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NORTHERN RIVER OTTER POPULATION ASSESSMENT AND CONNECTIVITY IN
WESTERN MONTANA

by

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B.S., University of Montana, Missoula, MT, 2009

Thesis

presented in partial fulfillment of the requirements
for the degree of

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Northern river otter population assessment and connectivity in western Montana

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Northern river otters (*Lontra canadensis*) are elusive and difficult to monitor, and little is known about their movement patterns or how populations are structured on the landscape. Otters are sensitive to degradation of aquatic systems, mostly due to loss of prey. This is evident in the Upper Clark Fork River (UCFR), Montana, which has been polluted from decades of mining activity. My objectives for this project were to determine otter population substructuring and connectivity in western Montana and Idaho, and produce a habitat assessment and population estimate for otters in the UCFR.

I used genotypic data from otter tissue samples collected in Montana and Idaho to determine otter population structuring and connectivity. I found no evidence that otter movements are restricted to streams, and there appears to be movements between otter populations in MT and ID over a mountain range. Pair-wise F_{ST} values were highest between ID and MT rivers, but were still within the range shown in otter populations with no physical barriers. Four possible first generation migrants were detected, with two of those migrants between ID and MT. Least-cost path analyses revealed that genetic distances between pairs of otter samples were more correlated with Euclidean distances than stream distances.

I characterized vegetation at 310 random locations and 35 otter latrine sites along the UCFR. Random sites were characterized by low overstory and understory cover, and tended to have ground cover dominated by grass, shrub, and dirt/sand. Latrine sites were located in areas with significantly higher bank heights and medium levels of understory cover. Latrine sites also tended to be located near beaver activity more than random sites. In addition to the habitat assessment, I non-invasively collected hair and scat samples for use in genetic population estimates. I was able to genotype 11 scat and hair samples, for a total of 8 individuals. I was unable to calculate a population estimate because of low sample size. It is not recommended to use non-invasive sampling measures to estimate population until other genetic markers (e.g. SNPS) are developed for otters that can utilize low-quality DNA from otter scat.

Otters can be found in multiple aquatic habitat types, and my habitat analyses reveal little to guide restoration in the river. Therefore, otters may not be an appropriate tool to guide restoration efforts on the UCFR. However, continuing to monitor otter latrine sites can be an effective way to track ecological responses to restoration.

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CHAPTER 1

INTRODUCTION

Furbearers have been an important resource in North America, and have helped shape the development of the continent since the 1490s (Ray 1987). One important furbearer throughout the history of trapping is the Northern river otter (*Lontra canadensis*; hereafter otters). Otters are mid-sized (7-10 kg) members in the family Mustelidae highly adapted for aquatic environments (Melquist and Dronkert 1987, Larivière and Walton 1998). Otters are a top predator in aquatic systems (Melquist and Hornocker 1983, Kruuk 2006), and as a furbearer, are an important resource for trappers in some states (Melquist and Dronkert 1987). Otters historically occurred throughout North America in any area a permanent water source was available, excluding the Arctic and arid southwestern U.S. (Hall 1981, Melquist and Dronkert 1987). The intense fur trade of the 19th and early 20th centuries led to over-harvesting of otters across much of their range (Melquist and Dronkert 1987). As a result, they saw population declines in all states and Canadian provinces, including the extirpation of the species from at least 6 states (Raesly 2001). Conservation efforts began in the 1970s (Raesly 2001) resulting in otter recovery in many areas. These efforts included reintroduction or translocation programs in 22 states and 1 National Park. Otters can now be found in every state in the continental U.S. (Raesly 2001).

In Montana, the otter trapping season was closed in 1949 for 7 years because the state population was too low to sustain harvest (Newby 1957). Today, otters are assumed to be relatively abundant in the state, with a state-wide trapping quota of around 100 annually (FWP 2012). As is the case in many states, managers in Montana largely rely on harvest data to set otter quotas (Organ et al. 2001, MFWP 2011).

Monitoring

There is surprisingly little information on otters in North America. Most studies have monitored otters after a reintroduction event (e.g. Serfass et al 1993, Johnson and Berkley 1999). There is a clear need for a better understanding of basic otter ecology to direct sound management.

Otters are difficult to monitor due to their elusive nature and low population densities (Kruuk and Conroy 1987). Numerous techniques have been used to monitor populations. In Canada (for Northern river otters) and Finland (for Eurasian otters [*Lutra lutra*]) snow tracking was reported to be a relatively inexpensive and useful method for determining presence across large areas, but required substantial manpower to be effective (Reid 1987, Sulkava 2007). However, snow tracking was found to estimate only half of known individuals when compared to genetic techniques (Arrendal et al. 2007). Surveys for scat and latrine sites (i.e. places where otters scent mark, urinate, and defecate repeatedly) have also been used, although there is controversy regarding the relationship between number of scat piles and otter density and activity (Kruuk and Conroy 1987, Mason and Macdonald 1987, Gallant et al. 2007). Many state and federal agencies use bridge-surveys to monitor otters. This involves surveying for otters for a specified distance up and downstream from randomly selected bridges (Gallant et al. 2008). There are discrepancies between studies using bridge crossing surveys. Some reported bridge surveys work as well as surveys of random locations to detect otter presence (Gallant et al. 2008, Roberts et al. 2008), while others reported random locations detected more otter presence than bridge-sites, possibly due to otter avoidance of human activity (Crimmins et al 2009). All of these methods, although inexpensive and good at detecting otter presence, fail to correlate sign detections with actual otter population numbers or density.

Genetic analysis is another method used to estimate population numbers. Genetic analyses from non-invasive samples (e.g. hair and scat) collected in the field are used to differentiate individuals and get population estimations using mark-recapture calculations (Mills et al. 2000). The method has been used for survey-difficult species such as grizzly bears (*Ursus arctos*; Kendall et al. 2008), mountain gorillas (*Gorilla beringei beringei*; Guschanski et al. 2009), and humpback whales (*Megaptera novaeangliae*; Palsboll et al. 1996), among many other species. Genetics can also shed light on movement patterns, population health, and help develop conservation strategies for species or populations (Blundell et al. 2002, Schwartz et al. 2006, Allendorf and Luikart 2007, Latch et al. 2008). Most otter non-invasive genetic studies have collected scat and primarily been done on Eurasian otters. DNA amplification rates as low as 12% from otter scat have inhibited many studies (Table 1.1).

Table 1.1. List of projects obtaining DNA from otter spraints and the genotyping success.

| Genotyping Success Rate | Success Definition | Species | Source |
|-------------------------|--------------------------------|----------------------|-----------------------|
| 20% (84/426) | 7-9 out of 9 microsatellites | <i>L. lutra</i> | Dallas et al. 2003 |
| 65% (222/343) | 7 out of 7 microsatellites | <i>L. lutra</i> | Hung et al. 2004 |
| 41% | 6 out of 6 microsatellites | <i>L. lutra</i> | Hajkova et al. 2006 |
| 43% (66/154) | 6-9 out of 9 microsatellites | <i>L. lutra</i> | Arrendal et al. 2007 |
| 85% (35/41) | 1 out of 4 microsatellites | <i>L. canadensis</i> | Hansen et al. 2008 |
| 18% (22/86) | NA | <i>L. canadensis</i> | McElwee 2008 |
| 46% (582/1265) | 7 out of 7 microsatellites | <i>L. lutra</i> | Koelewijn et al. 2010 |
| 12% (110/893) * | 7-8 out of 8 microsatellites | <i>L. canadensis</i> | Guertin et al. 2010 |
| 25.7% (124/483) * | 6 out of 6 microsatellites | <i>L. canadensis</i> | Brzeski 2010 |
| 24% (341/1421) | 7-10 out of 10 microsatellites | <i>L. canadensis</i> | Mowry et al. 2011 |

* These values explicitly include anal jelly samples, which have been shown to have higher amplification rates than spraints

No studies have used DNA extracted from hair to estimate otter populations, but this has been done successfully on multiple species (e.g. Kendall et al. 2009, Clevenger and Sawaya 2010, Wasserman et al. 2010, Henry and Russello 2011). Thus, using hair may be an effective

way to obtain genetic material to study otter populations, especially in areas with low otter populations.

Habitat

Otters are anatomically specialized to live in aquatic environments (Larivière and Walton 1998), but are habitat and environmental generalists. Otters preferred marshy areas in an Ohio Wildlife management area (Helon 2006), which is probably due to slow moving water, logjams, and prey congregation. Otters were found in all aquatic environment types in Massachusetts, ranging from shallow marshes to deeper lakes, and preferred areas with trees >30 cm, areas with beaver lodges or bank dens, and places near stream mouths (Newman and Griffin 1994). It was concluded that all freshwater aquatic environments had the potential to provide otter habitat in Massachusetts. In boreal Alberta, Canada, otters preferred streams with beaver ponds and lakeshores containing primarily organic substrates, with selection against large shore substrates such as sand and rocks (Reid et al. 1994). Otters preferred stream systems to lakes or marsh areas in central Idaho, most likely as an avoidance of human activity (Melquist and Hornocker 1983).

In collaboration with MFWP, I began a pilot project in summer 2009 documenting otter activity and distribution along 193 km of the Upper Clark Fork River (Foresman 2009), part of the largest EPA Superfund Site in the U.S. Thirteen latrine sites (defined as 2 or more otter scats) and 6 possible den sites were located. Otter sign was found sporadically along the entire stretch of the river and otter populations appeared to be much lower than populations in Southwest Montana (Zackheim 1982).

This thesis is a continuation of the previous work with MFWP and has 2 overall objectives. The first objective is to determine how otter populations are structured over large

landscapes. In Chapter 2, I use genotypic data obtained from tissues from otters in Montana and Idaho to determine connectivity of otters both between interconnected stream systems in Montana and between otter populations separated by mountains in Montana and Idaho. The second objective is to assess habitat availability and otter population size in a recovering riverine system. In Chapter 3, I present habitat and genetic data collected in the Upper Clark Fork River (UCFR), specifically addressing the recovering otter population from that area. I descriptively compare habitat data collected in the UCFR with habitat data collected in nearby river systems, and also evaluate otter latrine site selection. Lastly, I describe results of using hair samples to non-invasively estimate otter population size.

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CHAPTER 2
OTTER POPULATION SUBSTRUCTURING AND CONNECTIVITY IN WESTERN
MONTANA AND IDAHO

Abstract

Northern river otters (*Lontra canadensis*) are elusive and difficult to monitor. Therefore, little is known about their basic ecology, especially in mountainous areas. I used tissue samples collected from otters in Montana and Idaho (separated by the Bitterroot Mountain Range) to determine the geographic structure of otters. I genotyped tissue samples at 11 microsatellite loci, and evaluated substructure between river systems using pairwise F_{ST} values and the clustering programs GENELAND and STRUCTURE. I used least-cost path analyses to determine the likely route of gene flow between populations in Idaho and Montana. Pair-wise F_{ST} values were highest between Idaho and Montana rivers, but were still within the range previously shown in otter populations directly connected through stream networks. Both clustering programs indicated that the most likely number of otter populations was 2, with a strong division between the MT and ID otters. Four possible first generation migrants were detected, with 2 of those migrants likely between ID and MT. Least-cost path analyses suggest that Euclidean distance and models that allow for some overland movement better represent genetic comparisons between samples than models restricting otters to movements in stream networks. Collectively, these results suggest that otters are traveling over a mountain range between ID and MT. Because the heterozygosity values in MT and ID are similar to those found in much higher otter densities, it appears that the populations in MT and ID are historically either large or connected

enough to maintain genetic variation. However, large shifts in allele frequencies were detected over a short time-frame within some rivers, suggesting low population numbers.

