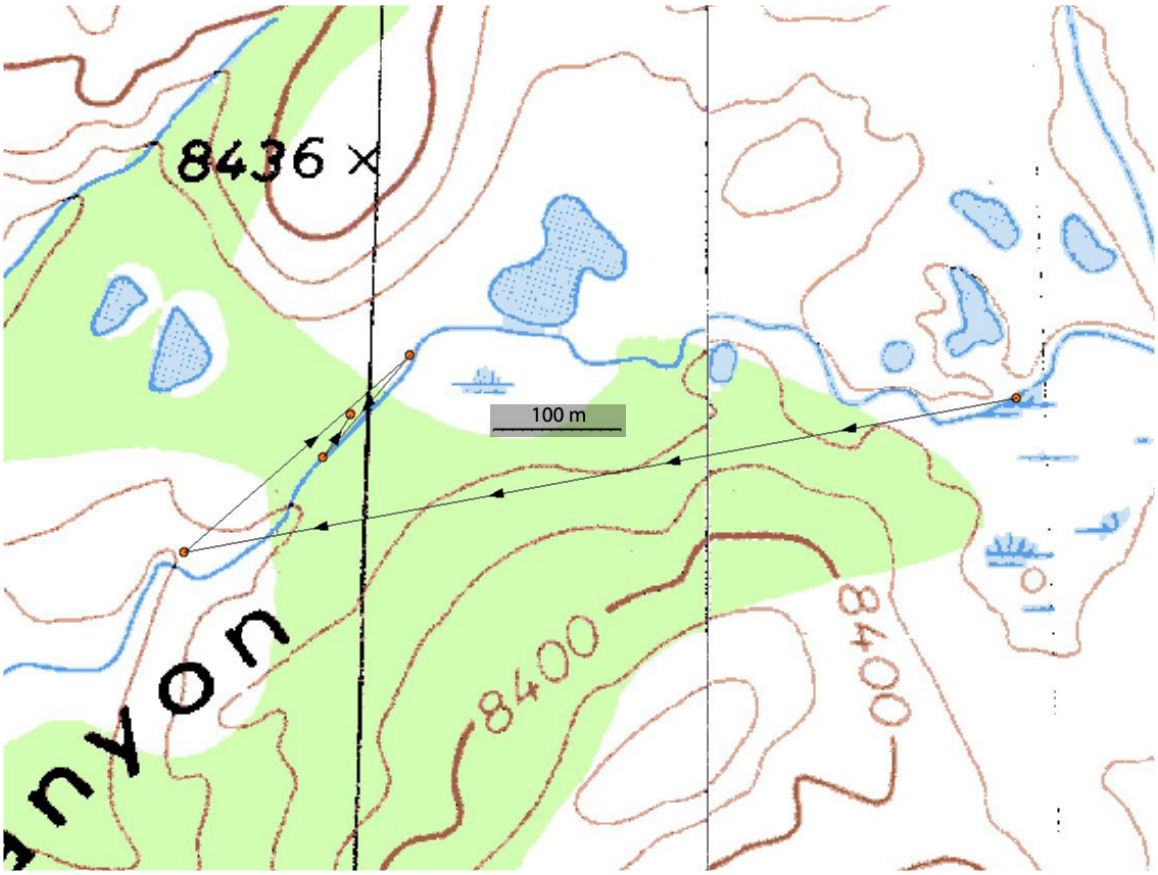


Figure 25.

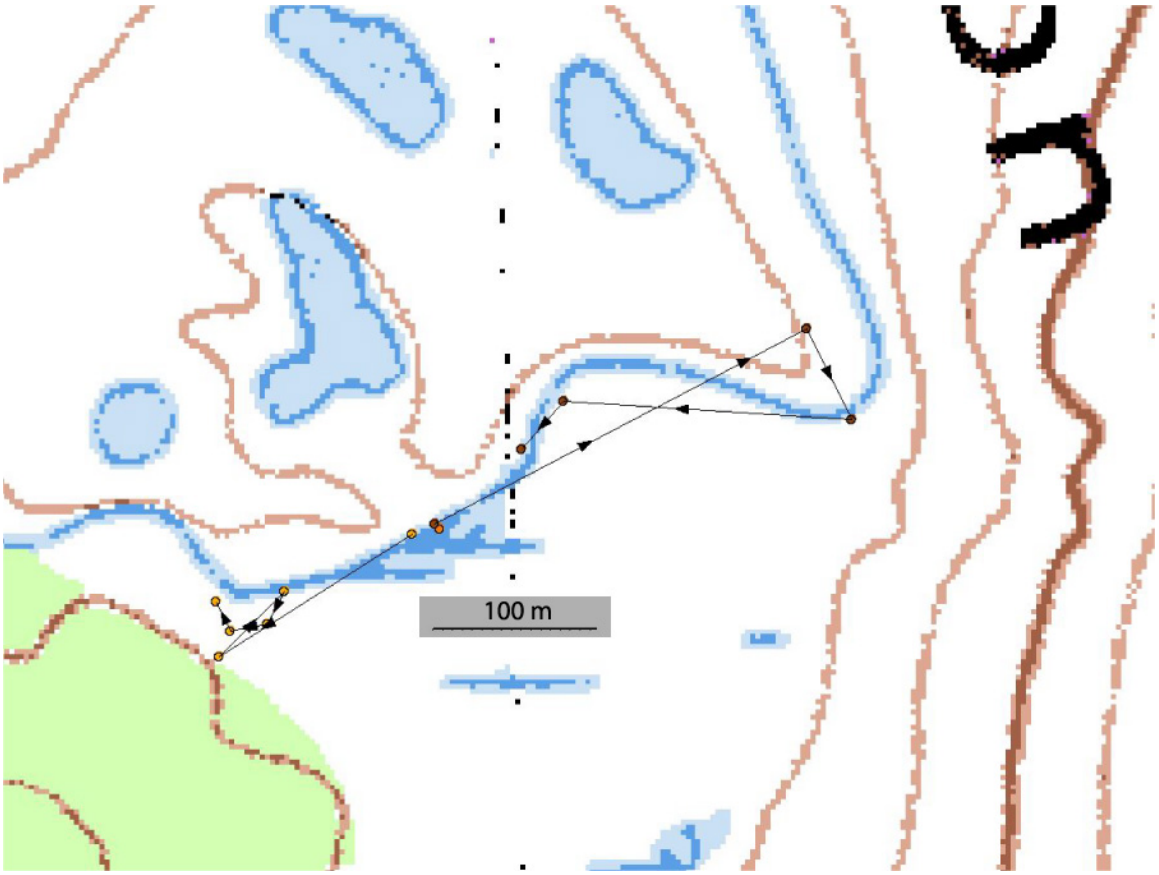


Figure 26.

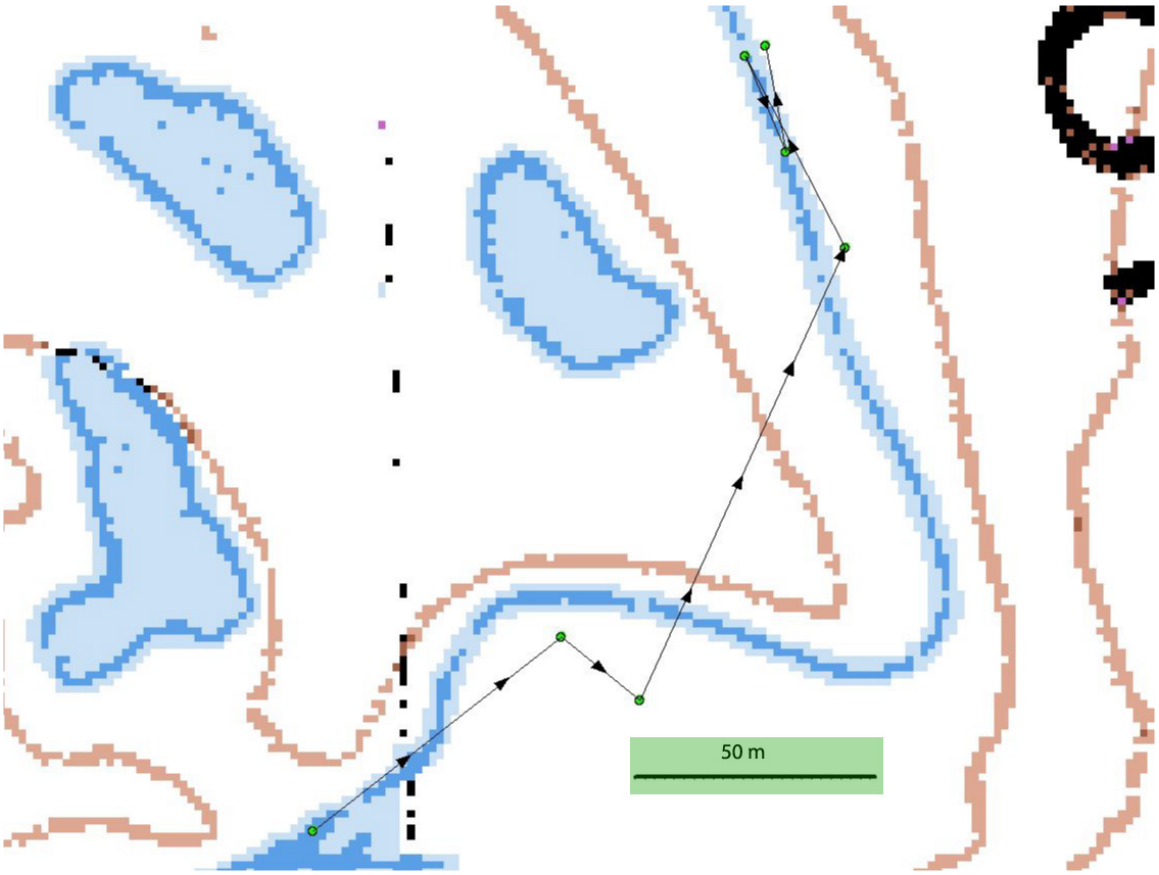


Figure 27.