Introduction

The study of population connectivity broadly deals with movements of animals between discrete populations or subpopulations. This movement of individuals can influence population vital rates, facilitate gene flow, and allow populations to persist over time (Mills 2007, Lowe and Allendorf 2010), but can also have negative impacts such as disease transmission (Hess 1994). Connectivity is the driving force behind conservation actions such as the creation of habitat corridors (Beier and Loe 1992) and the development of management units and reserves in fisheries (Fogarty and Botsford 2007). Knowing how animals move between subpopulations also helps determine the correct spatial scale for management (Fogarty and Botsford 2007), and guide managers when setting harvest quotas or units.

It is important to note two definitions of population connectivity: genetic and demographic connectivity. Demographic connectivity provides an understanding of how immigration and emigration of individuals affect vital rates and population growth rates (Runge et al. 2006, Waples and Gaggiotti 2006, Lowe and Allendorf 2010). Genetic connectivity simply implies that two or more populations are evolutionarily connected and exchange genes (Waples and Gaggiotti 2006, Lowe and Allendorf 2010). Commonly, F_{ST} values (Wright 1943) are used to assess genetic connectivity and gene flow between populations. With the rapid increase in technology and statistical tools, genetics can now be used to infer aspects of demographic connectivity. For instance, first generation migrants can be detected using multiple polymorphic genetic markers such as microsatellites (e.g. Piry et al. 2004).

There are two general approaches to estimate substructuring on a landscape, which is required to estimate connectivity between populations. First, subpopulations may be designated *a priori* using geographic distance and barriers to movement, or to use movement data from collared individuals (Amstrup et al. 2004). Genetic techniques now allow us to delineate possible groupings of individuals using multilocus markers. Common structuring programs use Bayesian clustering algorithms to determine the most likely number of populations, K (e.g. Pritchard et al. 2000, Guillot et al. 2005a).

Genetic tools that help determine population substructuring and connectivity are useful for species that are difficult to monitor otherwise. One such species is the northern river otter. Otters act as top predators in aquatic systems and can act as indicators of aquatic health (Beazley and Cardinal 2004), particularly pollutants in aquatic systems (e.g. Kucera 1983, Mayack 2012). They are also an important source of recreation and income for trappers (Melquist and Dronkert 1987, Melquist et al. 2003).

Little is known about otter populations, especially in mountainous regions. Otters are elusive and difficult to monitor (Swimly 1998), and seem to be at lower population densities in mountainous regions than in lowlands and coastal areas (Zacheim 1982, Melquist and Hornocker 1983, Shackelford and Whitaker 1997, Depue 2007, Mowry et al. 2011). To gain insights into otters in mountainous areas, I used genomic DNA extracted from tissue samples collected from trapped otters in Montana (MT) and Idaho (ID) to better understand otter population genetics, substructuring, and connectivity.

Methods

Study Area and Sample Collection

I collected 85 otter tissue samples from Montana and 22 samples from Idaho. Samples from Montana came from Montana Fish, Wildlife and Parks Region 2 (Figure 2.1), and were provided by MFWP, who collect and store the carcasses of all legally trapped otters in the state. MT samples came from 5 rivers: Bitterroot River, Blackfoot River, Clearwater River, Lower Clark Fork River, and Upper Clark Fork River. Samples from Idaho were provided directly by

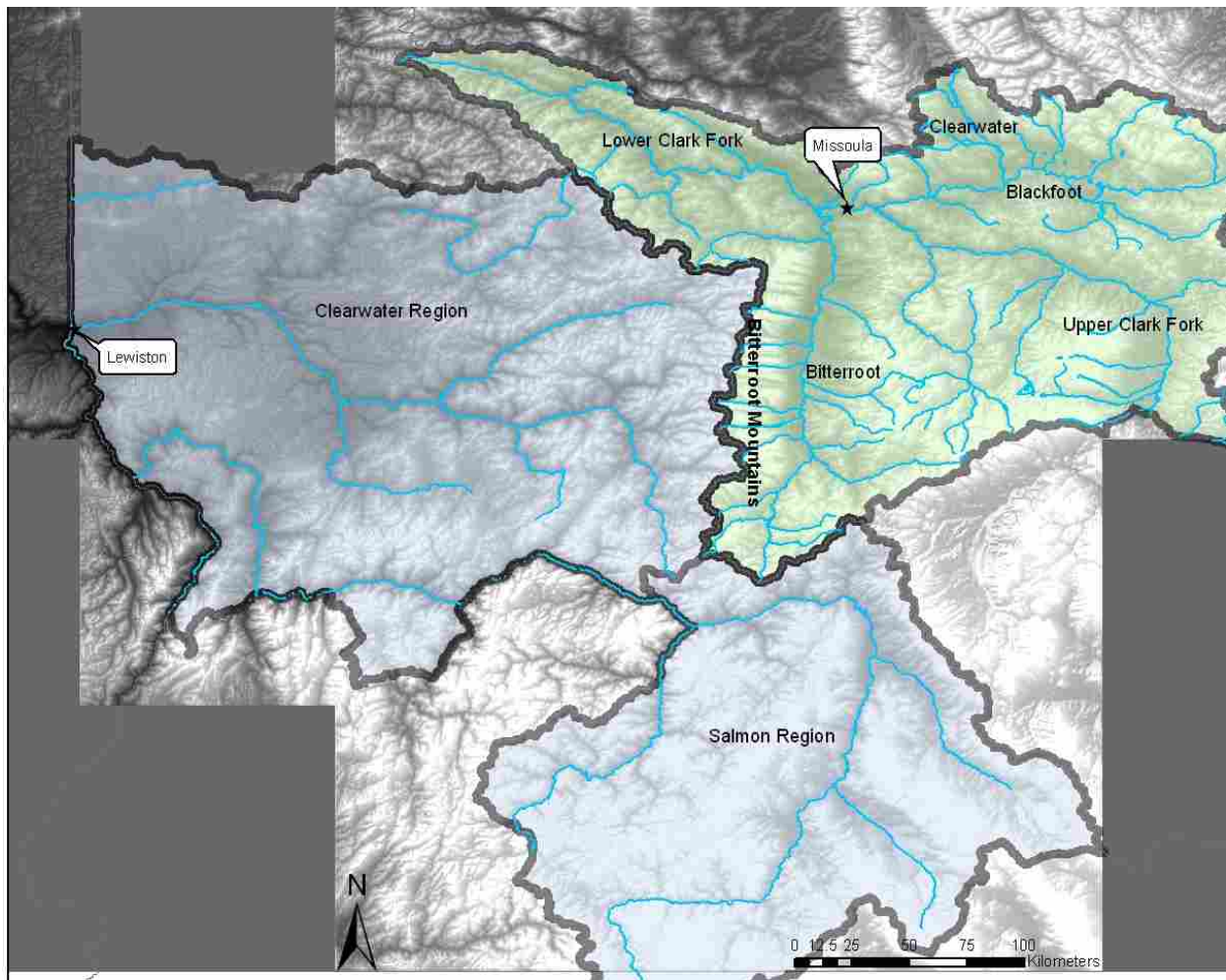


Figure 2.1. Map showing the sampling areas of otter tissues from Idaho and Montana. The green area represents Montana Fish, Wildlife and Parks Region 2, while the blue areas represent Idaho Fish and Game's Clearwater (referred to as the Lochsa area in analyses) and Salmon Regions. The Bitterroot Mountains separate Idaho and Montana samples.

trappers when they brought otter pelts to Idaho Fish and Game to be tagged. These samples came from the Clearwater and Salmon regions of Idaho (Figure 2.1). The Clearwater samples will be referred to as “Lochsa” samples in analyses to avoid confusion with MT samples from the Clearwater River, MT. Idaho samples are separated from those in MT by the Bitterroot Mountains and roughly 1500-2000 river kilometers (depending on the ID Region and the MT river), and the 2 regions in ID are separated by roughly 250 miles of the Salmon and Snake Rivers. There are no known major physical barriers to otter movement between the MT samples.

DNA Extraction and Sequencing

I extracted genomic DNA from the tissue samples using DNeasy Blood and Tissue kits (QUIAGEN Inc., Valencia, CA) and followed the kit protocols with only slight modifications. Samples were analyzed using 11 microsatellite loci published for mustelids: *Lut435*, *Lut457*, *Lut604*, *Lut701*, *Lut733*, *Lut782*, *Gg7*, *Gg14*, *Tt1*, *Mer022*, and *Mvis075* (Dallas and Piertney 1998, Davis and Strobeck 1998, Fleming et al. 1999). The reaction volume (10 µl) contained 1.0-3.0µL DNA, 1x reaction buffer (*Applied Biosystems*), 2.0 mM MgCl₂, 200µM of each dNTP, 1µM reverse primer, 1µM dye-labeled forward primer, 1.5 mg/ml BSA, and 1U *Taq* polymerase (*Applied Biosystems*). The PCR profile was 94°C/5 min, [94°C/1 min, 55°C/1 min, 72°C/30s] x 36 cycles. PCR products were run in a 6.5% acrylamide gel for 2 hours on a *LI-COR DNA analyzer* (*LI-COR Biotechnology*).

Genetic Variation and River-based Comparisons

I considered each river in MT and each Region in ID as a sampling area, for a total of 7 areas (Upper Clark Fork River, Blackfoot River, Clearwater River, Lower Clark Fork River, and Bitterroot River in MT; Salmon Region and Clearwater Region in ID). I will loosely refer to these as populations for simplicity. I tested for deviations from Hardy-Weinberg proportions and

linkage disequilibrium in GENEPOP (version 4.1; Rousset 2008) and calculated observed (H_O) and expected (H_E) using GENALEX (version 6.41; Peakall and Smouse 2006). I corrected for multiple tests using the sequential Bonferroni adjustments (Rice 1989). I also calculated pairwise- F_{ST} values (Weir and Cockerham 1984) between the 7 populations using the program FSTAT (version 2.9.3.2; Goudet 1995), and R_{ST} values with GENALEX. Allelic richness corrected for population size and F_{IS} values were calculated using FSTAT. Population clusters were visually inspected using principle component analysis (PCA) in program PCAGEN (<http://www2.unil.ch/popgen/softwares/pcagen.htm>).

Individual-based Analyses

I used individual-based methods to look at possible otter population structuring in the study area. I ran simulations including MT and ID samples, and also with samples only from MT. Specifically, I used the programs GENELAND (version 3.2.4; Guillot et al. 2005b) and STRUCTURE (version 2.2.3; Pritchard et al. 2000, Falush et al. 2003). Both of the programs use Bayesian clustering to estimate “K”, the most likely number of population clusters. GENELAND uses a spatial model and allelic differences to locate discontinuities in genetic data, while STRUCTURE uses an algorithm solely based on allelic differences. Each program also calculates a probability of population membership for each sample.

In GENELAND, I used 10 replicates of the spatially explicit model with uncorrelated allele frequencies and the null allele model set to “False”. I used the following parameters: maximum rate of Poisson process set to the number of samples (107 for ID and MT and 85 for MT only; recommended in Guillot et al. 2005a), uncertainty on coordinates at 0.05 degrees (~5 kilometers), number of K from 1-8, maximum number of nuclei set at 3 times the sample size (321 for MT and ID and 255 for MT only; Guillot et al. 2005b), number of MCMC iterations set

at 500,000 with a thinning of 100 and a burn-in of 200 saved iterations (or 20,000 total iterations).

In STRUCTURE, I ran the admixture model with correlated allele frequencies among populations. I ran 500,000 iterations after a burn-in of 20,000 for $K=1 - 8$. I replicated each K 10 times, for a total of 80 simulations. I then averaged the $\log \Pr(X|K)$ across the 10 runs for each K to determine which K value maximized $\Pr(X|K)$. I also calculated the ΔK values for each K as recommended in Evanno et al. (2005), which is the secondary rate of change between successive values of $\log \Pr(X|K)$.

I used the program GENECLASS 2 (Piry et al. 2004) to identify possible migrants between river populations, specifically between ID and MT populations. I used the detection of first generation migrants and individual assignment options with the Rannala and Mountain criterion for computation, and allowed probability computation using Monte-Carlo simulations following Paetkau et al. (2004).

Least-cost Path Analyses

I used least-cost path analyses to determine the most likely routes of population connection in my study area. I tested 3 competing landscape hypotheses to determine what best describes the pattern of genetic variation in the otter samples. 1) Euclidean Distance: genetic variation between points is explained by straight-line distance, 2) River Distance: genetic variation is explained by distances between points following streams only, 3) Landscape Resistance: genetic variation is best explained by a cost to otters traveling over land, but land does not represent a complete barrier to otter movement. I created simple cost-layers (Figure 2.2) using ArcMap in ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, California) where stream pixels had a cost of one and land pixels had varying levels of cost

values, ranging from 1 (simple Euclidean distance) to 10,000 (which restricts otter movements to streams; Table 2.5). I used the PATHMATRIX extension (Ray 2004) for ArcView (Environmental Systems Research Institute, Redlands, California) to calculate the “cost” of movements between any 2 of the otter sample locations, which is the sum of the pixels along a path between the locations. The program outputs a least-cost “distance” between the locations, depending on how the cost-layer is set. If the cost of land travel is low, the least costly route between 2 points may include some overland movements. However if the cost land travel is high, the least costly route may only follow streams between 2 points.

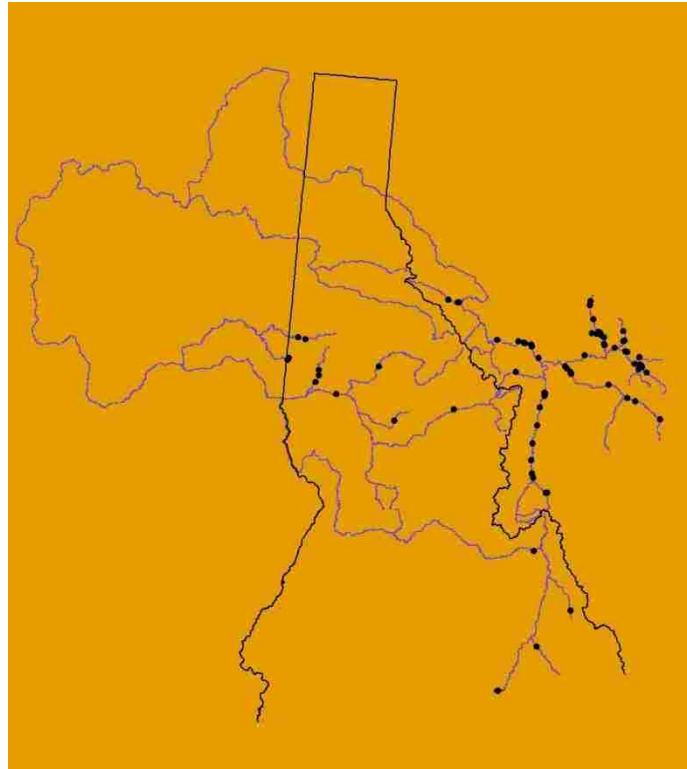


Figure 2.2. Sample cost layer from least-cost path analysis. In all analyses, stream pixels (blue) had a cost value of 1 while land pixels (orange) varied between models. The least-cost path is that which minimizes the sum of the pixels between 2 points on the map. With low land costs, least-cost paths may include some overland movements between points, while higher levels restrict movements to streams.

I used Mantel and partial Mantel tests to determine correlations between the cost-distance matrices and pairwise genetic distances (Smouse and Peakall 1999) of the otter samples following Cushman et al. (2006). Partial Mantel tests determine correlation significance between the cost and genetic matrices while accounting for the effects of isolation by distance (by

removing the correlation explained by Euclidean distances). Mantel tests were performed using the *ecodist* package (Goslee and Urban 2007) in program R (R Core Team 2012).

Results

Temporal Stability

The MT otter samples totaled 185 and dated from 1996 to 2011. To look at the possible effects of using samples from such a long time-frame, I used the oldest and newest (based on year collected) 10 samples from each MT population and calculated F_{ST} values between each of these pairs. All 5 comparisons within each river had significant F_{ST} values between the oldest and newest samples, which ranged from 0.021 to 0.113 (Table 2.1). This would suggest that otter populations within each river are fairly small, and drift has changed allele frequencies significantly since 1996 (roughly 5 otter generations). The highest F_{ST} value (0.113) came from the Upper Clark Fork River. Also, observed heterozygosity increased from 0.500 to 0.655 and the average number of alleles per locus increased from 3.18 to 4.46. Due to pollution from mining activities at the headwaters, it was thought that otters were absent from this river after no otter sign was detected during surveys in 1992 (Bergman and Szumski 1994). Thus, it is not surprising that allele frequencies changed in this population as it recovered from either being absent or at low numbers. Because of concerns with these changes in allele frequencies I only used samples from 2004 onward in my analyses.

Table 2.1. F_{ST} values for comparisons between the oldest and most recent (by collection year) 10 tissue samples within each river in Montana. BR = Bitterroot, BF = Blackfoot, CW = Clearwater, LCF = Lower Clark Fork, UCF = Upper Clark Fork

| River | F_{ST} | p -value |
|-------|----------|------------|
| BR | 0.033 | 0.003 |
| BF | 0.021 | 0.015 |
| CW | 0.047 | 0.001 |
| LCF | 0.046 | 0.002 |
| UCF | 0.113 | 0.001 |

Genetic Variation and River-based Comparisons

I detected one locus out of Hardy-Weinberg proportions (*Lut733*) and no pairs of loci were significant for linkage-disequilibrium (after sequential Bonferroni corrections). Across all samples, observed heterozygosity was 0.637 and ranged from 0.556 to 0.682 for each sample area. In general, MT populations had higher heterozygosity and allelic richness values than ID populations (Table 2.2), although the Clearwater River in MT had the 2nd lowest H_E and allelic richness (0.582 and 3.46, respectively) after the Salmon River. Pairwise F_{ST} and R_{ST} values show that, in general, MT populations are more closely related to each other than to ID populations, and vice-versa (Table 2.3). These values do not indicate a substantial barrier between ID and MT otters. For instance, the F_{ST} and R_{ST} values between the Lochsa area in ID and the Bitterroot River in MT were lower than those between the Lochsa and Salmon areas of ID. There were 3 main clusters in the principle component analysis: 1) Salmon and Lochsa; 2) Bitterroot, Blackfoot, Clearwater, and Upper Clark Fork; 3) Lower Clark Fork (Figure 2.3).

Table 2.2. Genetic diversity of otters sampled from 7 river systems in MT and ID. Presented are number of individuals sampled (n), F_{IS} , expected heterozygosity (H_E), and allelic richness (AR).

| | n | F_{IS} | H_O | H_E | AR |
|-----------------------|-----|----------|-------|-------|-------------|
| Bitterroot (MT) | 19 | 0.104 | 0.612 | 0.684 | 4.14 (0.77) |
| Blackfoot (MT) | 19 | 0.031 | 0.646 | 0.666 | 4.06 (1.09) |
| Clearwater (MT) | 18 | 0.074 | 0.556 | 0.600 | 3.46 (0.96) |
| Lochsa(ID) | 15 | -0.035 | 0.636 | 0.615 | 3.65 (0.83) |
| Lower Clark Fork (MT) | 15 | 0.02 | 0.667 | 0.680 | 4.21 (1.14) |
| Salmon (ID) | 7 | -0.148 | 0.662 | 0.577 | 3.27 (0.62) |
| Upper Clark Fork (MT) | 14 | -0.013 | 0.682 | 0.673 | 4.04 (0.69) |
| All Samples | 107 | 0.025 | 0.637 | 0.620 | 3.83 (0.95) |

Table 2.3. Table showing pairwise F_{ST} values (below diagonal) and R_{ST} values (above diagonal) for 7 river systems in MT and ID. Significant values are indicated in bold and with asterisks. BR = Bitterroot, BF = Blackfoot, CW = Clearwater, LS = Lochsa, LCF = Lower Clark Fork, SA = Salmon, UCF = Upper Clark Fork.

| | BR | BF | CW | LS | LCF | SA | UCF |
|-----|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|---------------|
| BR | | 0.005 | 0.000 | 0.039* | 0.018 | 0.112* | 0.000 |
| BF | 0 | | 0.000 | 0.094** | 0.020 | 0.167** | 0.001 |
| CW | 0.0157* | 0.0221* | | 0.049 | 0.050* | 0.131* | 0.001 |
| LS | 0.0416** | 0.0832** | 0.084** | | 0.133** | 0.084* | 0.080* |
| LCF | 0.016* | 0.0424** | 0.0586** | 0.086** | | 0.202* | 0.044 |
| SA | 0.0736** | 0.1051** | 0.1263** | 0.0439* | 0.1023** | | 0.117* |
| UCF | 0.013 | 0.0139 | 0.0409** | 0.0871** | 0.0593** | 0.0958** | |