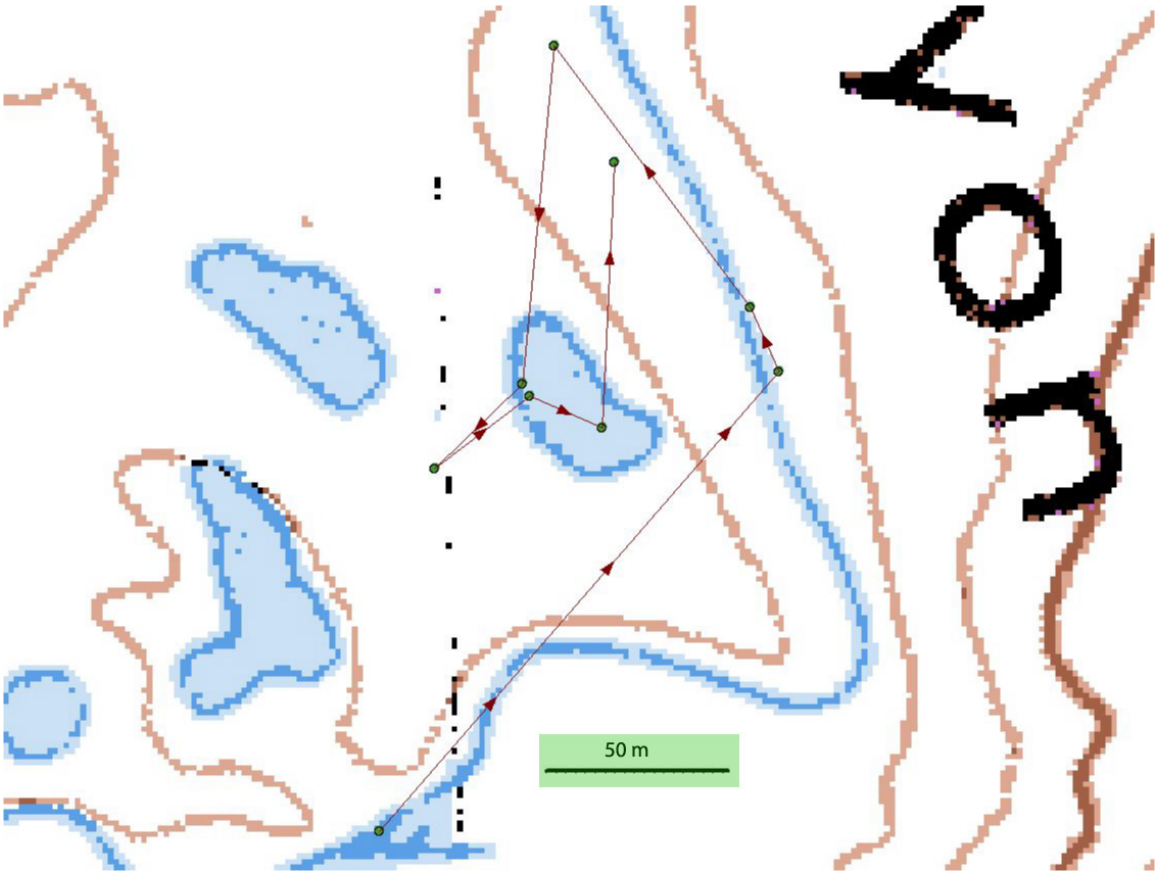


Figure 28.

One frog at Cow Creek.

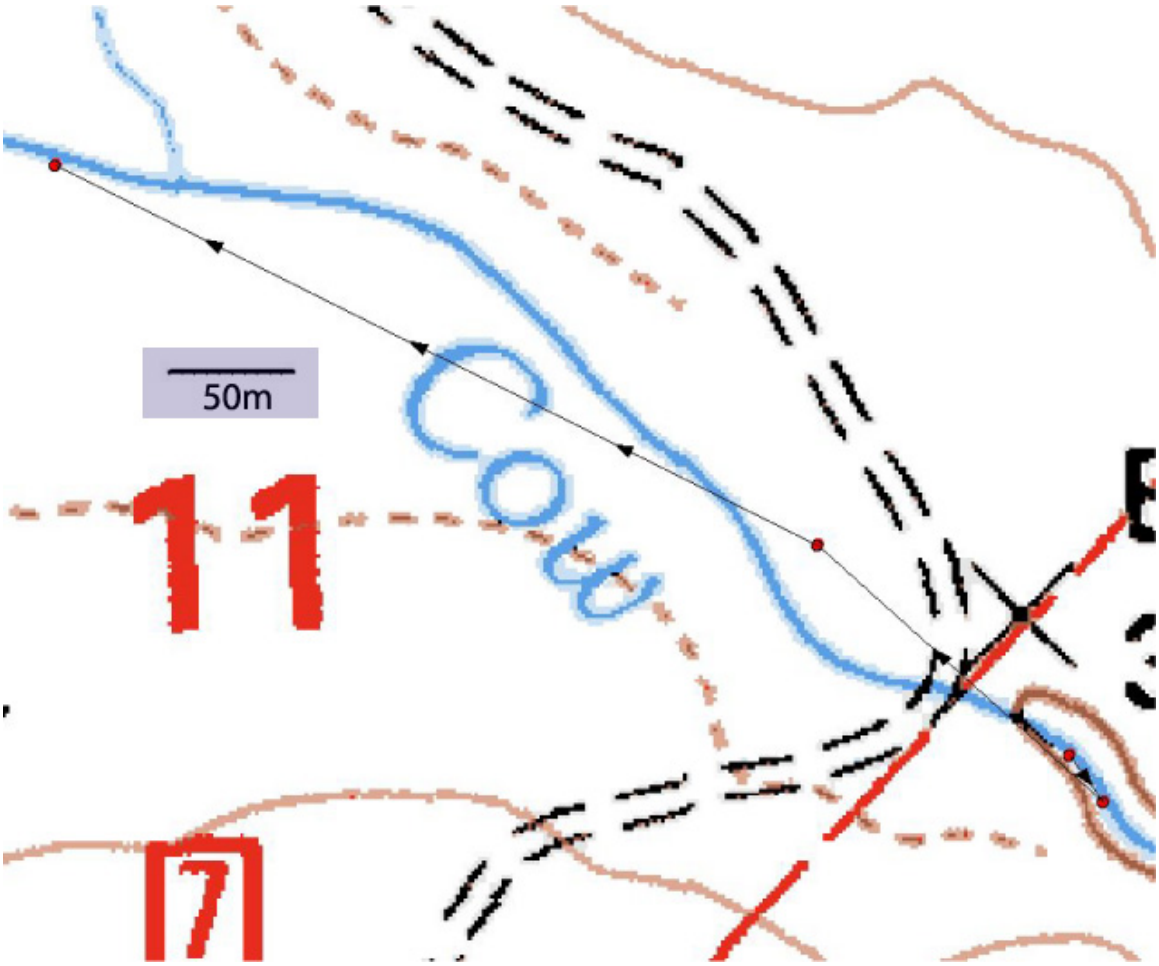


Figure 29.

Two frogs at Cow Creek.

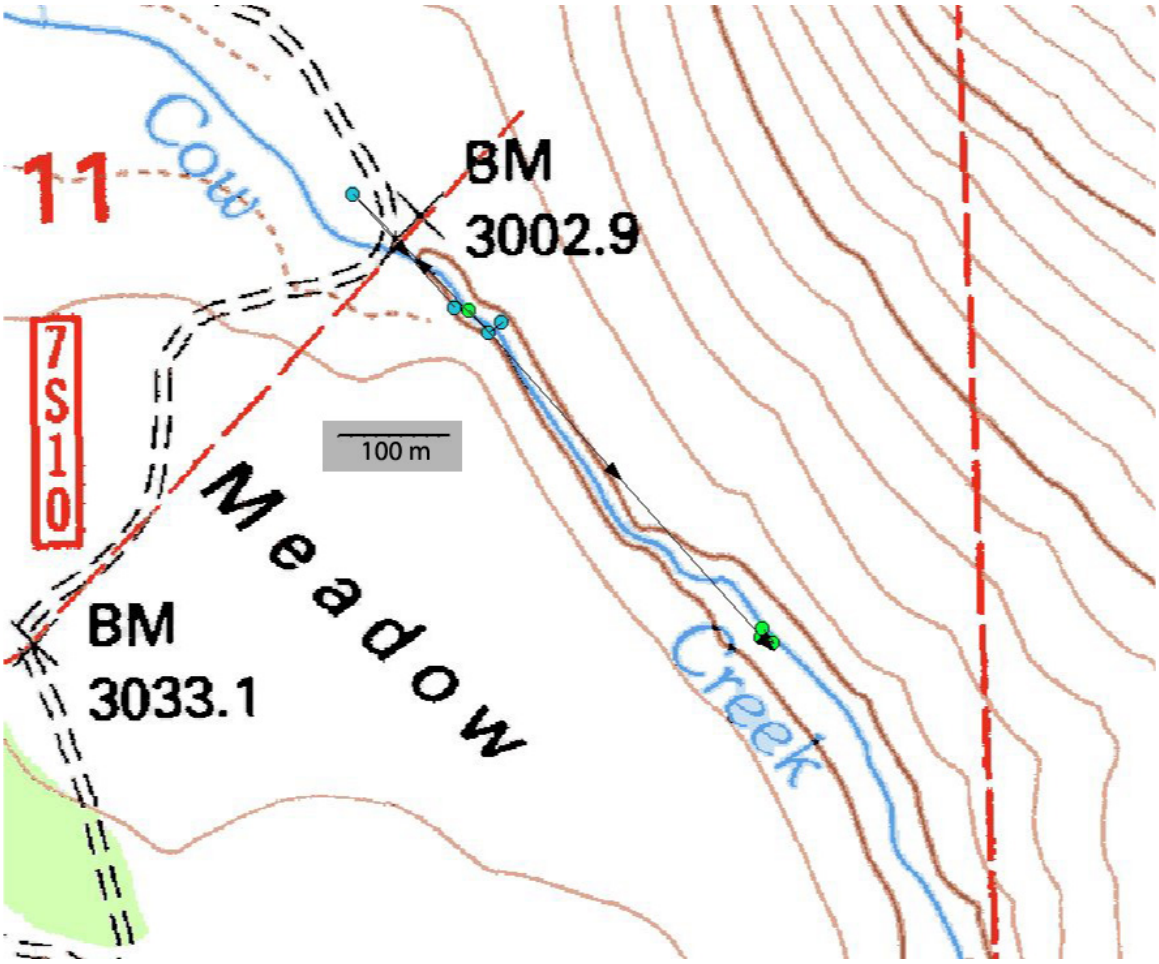


Figure 30.