* $p < 0.05$; ** $p < 0.01$

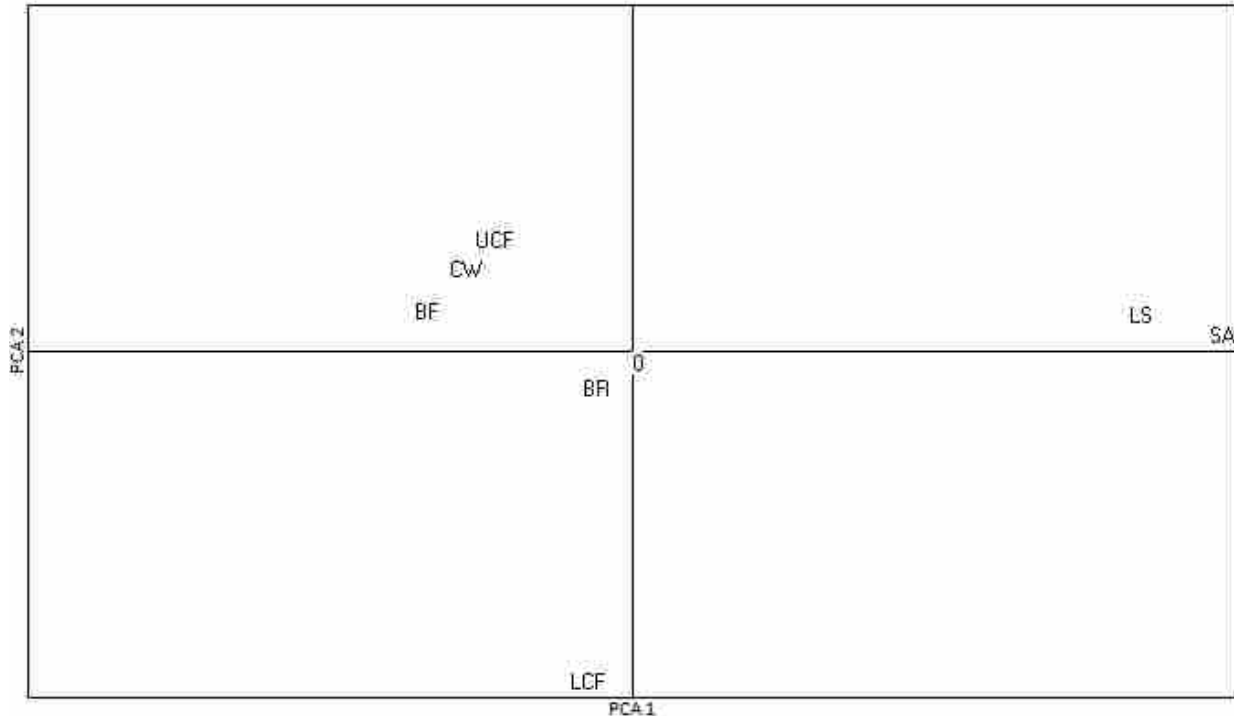


Figure 2.3. Principle Component Analysis visualizing clustering of 7 otter populations. PC1 = 39%, PC2 = 21%. BR = Bitterroot, BF = Blackfoot, CW = Clearwater, LS = Lochsa, LCF = Lower Clark Fork, SA = Salmon, UCF = Upper Clark Fork.

Individual Based Analyses

GENELAND identified two populations in my dataset, while STRUCTURE gave conflicting results. The STRUCUTRE log-likelihood values plateaued at K=4, while the ΔK were highest for K=2 (Figure 2.4). The GENELAND simulations divided the samples almost exclusively between ID and MT samples, with a few exceptions (Figure 2.5A). All 10 GENELAND runs identified 2 populations (K=2) as the highest probability of K. The F_{ST} value between the 2 populations was 0.070 ($p = 0.001$), and the F_{IS} values for the 2 populations were 0.061 and -0.031, respectively. A map showing the probability of population membership for each population is shown in Figure 2.6. The STRUCTURE output was less distinct and lacked a clear pattern of population designations in the MT samples (Figure 2.5B).

GENECLASS 2 detected 4 possible first generation migrants (Figure 2.5A, Table 2.4) with $P < 0.01$. Two of these individuals would be migrants between ID and MT. One individual is indicated as a migrant from the Lower Clark Fork River in MT to the Lochsa area of ID, and one is indicated as a migrant from the Lochsa to the Bitterroot River in MT.

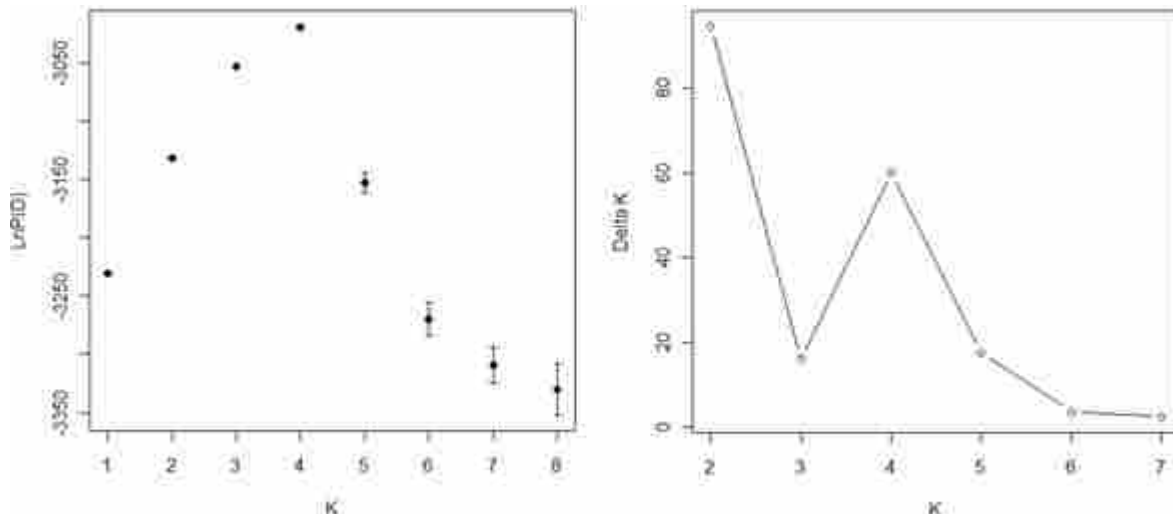


Figure 2.4. Graphs showing results from STRUCTURE simulations. Graph (A) shows mean log-likelihood values for 10 replicated runs at $K=1-8$ with 95% CIs. Graph (B) shows ΔK values for $K=2-7$ (Evanno et al. 2005).

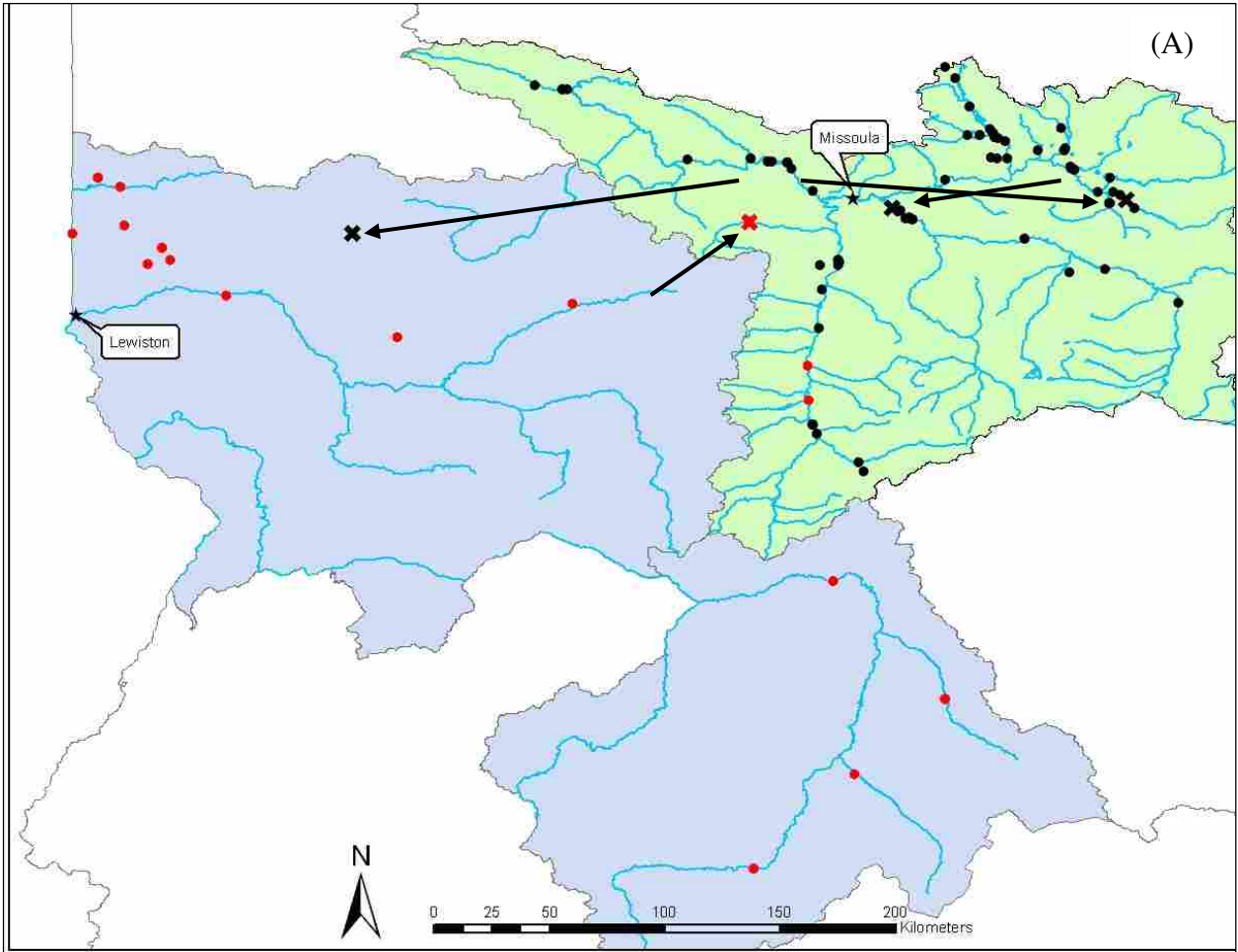


Figure 2.5A

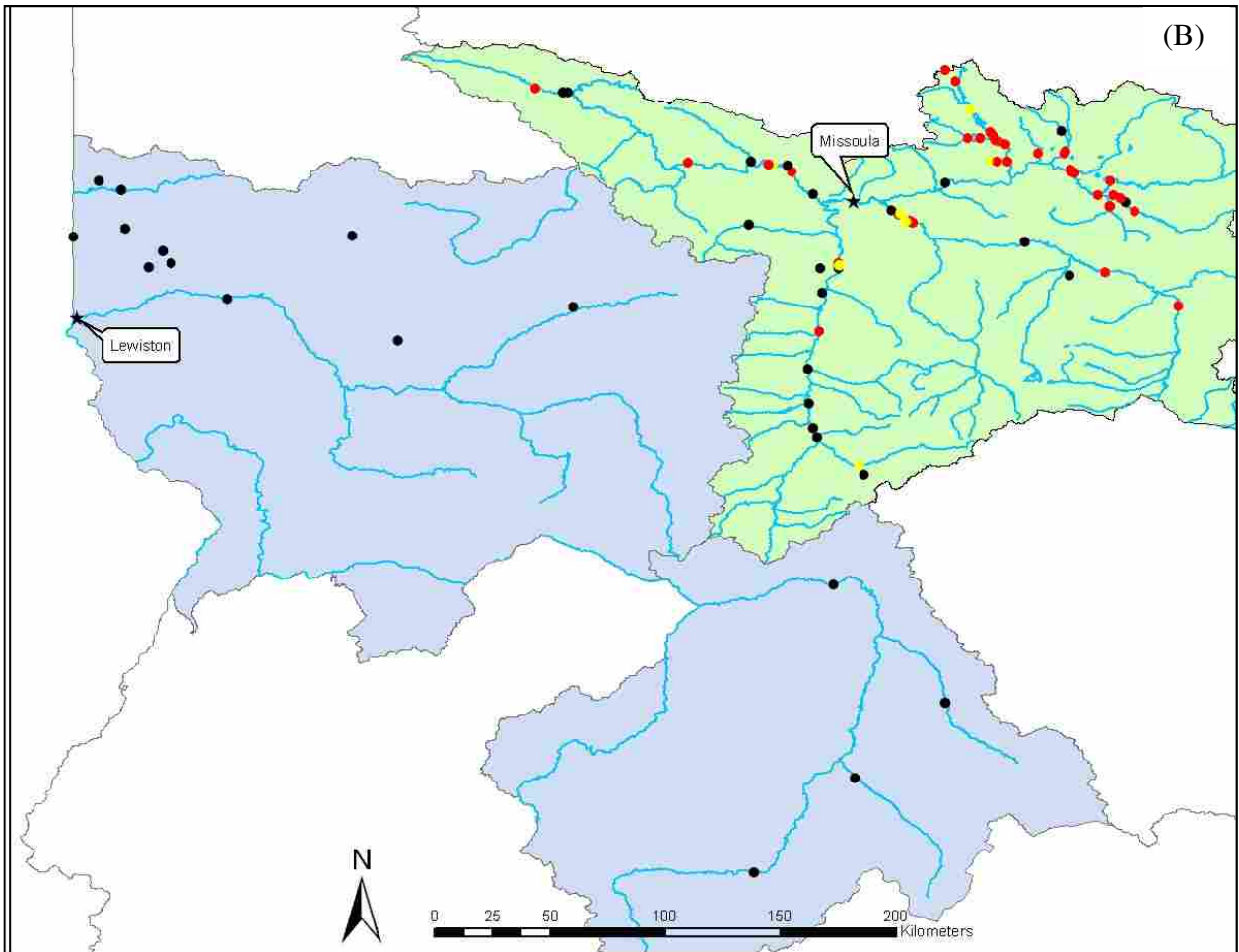


Figure 2.5. Locations of tissue samples collected from trapped otters in Idaho (blue area) and Montana (green area). (A) shows the designation of samples into 2 groups based on GENELAND outputs, while (B) shows the designation based on STRUCTURE outputs. The red and black circles represent the 2 populations, while the yellow circles in (B) represent those individuals that had a probability of population designation between 0.40 and 0.60. The “X” symbols in (A) represent the 4 otters indicated as possible first generation migrants using GENECLASS 2. Arrows point to the possible migrants, and come from the most likely source population.

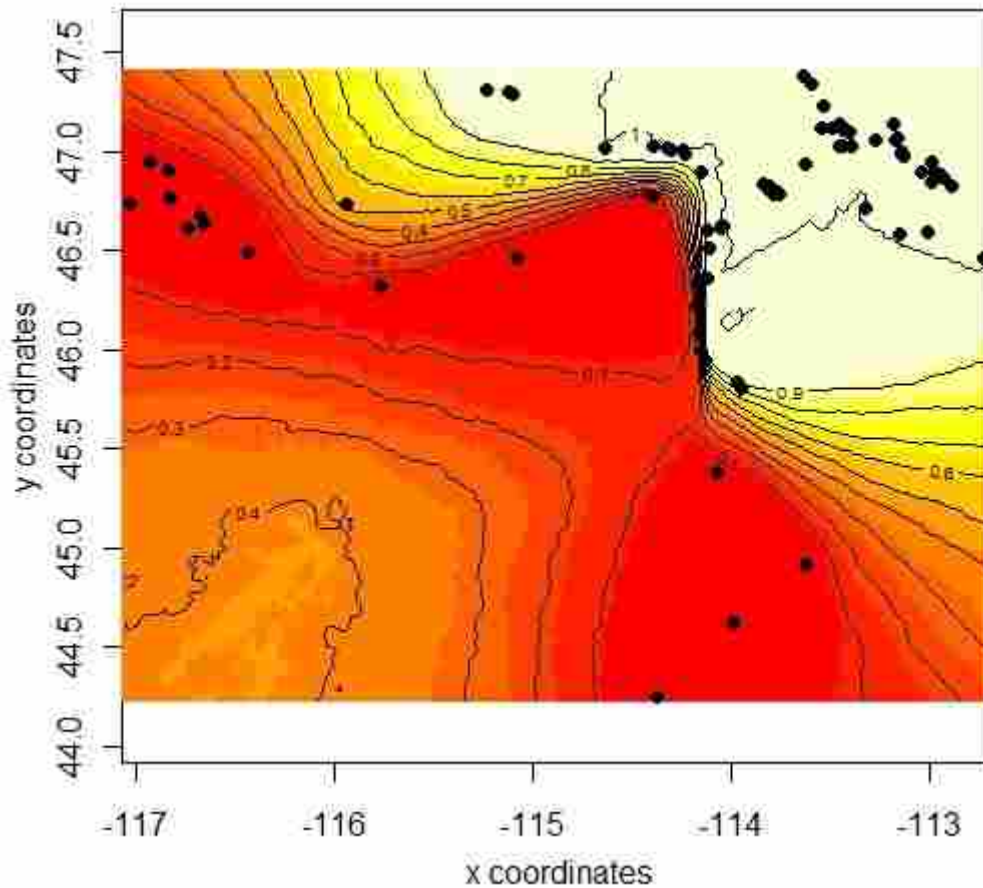


Figure 2.6. GENELAND output showing the posterior probability of population membership for 2 populations. The light areas indicate high probability of belonging to population 1, while dark areas indicate low probabilities of membership in population 1 (and thus high probability of membership in population 2).

Table 2.4. Results from the first generation migrant detection simulations run in GENECLASS 2 (Piry et al. 2004). Four otters were indicated as probably migrants. The p -value indicates the probability that an individual is a migrant, and the Pr(MLOP) value is the probability that the migrant originated in the likely population origin (e.g. there is a 0.281 probability that individual 183999 originated in the Lower Clark Fork River).

| Individual | Sampled Population | Likely Population Origin | p -value | Pr(MLOP) |
|-------------|-----------------------|--------------------------|------------|----------|
| OT_091124_9 | Bitterroot (MT) | Lochsa (ID) | 0.003 | 0.585 |
| IDFG00509 | Lochsa (ID) | Lower Clark Fork (MT) | 0.000 | 0.228 |
| 183999 | Blackfoot (MT) | Lower Clark Fork (MT) | 0.004 | 0.281 |
| 184202 | Upper Clark Fork (MT) | Blackfoot (MT) | 0.003 | 0.581 |

Least-cost Path Analysis

There were significant correlations among genetic distance and cost distance in all of our resistance models (Table 2.5). The greatest correlation was between genetic distance and Euclidean distance (cost of 1), with a decreasing Mantel r value as cost was increased (Table 2.5, Appendix 1). Partial Mantel tests show that there are no significant correlations between genetic distance and any of the cost distances once Euclidean distance is partialled out of the model (Table 2.6), while the correlation between genetic distance and Euclidean distance was only significant when the 2 highest land cost values were partialled out (Table 2.6).

Table 2.5. Results from Mantel tests comparing matrices of genetic distances between all pairs of otter samples and matrices of least cost paths between the samples. The land cost “Streams” had a cost of 10,000 which resulted in the distance between samples following streams only, with no land movements.

| Land Cost | Mantel r | p |
|------------------|------------|-------|
| Euclidean (1) | 0.108 | 0.007 |
| 5 | 0.107 | 0.008 |
| 10 | 0.106 | 0.011 |
| 15 | 0.106 | 0.010 |
| 20 | 0.106 | 0.012 |
| 25 | 0.106 | 0.010 |
| 50 | 0.106 | 0.011 |
| 100 | 0.103 | 0.014 |
| 250 | 0.099 | 0.019 |
| 500 | 0.086 | 0.034 |
| Streams (10,000) | 0.075 | 0.065 |

Table 2.6. Partial Mantel results from comparisons between genetic distance matrix between all pairs of otter samples, a matrix of Euclidean distances between samples, and least-cost paths through cost layers. The land cost “Streams” had a cost of 10,000 which resulted in the distance between samples following streams only, with no land movements.

| Correlation | Partialled Out | Mantel <i>r</i> | <i>P</i> |
|------------------------------|--------------------|-----------------|----------|
| GenDist X Euclidean distance | Streams | 0.082 | 0.001 |
| GenDist X Euclidean distance | Land Cost = 5 | 0.019 | 0.597 |
| GenDist X Euclidean distance | Land Cost = 10 | 0.025 | 0.493 |
| GenDist X Euclidean distance | Land Cost = 15 | 0.027 | 0.467 |
| GenDist X Euclidean distance | Land Cost =20 | 0.029 | 0.422 |
| GenDist X Euclidean distance | Land Cost = 25 | 0.029 | 0.424 |
| GenDist X Euclidean distance | Land Cost = 50 | 0.029 | 0.421 |
| GenDist X Euclidean distance | Land Cost = 100 | 0.035 | 0.321 |
| GenDist X Euclidean distance | Land Cost = 250 | 0.044 | 0.185 |
| GenDist X Euclidean distance | Land Cost = 500 | 0.066 | 0.041 |
| GenDist X Streams | Euclidean Distance | -0.026 | 0.335 |
| GenDist X Land Cost = 5 | Euclidean Distance | 0.013 | 0.739 |
| GenDist X Land Cost = 10 | Euclidean Distance | 0.016 | 0.666 |
| GenDist X Land Cost = 15 | Euclidean Distance | 0.019 | 0.628 |
| GenDist X Land Cost =20 | Euclidean Distance | 0.021 | 0.586 |
| GenDist X Land Cost = 25 | Euclidean Distance | 0.021 | 0.579 |
| GenDist X Land Cost = 50 | Euclidean Distance | 0.022 | 0.577 |
| GenDist X Land Cost = 100 | Euclidean Distance | 0.016 | 0.670 |
| GenDist X Land Cost = 250 | Euclidean Distance | 0.010 | 0.778 |
| GenDist X Land Cost = 500 | Euclidean Distance | -0.010 | 0.761 |

Discussion

Gene Flow Among Populations

Collectively, my data indicate that there is gene flow between otter populations in Montana and Idaho, and populations in MT exhibit low differentiation. There was no evidence that otters in MT and ID are connected by streams only, or that land is a complete barrier to otter movement. I detected 2 possible first generation migrants between ID and MT. These individuals were detected using GENECLASS 2, and were also indicated in the GENELAND output (Figure 2.4A). Least-cost path analyses show there is more correlation between genetic distance between pairs of otter samples and Euclidean distance between the samples (low cost of moving over land) than genetic distance and high levels of resistance surfaces (high cost of moving over land). Otters are most likely crossing between the Lochsa area of ID and the Bitterroot River, MT more than between the Salmon area of ID and the Bitterroot River. It is roughly 5 linear km from Pack Creek, ID (a tributary of the Lochsa River) up and over the Bitterroot Mountains to the headwaters of Lolo Creek (a tributary of the Bitterroot River), and reaches an elevation around 1595 m. An otter would have to travel overland over the Bitterroot Mountains at least 14 linear km, and reach an elevation of 2138 m to travel from a tributary of the Salmon River, ID to a tributary of the Bitterroot River, MT. This is shown in the F_{ST} values, with the Bitterroot having a lower pairwise F_{ST} value of 0.0416 with the Lochsa, compared to 0.736 with the Salmon Region. One otter sampled near Dwarshak Reservoir, ID was indicated as originating in the Lower Clark Fork River, MT. Straight line distance between the sampling location and the Lower Clark Fork is roughly 100 linear km. This is likely far less than the actual distance an otter would probably travel following streams up to the pass. It is possible that this individual originated in a population that was not sampled.