Three frogs at Baker Creek.

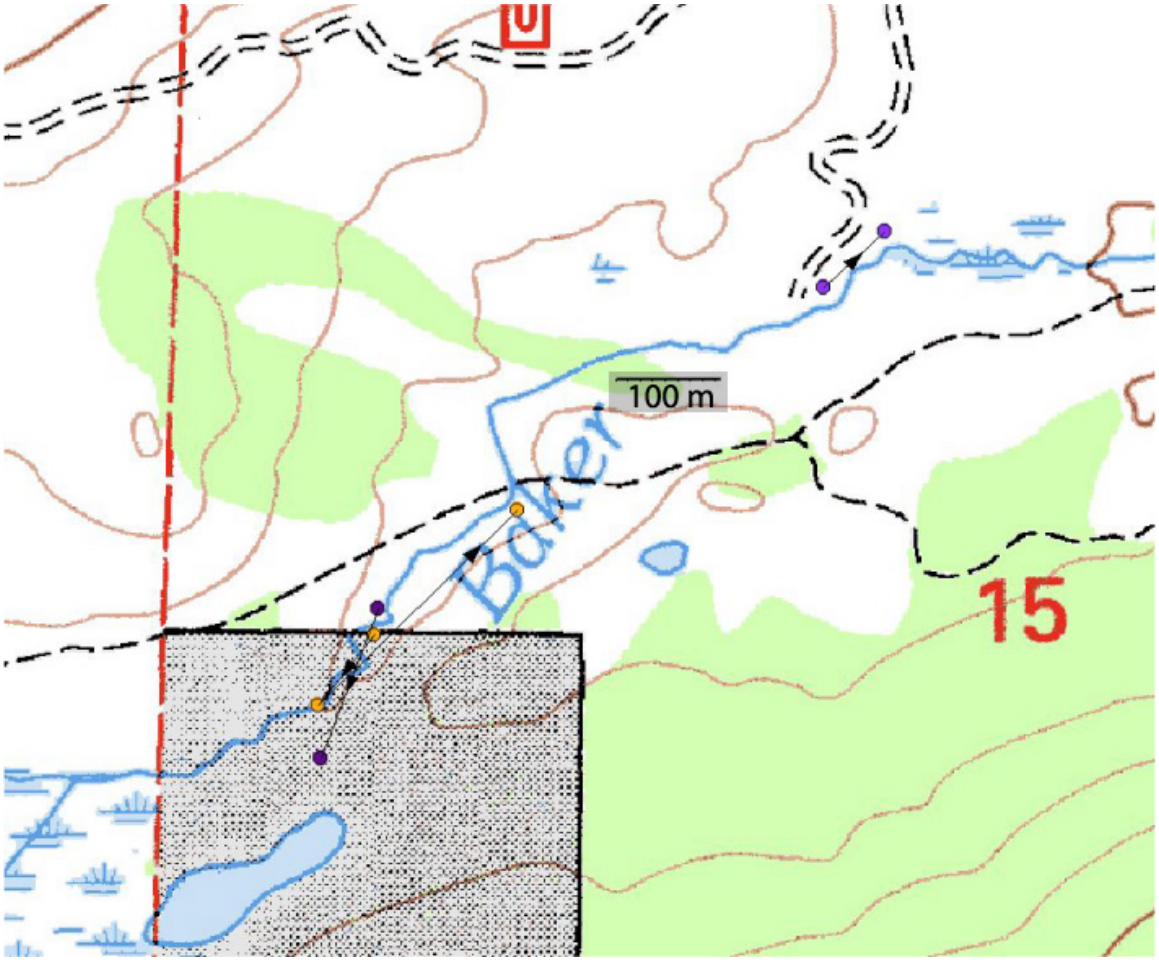


Figure 31.

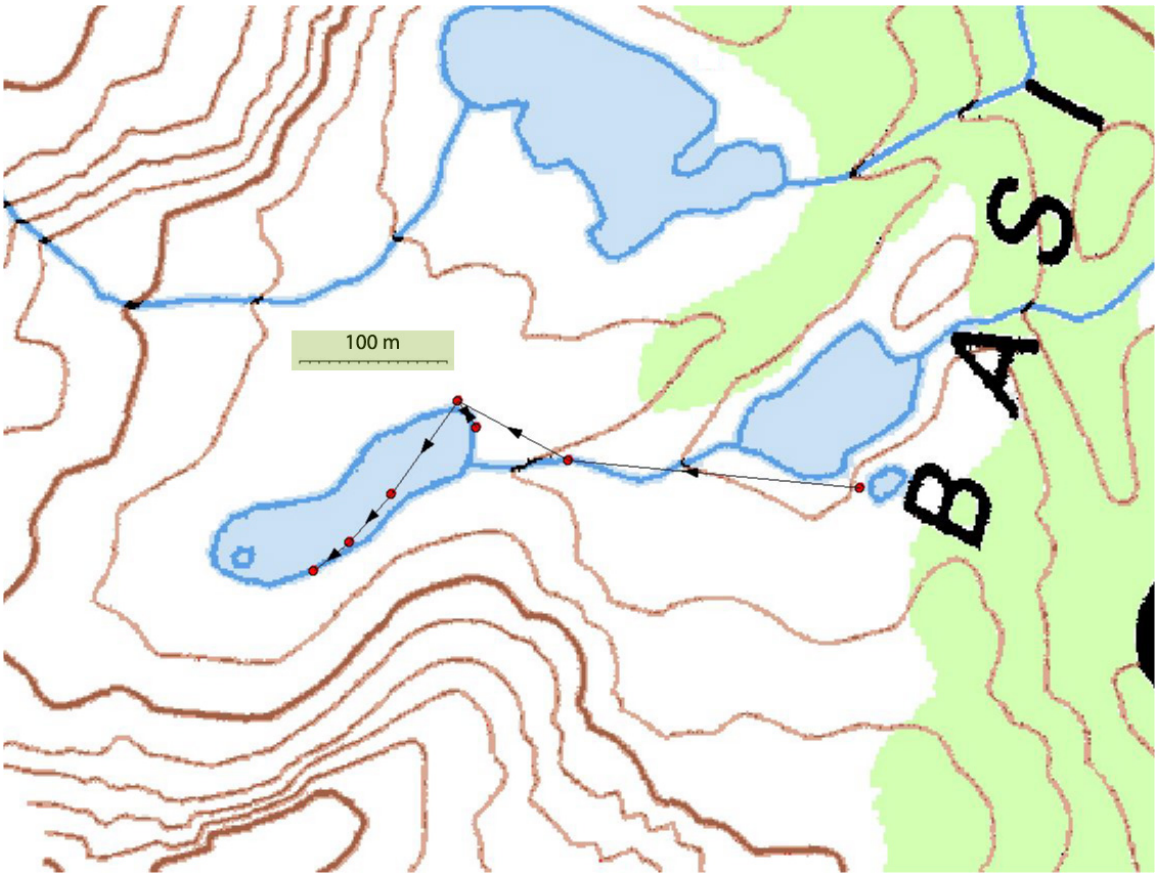


Figure 32.