The levels of connectivity seen in the MT and ID otters are similar to those found in other otter populations. Otters in coastal Louisiana had pair-wise F_{ST} values ranging from 0.0370-0.0524 between sampling areas (Latch et al. 2008). All 3 of these populations were connected by water, and no known physical or behavioral barriers exist between them. In Colorado, there was distinct clustering of otters on the Gunnison River, even though the 2 sample areas were only separated by 45 stream km (Depue 2007). This was attributed to low otter densities and possible habitat degradation (pollution) acting as a behavioral barrier.

Data on otter movements is sparse, but they have been shown to have long dispersal capabilities (Melquist and Hornocker 1983, Blundell et al. 2002, Spinola et al. 2008). There is also evidence that otters have moved across mountain ranges (Laughlin 1955, Morejohn 1969, Magoun and Valkenburg 1977, Melquist and Hornocker 1983). Thus, it is not surprising that otters are making overland movements between ID and MT. Yet, it is important to realize the overland movement capabilities of otters. For instance, otter groups were delineated using geographical features, such as mountains, in a study looking at genetic variation in central and eastern U.S (Serfass et al. 1998). The authors assumed that these features were barriers to gene flow, which may have influenced their interpretations of possible otter subspecies designations.

Genetic Variation

Otters sampled in ID and MT had heterozygosity values similar to those found in other otter populations. Heterozygosity values in Missouri populations ranged from 0.547 to 0.875 (Mowry et al. 2011), although the higher values are most likely due to very small sample sizes. In Louisiana, heterozygosity values ranged from 0.539 to 0.625 (Latch et al. 2008). The otter populations in these states are higher than in MT and ID. For instance, around 3300 otters are harvested in Louisiana annually (Latch et al. 2008), while only 100 are harvested in MT.

Because the heterozygosity values in MT and ID are similar to those found in much higher otter densities, it appears that the populations in MT and ID are either large or connected enough to maintain high levels of heterozygosity. However, allelic richness values were lower in MT and ID than those in Louisiana, a result of the much lower population numbers in my study area.

Population Substructuring

Both STRUCTURE and GENELAND indicated that the most likely number of groups of otters was 2 ($K=2$). The designation of individuals into populations differed between the two programs, however. Individual designations in GENELAND conformed to what was expected: a separation of otters between ID and MT. The STRUCTURE results were less clear, with no pattern in the MT samples. This could be due to the fact that STRUCTURE can suffer problems when an isolation-by-distance (IBD) pattern represents genetic data (Frantz et al. 2009). Also, with lower levels of population differentiation ($F_{ST} < 0.05$), STRUCTURE tends to estimate the correct number of populations, but is ambiguous with designation of individual samples to populations (Latch et al. 2006).

Conclusions and Management Implications

It appears that otters are crossing over mountainous areas to travel from one watershed to another. This dispersal maintains gene flow between otters in ID and MT, and may also have effects on demographic rates depending on the rate of migration and the relative contribution of these migrants (Lowe and Allendorf 2010). It is crucial that managers on both sides of the border take into consideration this exchange of individuals from one state to another. A logical next step would be to apply this methodology to all of MT, not just one region. This may help managers in delineating trapping units for otters and setting quotas within each unit.

Based on large changes in allele frequencies over time, it appears that some river systems in the study area have low otter numbers. This is especially apparent in the Upper Clark Fork River. Managers may want to shift trapping focus away from these populations to areas that may have higher numbers, and encourage people trapping for beaver to modify their traps to minimize incidental otter take. For instance, 4 otters were trapped in the Upper Clark Fork during the 2010-2011 trapping season, which may represent a large proportion of the total number. This type of pressure in the Upper Clark Fork may curb otter recovery. However, the increase in heterozygosity and number of alleles over time in the Upper Clark Fork suggests an increase in population size, or higher connectivity to other rivers. This may indicate that otters are seeing a benefit from restoration efforts in the basin.

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CHAPTER 3
OTTER HABITAT ASSESSMENT AND POPULATION ESTIMATION IN A
RECOVERING RIVER SYSTEM

Abstract

Northern river otters (*Lontra canadensis*) were severely impacted in the Upper Clark Fork River (UCFR), MT, due to decades of mining activities. They were thought to be either absent or at very low numbers in the early 1990s. The goal of this project was to document otter activity along the UCFR, evaluate habitat availability to otters, and estimate population size using non-invasive tools. I measured 7 habitat and 5 human-distance variables at random locations along the UCFR and at latrine sites, and used χ^2 , *t*-tests, and logistic regression to determine which variables differed between latrine and random sites. I also collected hair and scat samples for genetic material to obtain a population estimate. Understory cover, bank height, and distance to abandoned mines were the only habitat variables that differed ($\alpha = 0.05$) between latrine and random sites, while beaver activity was marginally significant. Random sites were characterized by low overstory and understory cover, and tended to have ground cover dominated by grass, shrub, and dirt/sand. I was able to obtain 11 genotypes from the hair and scat samples, representing 8 individuals (3 recaptures). Due to low sample size I was unable to estimate otter population size in the UCFR. However, based on latrine densities, it appears that otters are at a low density compared to other populations in Montana and Pennsylvania. Because few habitat variables modeled otter locations well, it is recommended that restoration efforts focus on restoring other aspects of the ecology in the Upper Clark Fork River, such as fisheries. Continuing to monitor otter latrine sites can be an effective way to track ecological responses to restoration.

Introduction

The Upper Clark Fork River Basin (UCFRB) encompasses the largest EPA Superfund site in the U.S. The Superfund program was initiated to clean up the uncontrolled hazardous waste sites throughout the U.S. (EPA 2010a). Decades of mining and smelting near the headwaters of the Clark Fork River resulted in the pollution of roughly 193 km of river with heavy metals, including arsenic, cadmium, copper, lead, and zinc (EPA 2010b). As a result, populations of fish and macroinvertebrates were either absent or at low numbers along this stretch and have only begun to recover in the last 15-20 years (Tetra Tech 2007). Also, species that depend solely on fish and macroinvertebrates for food, such as the Northern river otter (*Lontra canadensis*), declined (Bergman and Szumski 1994). No otters or otter sign were found in the entire UCFRB during semi-aquatic mammal surveys conducted for the Montana Natural Resource Damage Program (NRDP) in 1992 (Bergman and Szumski 1994). Recently, otters have increased in the UCFRB, as evidenced by trapping records from Montana Fish, Wildlife and Parks (MFWP) and public sighting. The reemergence of otters is likely due to the natural cleansing of the river following the cessation of mining activities, in addition to contaminant removal and vegetation rehabilitation that began in 2000.

Otters are highly influenced by habitat quality and food availability, and are useful as indicator species (Beazley and Cardinal 2004). As a result, the newly established otter population in the UCFRB provided an opportunity to monitor the progress and success of restoration efforts in the basin, and determine the feasibility of using otters to guide restoration efforts. Because otters did not inhabit landscapes with high contamination levels and habitat degradation, an increase in otter populations may be an indication of a recovery in the UCFRB. For this reason, the NRDP, the agency overseeing the restoration efforts, and MFWP, identified

otters as a focal species within the system. As a focal species, investigations into habitat availability and population estimates in the UCFRB are needed to determine the extent to which otters, and the watershed, have recovered. In this study I documented the habitat available to otters in the Upper Clark Fork River based on other studies of otter habitat selection and documented habitat variables associated with otter presence, specifically otter latrine sites. I also used non-invasive sampling to collect hair and scat samples to get a baseline population estimation of otters in the UCFRB.

This study was a continuation of a study conducted by MFWP and the University of Montana in 2009 documenting sign of semi-aquatic furbearers along the Upper Clark Fork River (Foresman 2009).

Methods

Study Area

The Upper Clark Fork River (UCFR; Figure 3.1) is designated as the stretch of the Clark Fork River that begins in the headwaters near Warm Springs, MT, and ends with the confluence of the Blackfoot River near Bonner, MT. It flows roughly 193 km in a Northwest direction. Highlands near the headwaters are dominated by grasses and sagebrush, with willow and cottonwood riparian areas. This transitions into a Douglas fir forest dominated upland, with similar willow and cottonwood riparian areas. Elevation ranges from 1460 m at the headwaters to 996 m near Bonner.

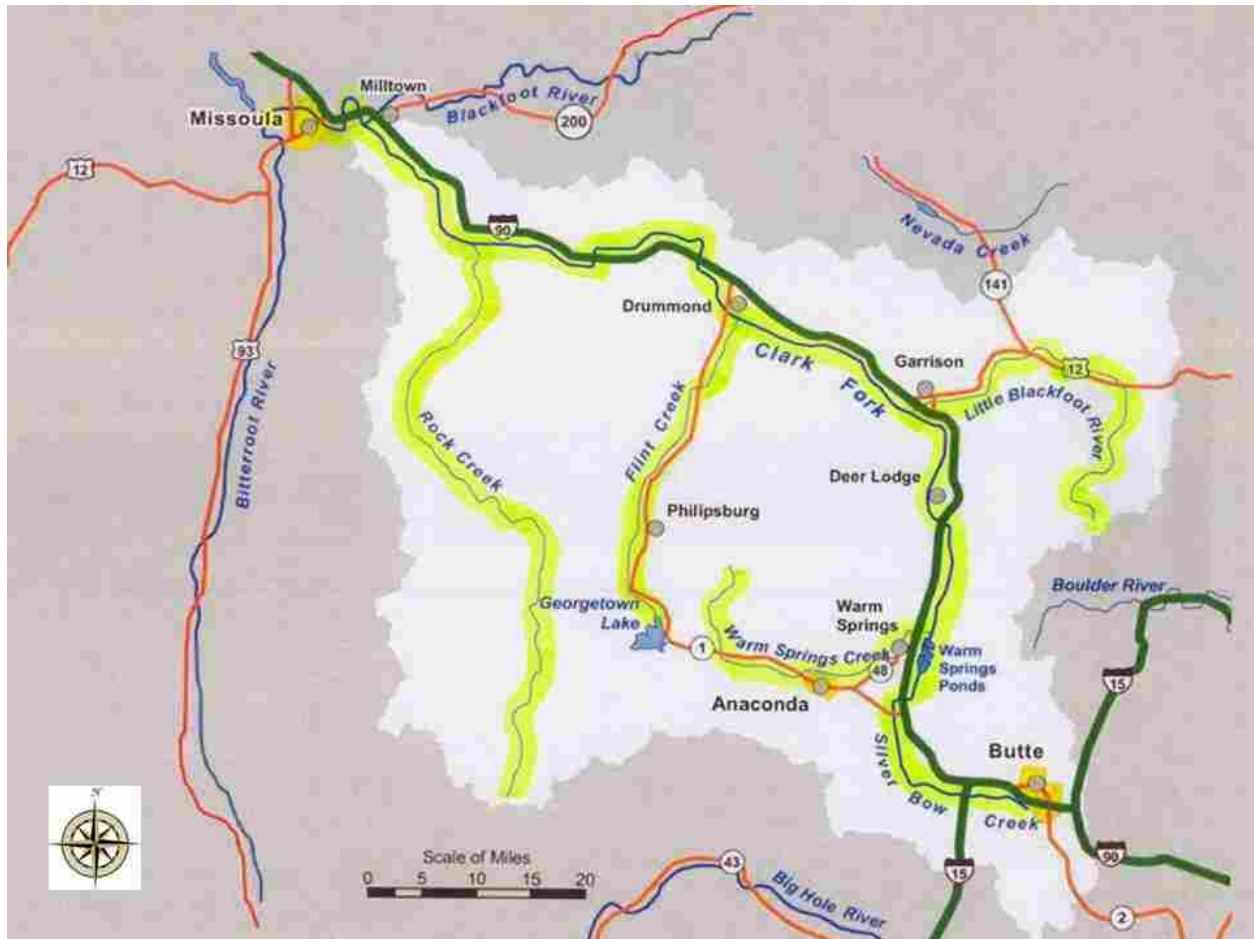


Figure 3.1. Map of the Upper Clark Fork River, which is designated as the stretch of the Clark Fork River from its headwaters near Warm Springs, MT to its confluence with the Blackfoot River.