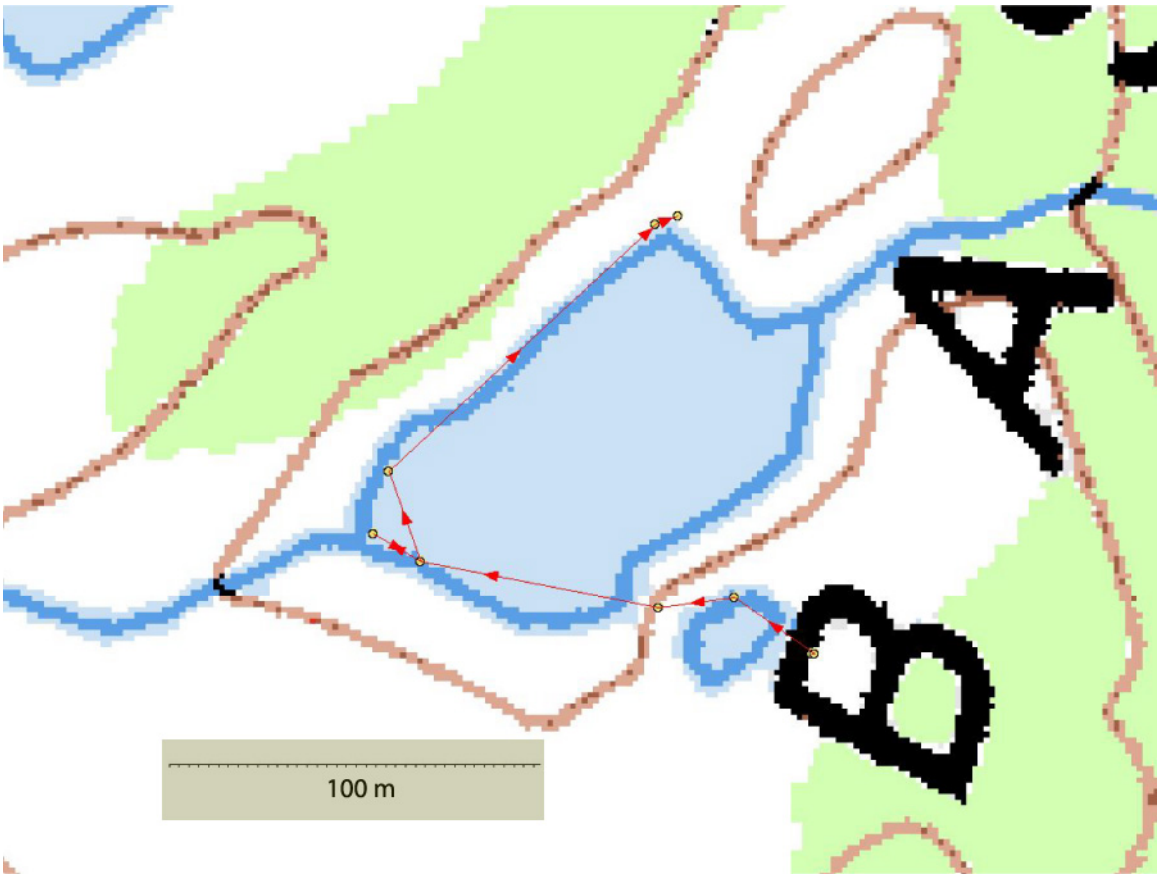


Figure 33.

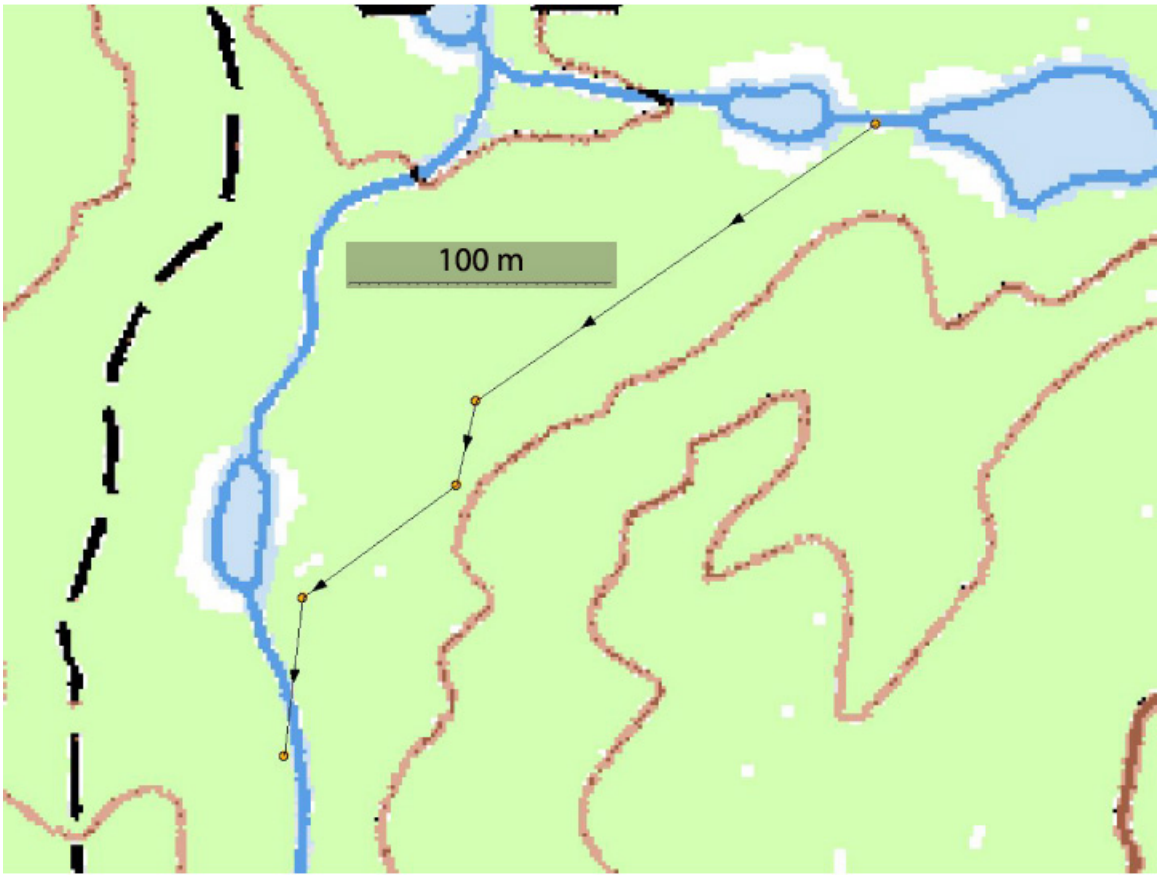


Figure 34.

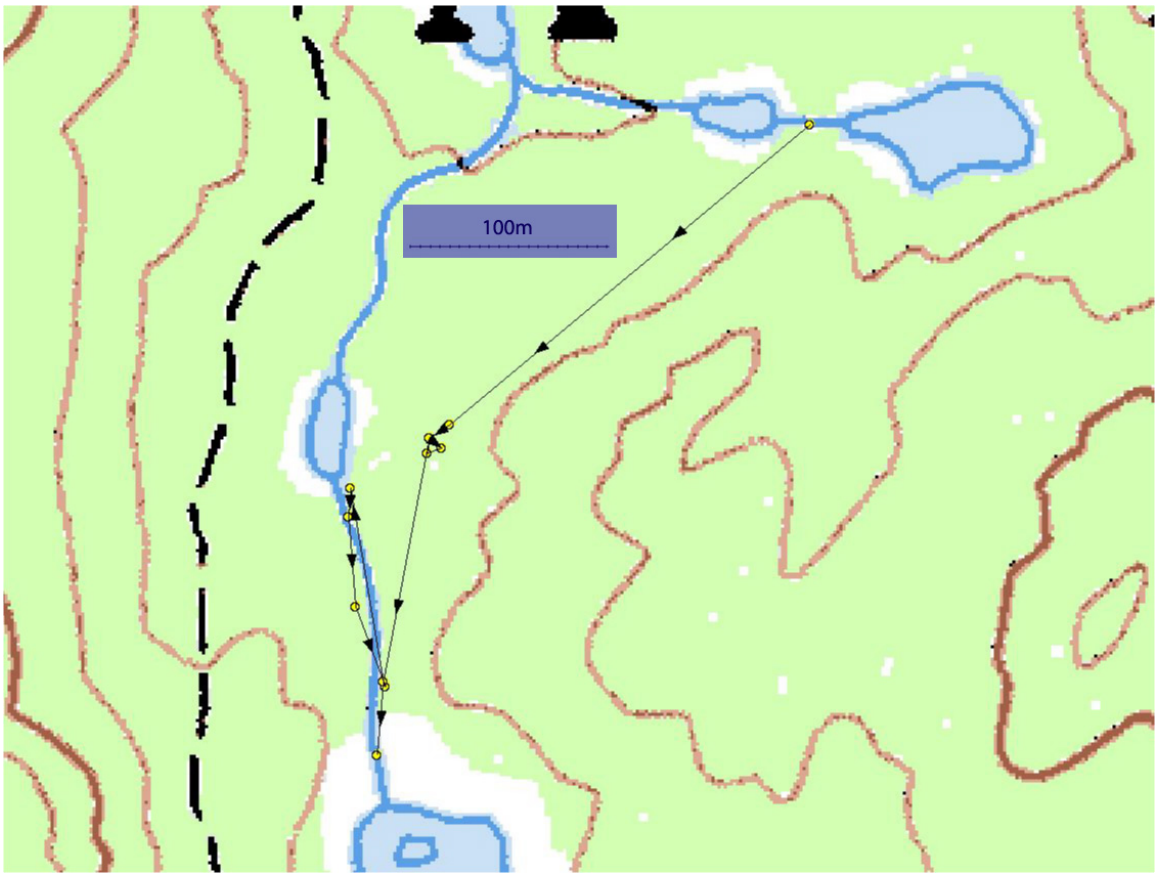


Figure 35.