Documenting Otter Activity Sites

In the summer of 2010 I canoed the entirety of the UCFR from Perkins Gulch Bridge near Warm Springs, MT to the Turah Fishing Access Site near Turah, MT. I stopped every 0.5 km (see description of 0.5 km locations below in the “Habitat Assessment” section). At each of these sites, I walked 50 m upstream and downstream looking for otter tracks and scat. The most noticeable indications of otter presence are latrine sites, which are areas where otters continually deposit scat, urine, and scent for communication. Latrine sites were defined as those sites with ≥ 2 scats (Swimley et al. 1998).

Habitat Assessment

I assessed habitat available to otters along the UCFR by measuring 7 habitat variables (Table 3.1). While canoeing, I continually measured beaver activity (a tally of beaver lodges and dams) for each 3 km stretch of river. For 5 variables (bank height, bank slope, % understory cover, % overstory cover, % ground cover), I stopped every 0.5 km of river to take measurements (not including starting and stopping points for each 3 km stretch), resulting in 5 measurements every 3 km stretch (Figure 3.2; although these locations are not technically random, I refer to them as random throughout the rest of this chapter). These 0.5 km locations were determined in advance using Google Earth software, and were recorded in the middle of the stream. I took measurements on the side of the river offering the seemingly best otter habitat. This usually included better vegetation, more complex banks, higher banks, and deeper water. The plot center was determined using a GPS unit. I walked the bank holding a GPS unit and determined the closest distance I could come to the GPS point. After that distance was determined, I made one more pass by the point, and the plot was placed as soon as that closest distance was reached. For instance, if the closest distance I could get to the GPS point was 13 m, I placed the point as soon as the distance on the GPS unit went from 14 to 13 m. The plot center was placed 1 m from the high water mark. For vegetation variables, percent cover was estimated visually within a 2.5 m arc and placed into 5 categories: 0-5%, 6-25%, 26-50%, 51-75%, and 76-100%. Percent ground cover was estimated for grass (defined as herbaceous plants), shrubs (woody plants), coarse woody debris (CWD), trees, rock, and sand/dirt. I used Google Earth software to determine sinuosity by measuring the straight line distance of each 3 km section and dividing that by 3 km, the actual river distance of each section. In addition to the random locations, I measured these habitat variables at all known otter latrine sites.

Table 3.1. Variables I used to assess available habitat for otters in the Upper Clark Fork River. Each stretch was 3 km of river. Understory, overstory, and ground cover were all measured visually in a 2.5 m arc as either 0-5%, 6-25%, 26-50%, 51-75%, or 76-100%.

| Variable | Measurement | Source |
|---------------------------------|--|--|
| Continuous Measurements | | |
| Beaver Activity | # of lodges and dams / stretch | Melquist and Hornocker 1983 ,Waller 1992 |
| Sinuosity | 3 km / straight line distance of stretch | |
| 5 measurements per 3 km stretch | | |
| Bank Height | Height in meters | Waller 1992 |
| Bank Slope | Degrees | Waller 1992 |
| Understory Cover | % cover of vegetation > 1 m and < 2 m | Dronkert-Egnew 1991 |
| Canopy Cover | % cover of vegetation \geq 1 m | |
| Ground Cover | % ground cover of 6 variables | |
| Human Variables | | |
| Bridge Distance | | |
| Mine Distance | Distance to abandoned mines | |
| Cropland Distance | | |
| Rangeland Distance | | |

To look at how human activity affects otter presence at a larger scale, I measured the distance of 5 human-related variables (Table 3.1) to both latrine and random locations using ArcMap in ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, California). We calculated pairwise Pearson's correlation statistics for all variables and dismissed variables that had $|r\text{-values}| > 0.4$ (Zar 1999).

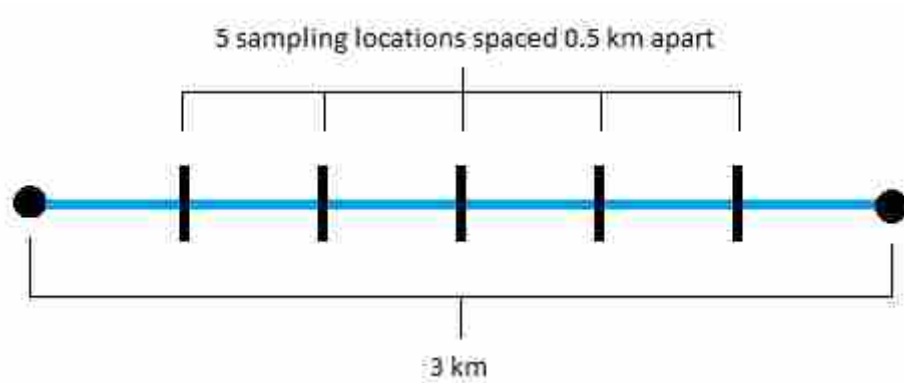


Figure 3.2. Habitat sampling scheme. I measured habitat variables at 5 locations per 3 km stretch. Sinuosity and beaver activity were measured per 3 km stretch.

I used chi-square tests for independence on categorical variables and Welch's *t*-test on continuous variables to test whether there were differences in the habitat variables between otter latrine sites and random locations along the river. Because some of the categorical variables had zeros in certain percentage categories in either the random or latrine sites, I pooled data together into new bins for the chi-square tests. The resulting bins for Understory Cover were 0-5%, 6-25%, 26-50%, and 51-100%; for Overstory Cover 0-5%, 6-25%, and 26-100%; for Shrub Ground Cover 0-5%, 6-25%, and 26-100%; for Grass Ground Cover 0-25%, 26-50%, 51-75%, and 76-100%. I also used logistic regression with all variables in a model (1 model for latrine site selection and 1 for human impacts) to determine whether otters are selecting or avoiding the variables.

Non-invasive Genetic Sampling

I collected otter scat samples (“spraints”) at known latrine sites in 2009 and 2010. I distinguished otter scat from other species by the presence of fish bones and scales, crayfish (*Pacifastacus leniusculus*) exoskeletons, and an overall fish smell (Halfpenny 2001). It can be separated from mink scat, which may contain the same prey remains, by circumference and shape (Halfpenny 2001). I stored individual scat samples in paper bags while in the field and transferred them to 50 mL polypropylene tubes filled with silica desiccant once in the lab. At latrine sites I opportunistically set hair snares. The snares consist of a conventional body snare modified to be non-invasive (Depue and Ben-David 2007), by fraying the wire to collect hair, and replacing the locking mechanism with a paper clip (Figure 3.3). As an otter walks through the snare, it tightens around the otter. However, with little effort from the otter, the paper clip bends and the snare falls apart, leaving behind a hair sample from only one individual. I

collected hair with sterilized tweezers (washed in 95% alcohol) and stored the samples in coin envelopes. These envelopes were placed in silica desiccant once in the lab. Hair identification followed that of Moore et al. (1974). I also searched for latrine sites on 3 tributaries of the UCFR: Flint Creek, Little Blackfoot River, and Rock Creek. However, I did not find sufficient areas to warrant placing hair

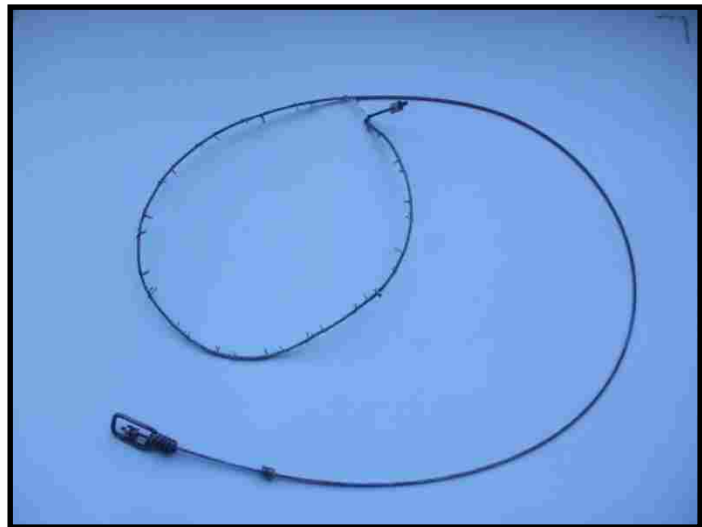


Figure 3.3. Body snare modified to non-invasively collect otter hair (Depue and Ben-David 2007) Wires are frayed, and the snare is held together with a paper clip.

snares.

All samples were sent to the Wildlife Genetics Lab at the Rocky Mountain Research Station (USDA, Missoula, MT). Genomic DNA from hair samples was extracted following the protocols in Mills et al. (2000). Genomic DNA was extracted from spraints by swabbing the outside with a sterile, polyester tipped applicator (Puritan Medical Products, Guilford, ME) and placing the swab in ATL buffer and following protocols for isolating DNA from tissues using the *QIAamp DNA Micro Kit* (Qiagen Inc.). Samples were analyzed using 9 microsatellite loci published for mustelids: *Lut435*, *Lut457*, *Lut604*, *Lut701*, *Lut733*, *Lut782*, *Gg14*, *Tt1*, and *Mer022* (Dallas and Piertney 1998, Davis and Strobeck 1998, Fleming et al. 1999). The reaction volume (10 μ l) contained 1.0-3.0 μ L DNA, 1x reaction buffer (*Applied Biosystems*), 2.0 mM MgCl₂, 200 μ M of each dNTP, 1 μ M reverse primer, 1 μ M dye-labeled forward primer, 1.5 mg/ml BSA, and 1U *Taq* polymerase (*Applied Biosystems*). The PCR profile was 94°C/5 min, [94°C/1 min, 55°C/1 min, 72°C/30s] x 36 cycles for tissue samples (45 cycles were used for non-invasive samples). PCR products were run in a 6.5% acrylamide gel for 2 hours on a *LI-COR DNA analyzer* (*LI-COR Biotechnology*). I used GENALEX 6.4 (Peakall and Smouse 2006) to calculate probability of identity (P_{ID}) and of siblings (P_{SIB}) to describe the ability of the loci to determine individuals.