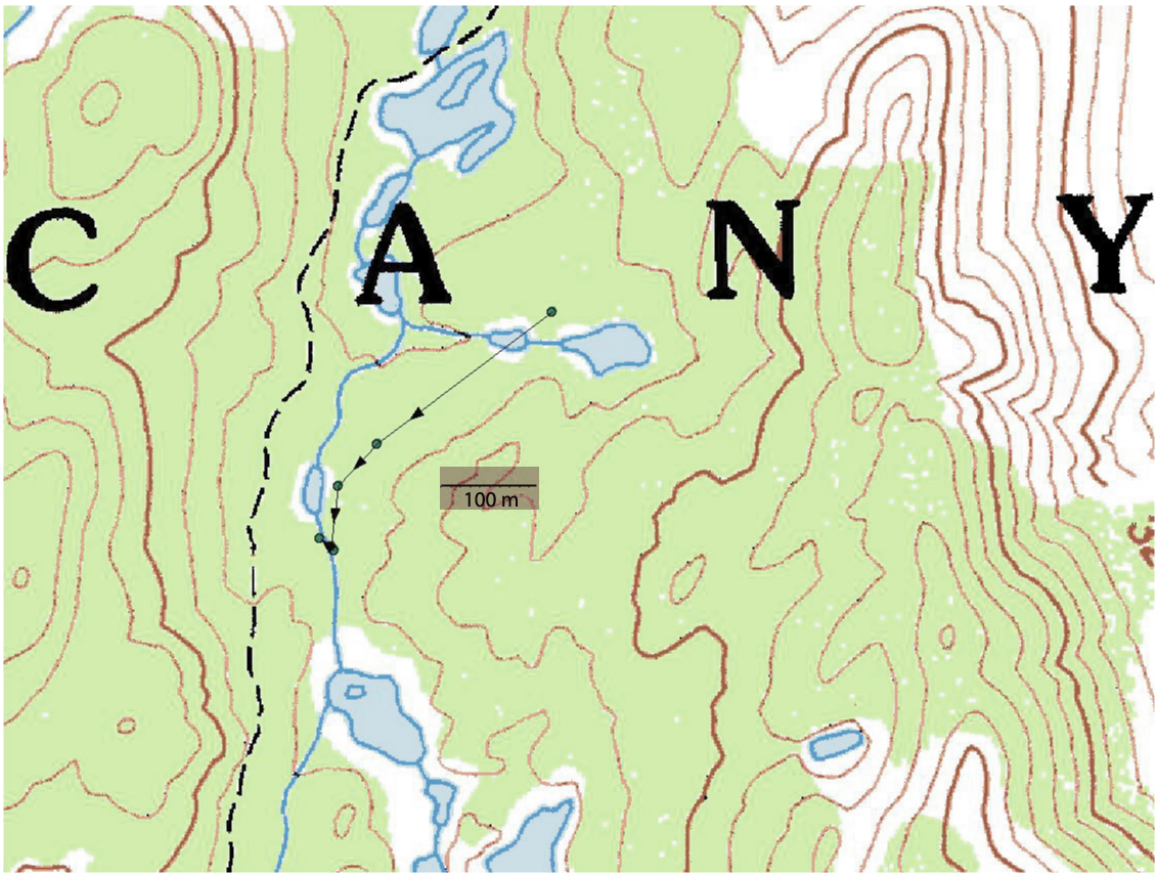


Figure 36.

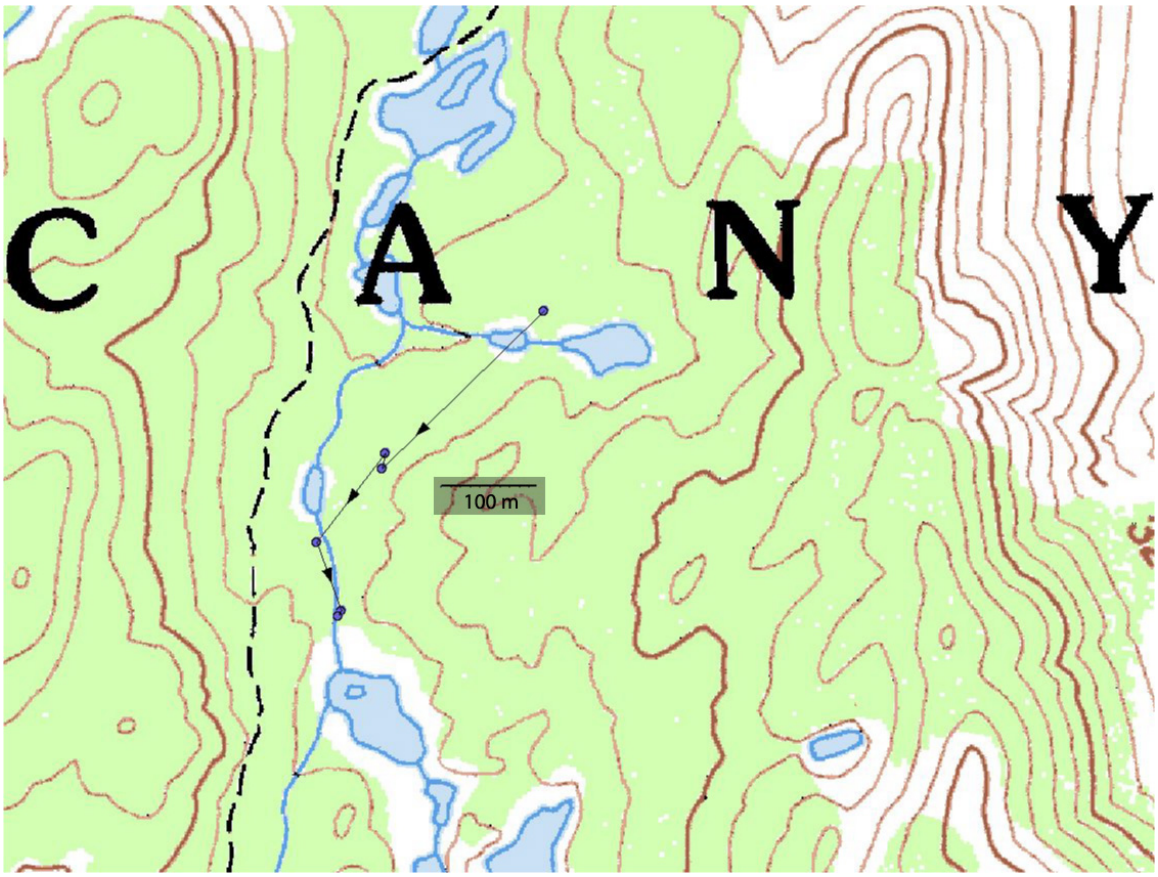


Figure 37.

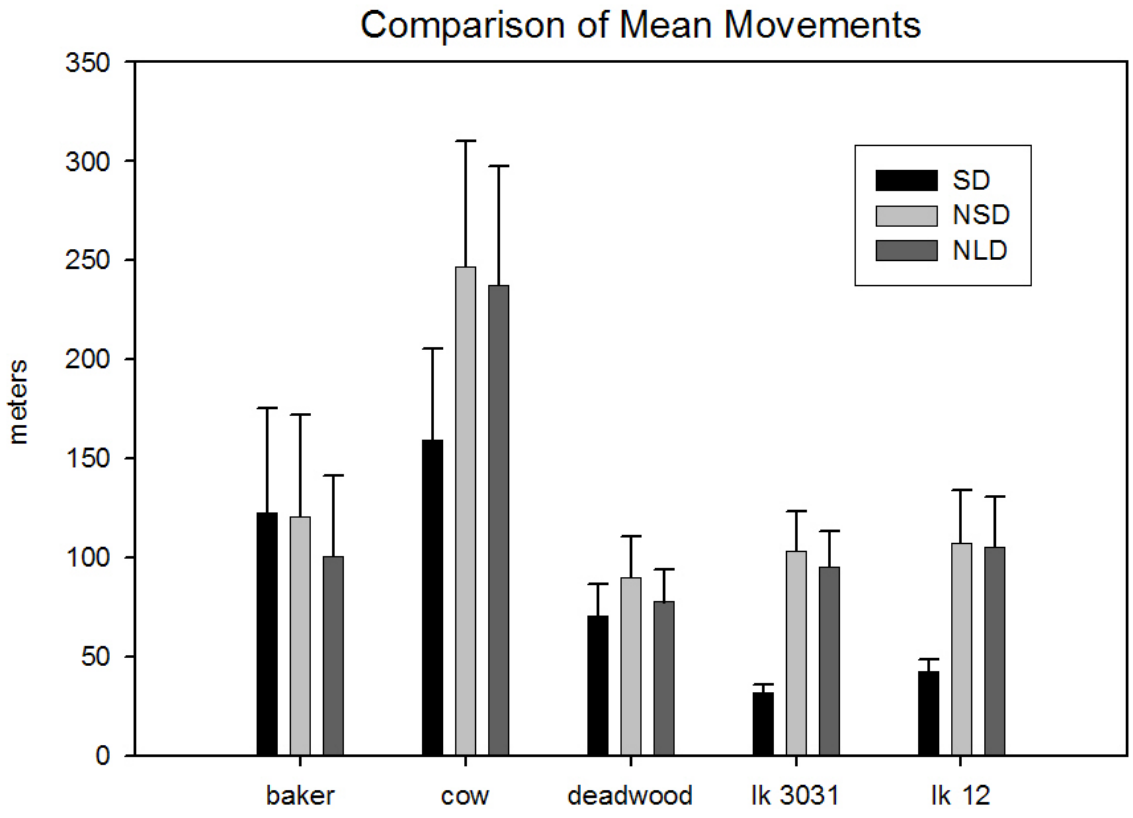

Comparison of Mean Movements
350
300
250
200
150
100
50
0
meters
SD
NSD
NLD
baker
cow
deadwood
lk 3031
lk 12

Figure 38.

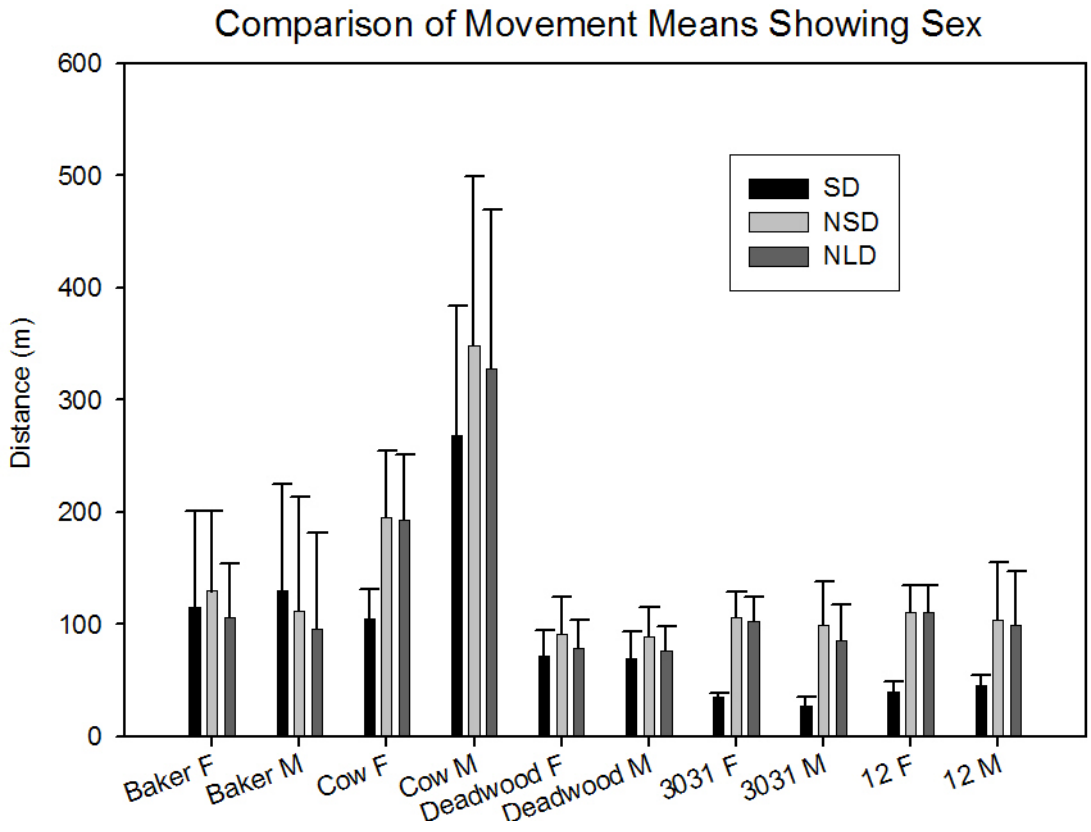

Comparison of Movement Means Showing Sex
Distance (m)
600
500
400
300
200
100
0
SD
NSD
NLD
Baker F
Baker M
Cow F
Cow M
Deadwood F
Deadwood M
3031 F
3031 M
12 F
12 M

Figure 39.

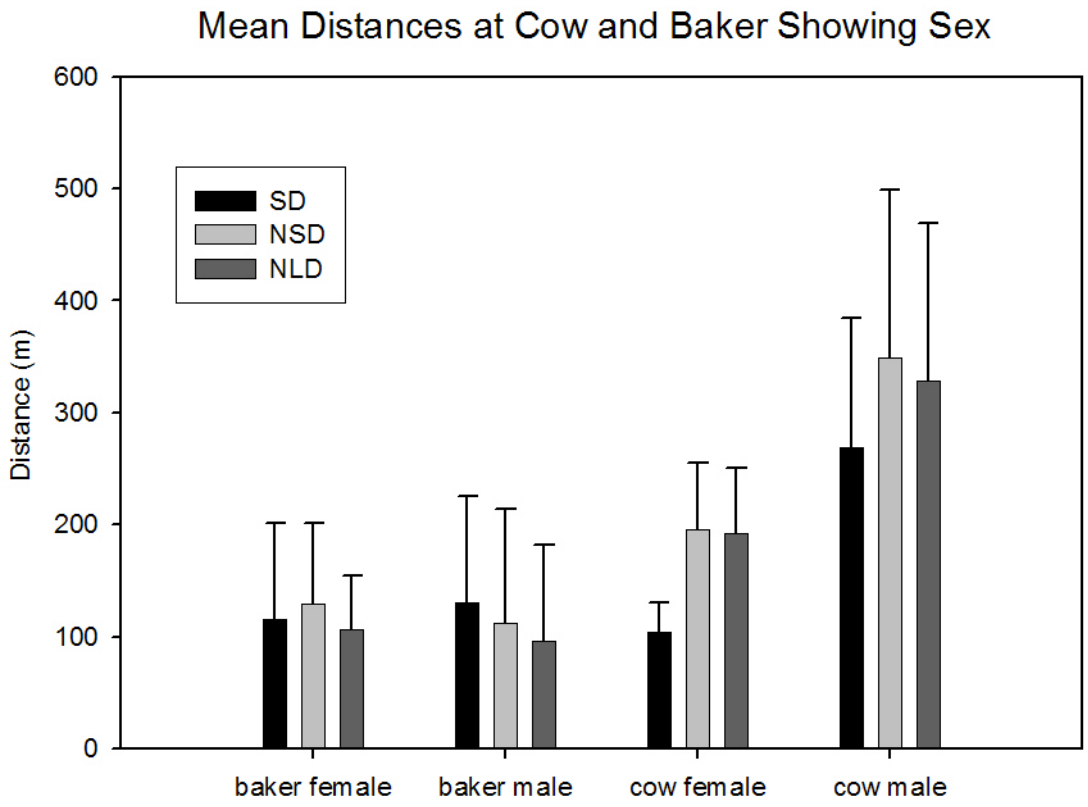

Mean Distances at Cow and Baker Showing Sex
Distance (m)
600
500
400
300
200
100
0
SD
NSD
NLD
baker female
baker male
cow female
cow male

Figure 40.

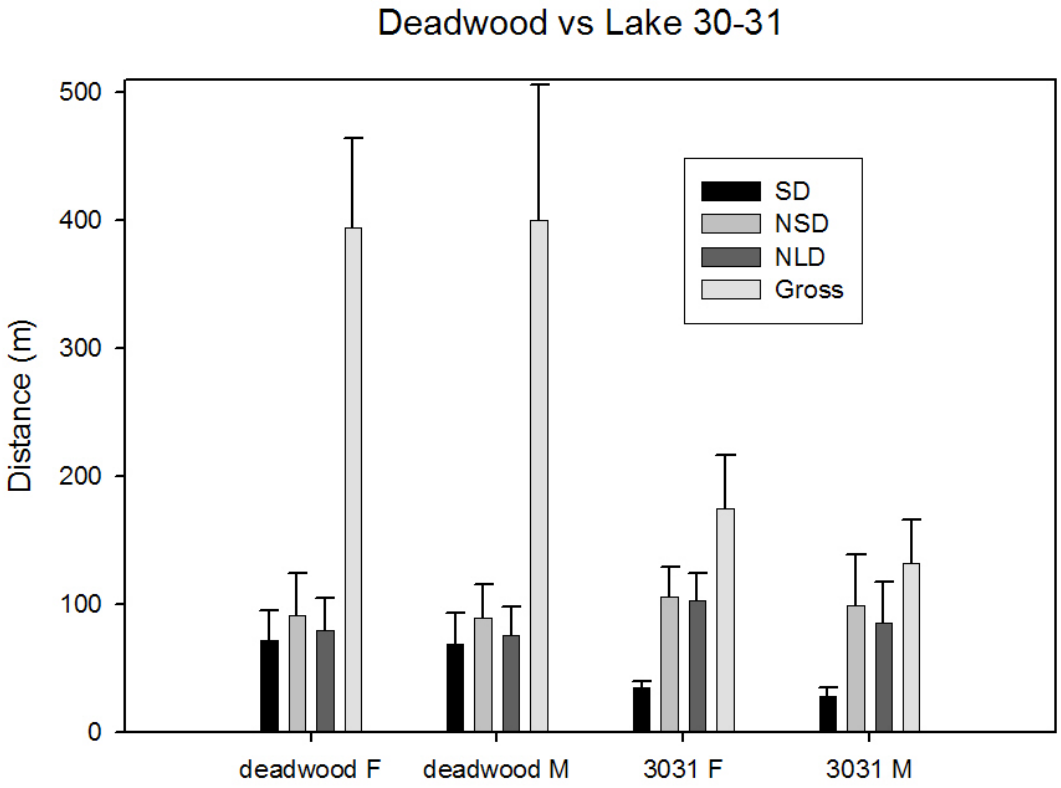

Deadwood vs Lake 30-31
Distance (m)
500
400
300
200
100
0
SD
NSD
NLD
Gross
deadwood F
deadwood M
3031 F
3031 M

Figure 41.

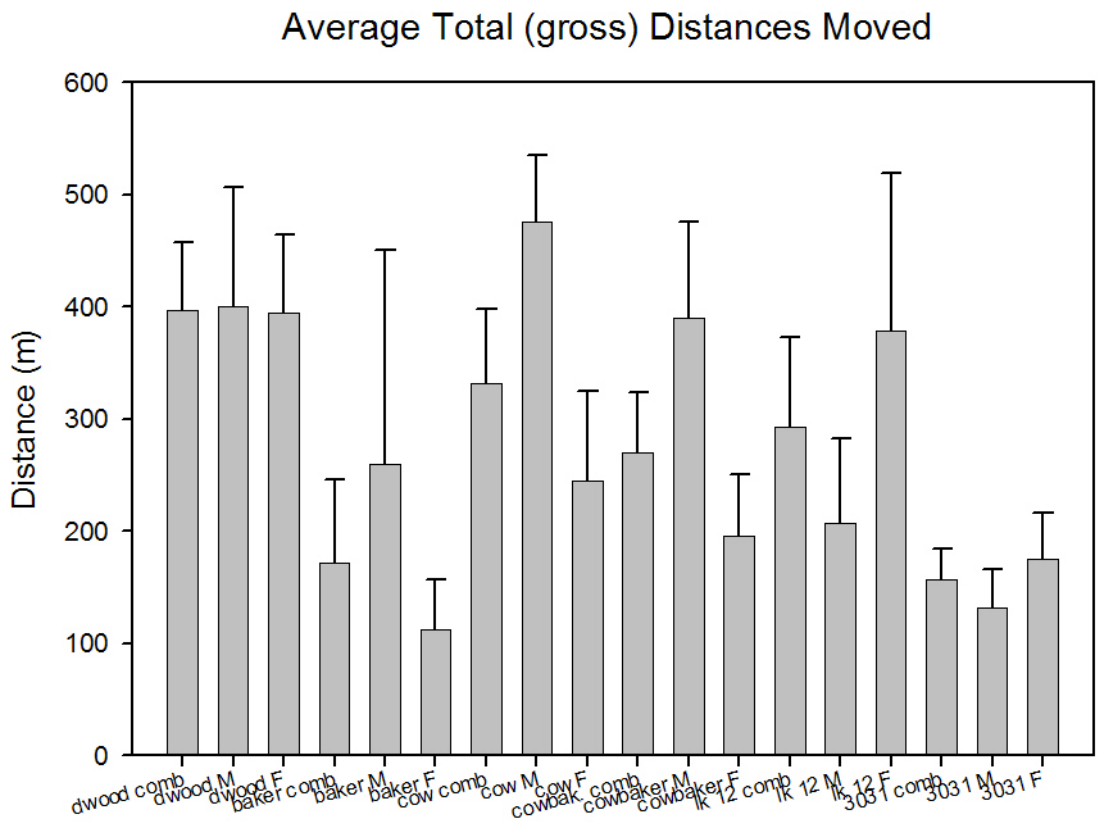

Average Total (gross) Distances Moved
Distance (m)
600
500
400
300
200
100
0
dwood comb
dwood M
dwood F
baker comb
baker M
baker F
cow comb
cow M
cow F
cowbak comb
cowbaker M
cowbaker F
lk 12 comb
lk 12 M
lk 12 F
3031 comb
3031 M
3031 F

Figure 42.

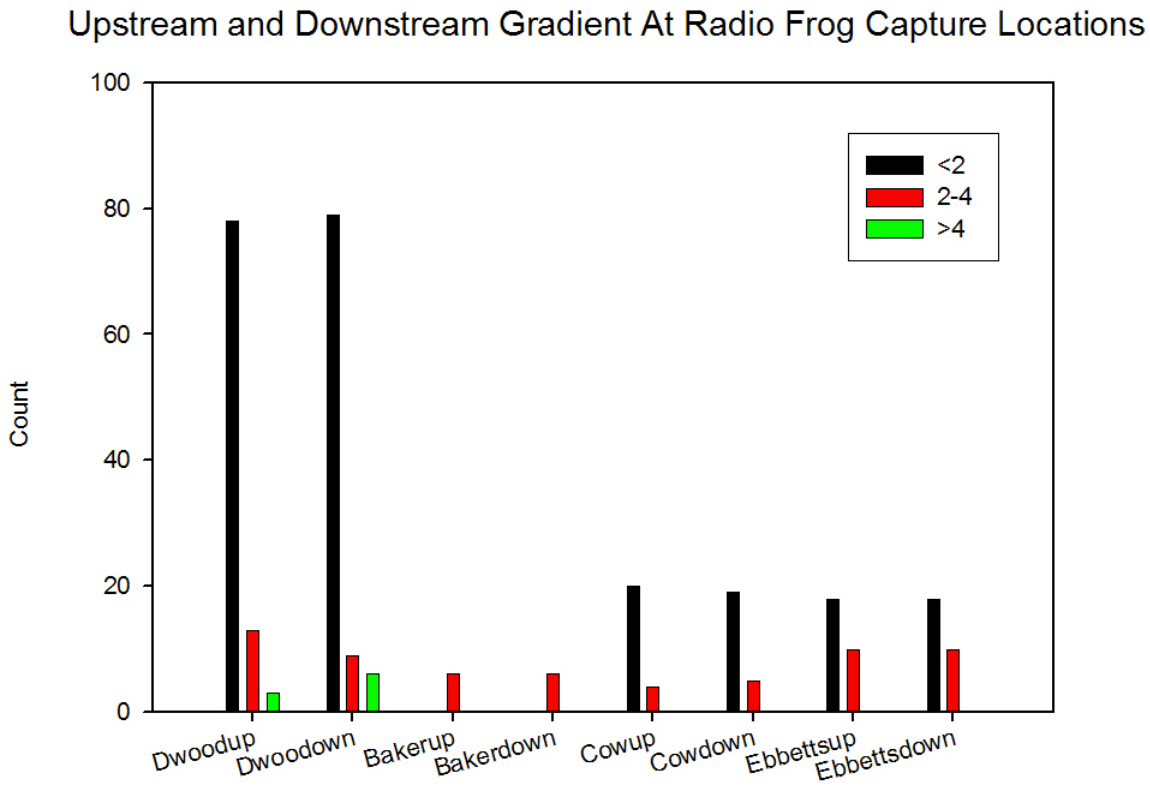

Upstream and Downstream Gradient At Radio Frog Capture Locations
Count
100
80
60
40
20
0
<2
2-4
>4
Dwoodup
Dwoodown
Bakerup
Bakerdown
Cowup
Cowdown
Ebbettsup
Ebbettsdown

Figure 43.

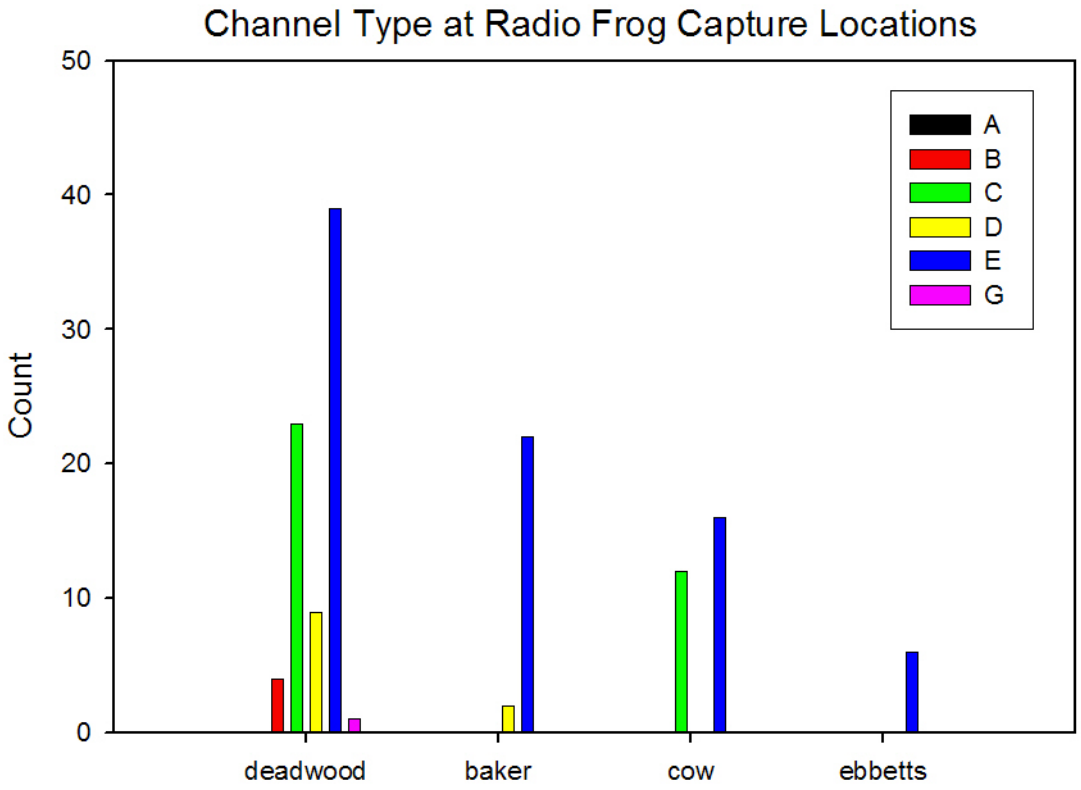

Channel Type at Radio Frog Capture Locations
Count
50
40
30
20
10
0
A
B
C
D
E
G
deadwood
baker
cow
ebbetts

Figure 44.

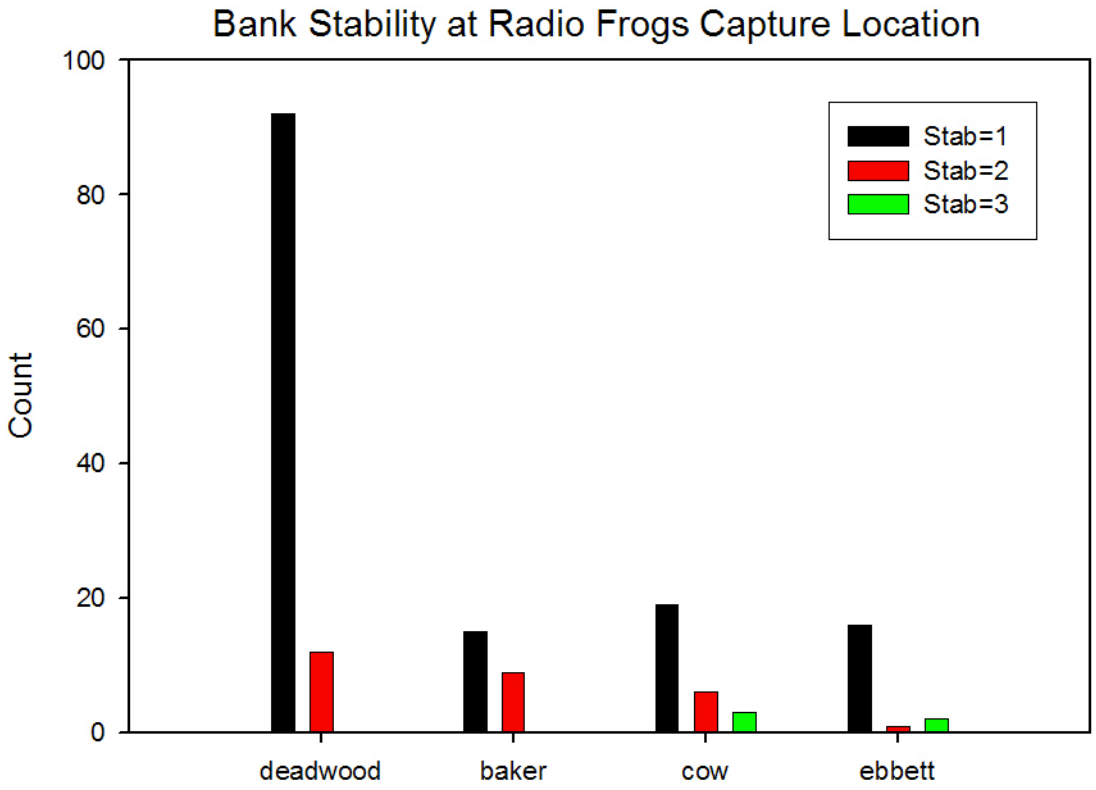
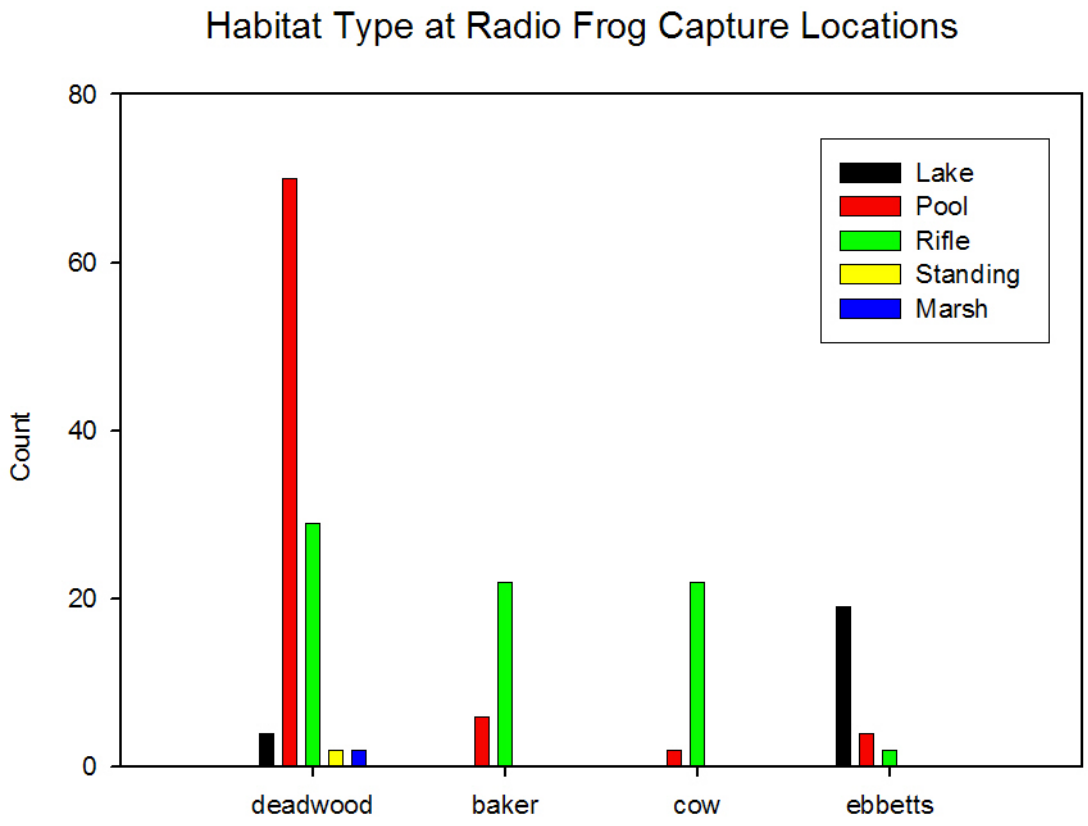

Bank Stability at Radio Frogs Capture Location
Count
100
80
60
40
20
0
Stab=1
Stab=2
Stab=3
deadwood
baker
cow
ebbett

Figure 45.


Habitat Type at Radio Frog Capture Locations
Count
80
60
40
20
0
Lake
Pool
Rifle
Standing
Marsh
deadwood
baker
cow
ebbetts

Appendix A

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Methods to prevent spread of disease:

To ensure that we did not spread potential disease between sites, we followed the standard cleaning protocol (below) whenever we traveled between sites that we either suspected of being infected or were > 500 m apart. With an extensive survey such as proposed in the monitoring program, there is a high risk of field crews spreading disease among amphibian populations. There is increasing evidence that the occurrence of disease, specifically chytrid fungus, is increasing in the Sierra Nevada. Therefore, crews will follow these protocols to clean equipment.

Surveys will begin at the top of the basin and crews work their way down.

Equipment was cleaned:

- immediately after visiting a site where animals appeared to be infected or if the site had a known history of infection, or,
- when moving to a new drainage.

Cleaning Procedures

1. We removed all wet or dried mud, vegetation, and other debris from boots, nets, and other equipment.

2. We mixed a solution of 32 parts water (=1 gallon) to 1 part bleach (= 1/2 cup). We soaked the equipment for at least 15 min.
3. We discarded the solution on site, well away from any water source.

Appendix B.

Stream classification reproduced from Rosgen (Rosgen 1996).

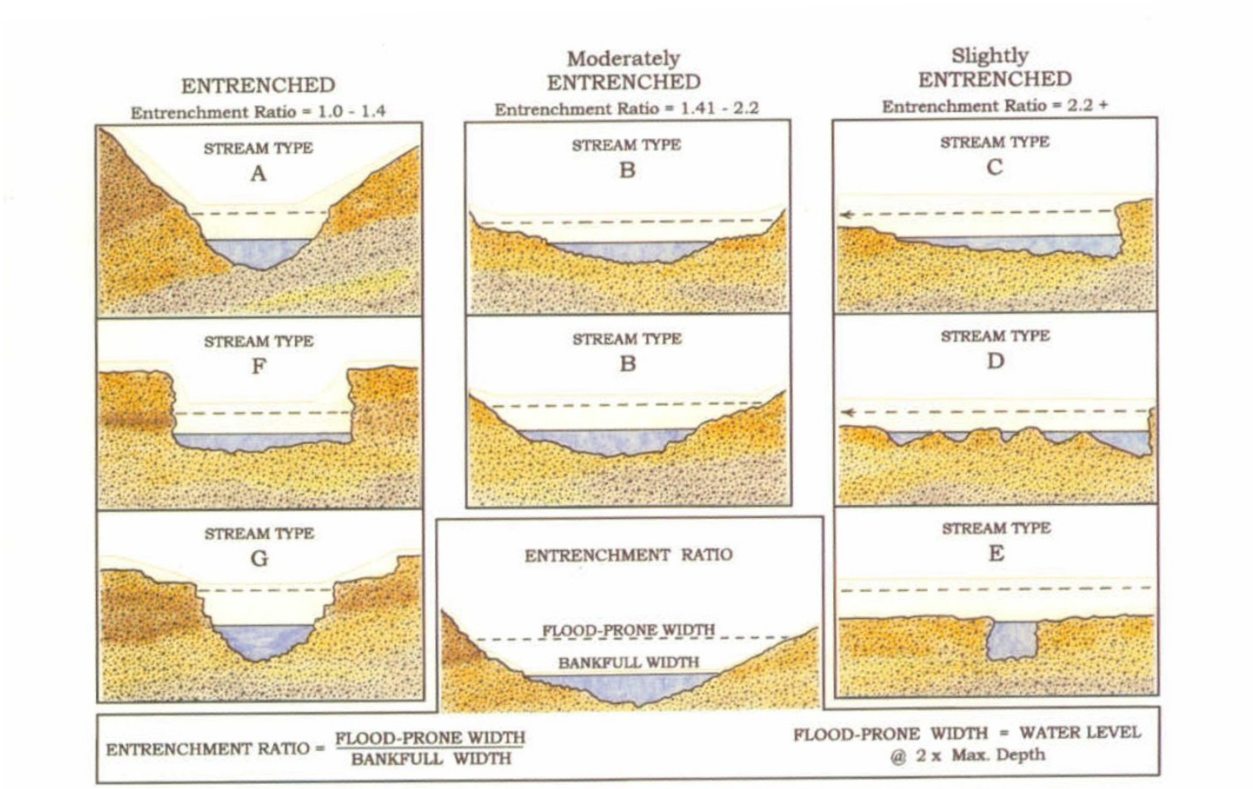

FIGURE 5-10. Representative entrenchment ratios for cross-sections of various stream types.

Appendix C. Reprinted from Macey et al 2001 (Fig 5). Map showing locations of *R. muscosa* populations sampled in California. The four clades are labeled on branches of the phylogenetic tree to the right. Suggested dates of divergence between the clades are derived by applying the pairwise rate of 1.3% sequence divergence per million years. Note the similarity in timing of divergence at approximately 1.5 million years within each of the two major clades.