Results

Habitat

I documented 35 latrine sites and 10 sets of tracks in 2010 along the Upper Clark Fork River. These locations were in similar areas along the river as those found in 2009 (Figures 3.4, 3.5). Twenty-one of the latrine sites and all of the track locations were found by floating the river and stopping every 0.5 km, while 14 latrine sites were found during other searches along

the river. I received numerous responses from fishing guides and the public, and all sightings were in stretches where I found otter sign.

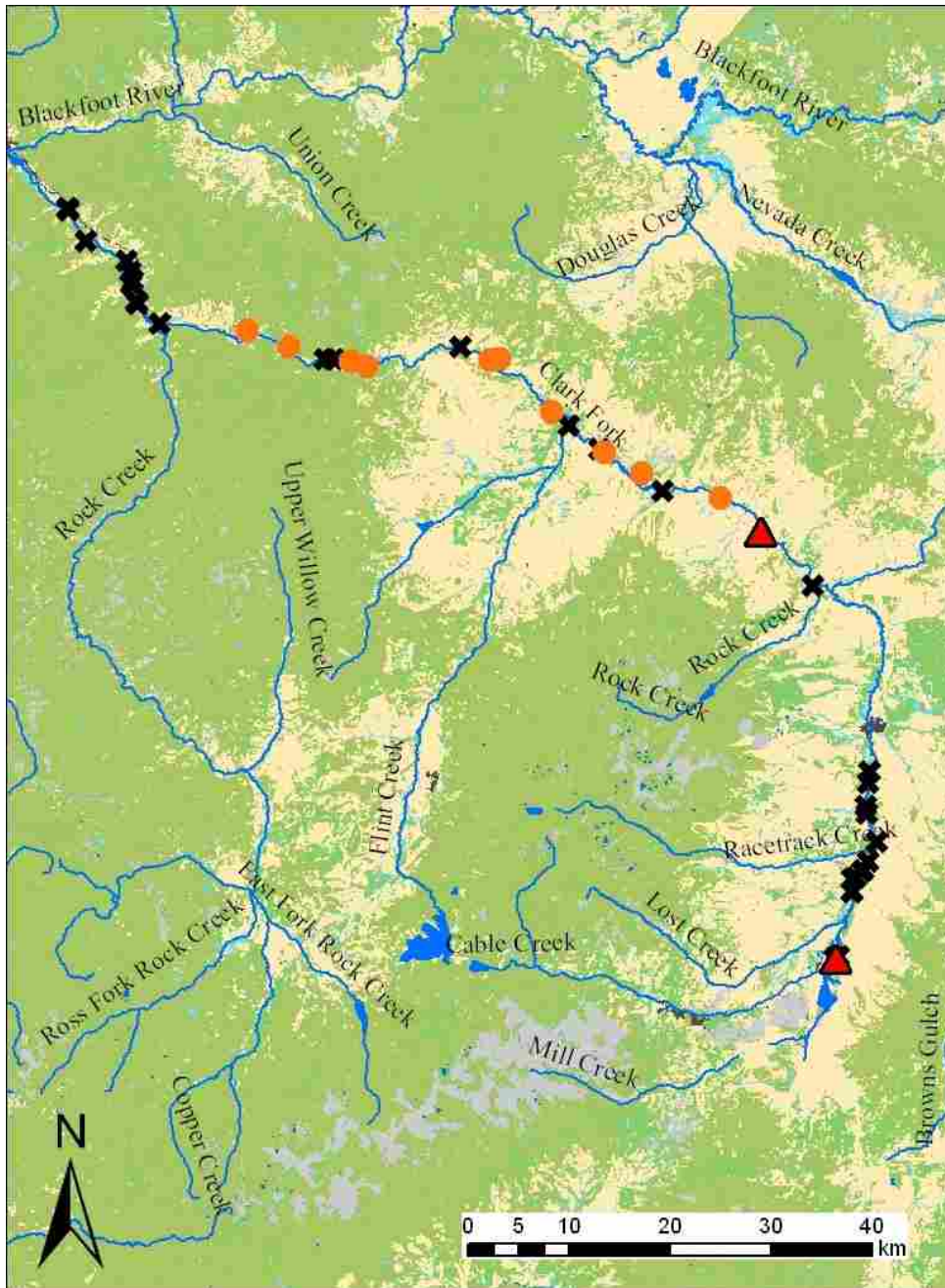


Figure 3.4. Map showing the Upper Clark Fork River with otter sign detected in 2010. Black “X” symbols represent latrine sites (> 1 otter scat), orange circles represent otter tracks, and red triangles represent single otter scats.

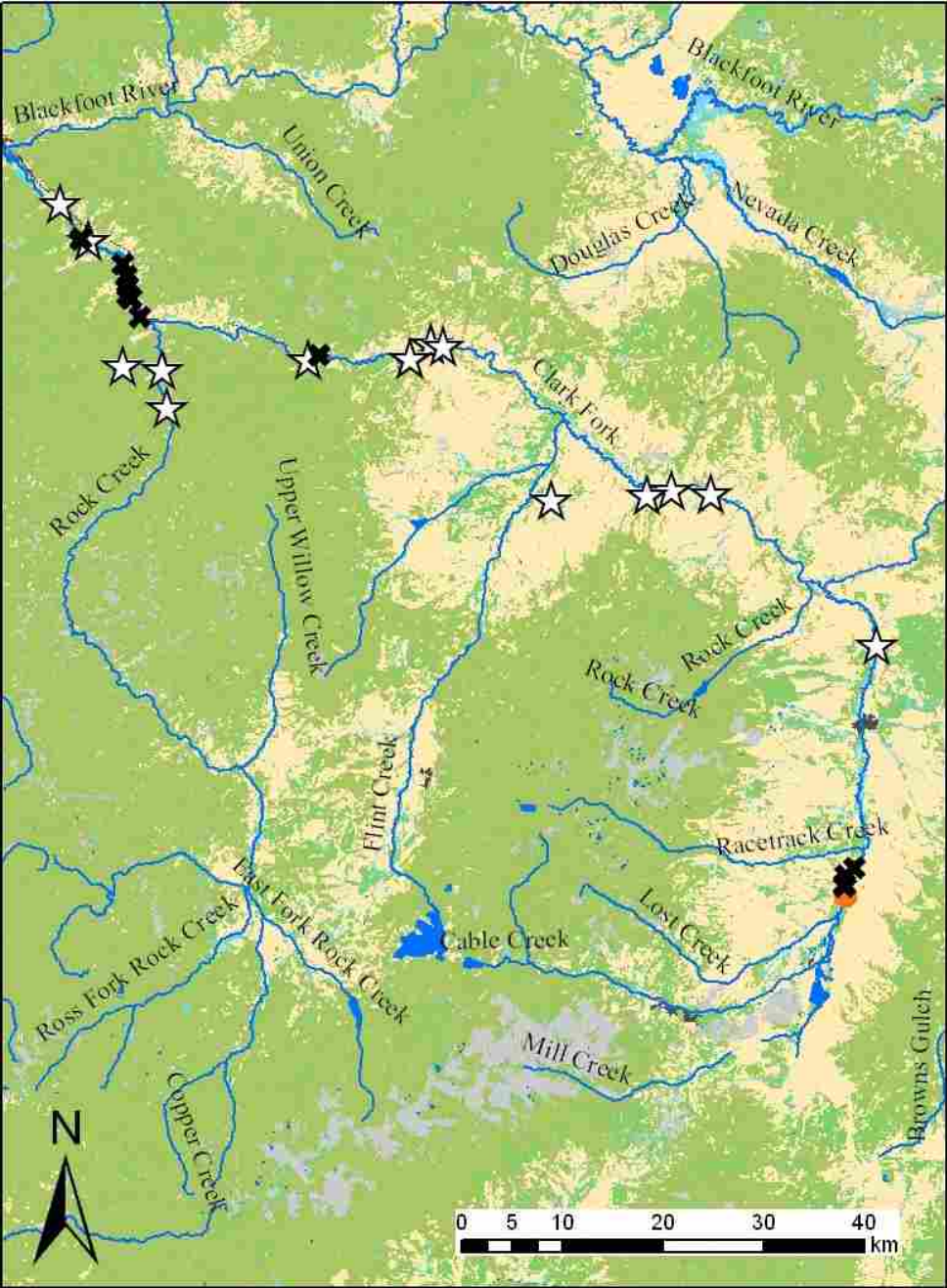


Figure 3.5. Map showing the Upper Clark Fork River with otter sign detected in 2009 during a project conducted by Montana Fish, Wildlife and Parks and the University of Montana documenting sign of semi-aquatic furbearers (Foresman 2009). Black “X” symbols represent latrine sites (> 1 otter scat), orange circles represent otter tracks, and white stars represent otters trapped between 2005-2008.

I measured habitat variables at 310 locations and documented beaver sign and sinuosity for 62 stretches. I measured habitat variables at 29 of the 35 latrine sites and assigned the sinuosity and beaver activity variables depending on which stretch the latrine was found in. Five variables (Bank Slope, Tree Ground Cover, Rock Ground Cover, Sand/dirt Ground Cover, Residential Distance) were dropped from analyses due to correlations with other variables (all had $|r| > 0.4$).

Understory Cover and Bank Height were the only habitat variables that statistically differed ($\alpha=0.05$) between latrine sites and random sites, while the difference in beaver activity was marginally significant (Table 3.2, Figure 3.6, Figure 3.7). The logistic regression model shows that otter latrine sites tended to be on higher banks and were more often located in areas with Understory Cover 6-25%, than random locations (Table 3.3). Random sites along the Upper Clark Fork River (UCFR) tended to have low overstory and understory cover, and tended to have ground cover dominated by grass, shrub, and dirt/sand (Figure 3.8). The number of beaver activity areas (lodges and dams) ranged from 0-8 per 3 km stretch, with a mean of 1.55 (Figure 3.10), while the mean sinuosity was 1.51 (the river distance is on average 1.51 times longer than the straight-line distance).

Table 3.2. List of habitat variables, their test statistics, and significance values comparing between latrine and random sites.

| Variable | Test | Test Statistic | <i>p</i> -value |
|-------------------------|------------------------------|-----------------------------------|-----------------|
| Beaver Activity | Welch's <i>t</i> -test | <i>t</i> = 1.9 | 0.067 |
| Bank Height | Welch's <i>t</i>-test | <i>t</i>= 2.23 | 0.033 |
| Sinuosity | Welch's <i>t</i> -test | <i>t</i> = 1.28 | 0.21 |
| Understory Cover | Chi-square | $\chi^2 = 8.00$ | 0.046 |
| Canopy Cover | Chi-square | $\chi^2 = 1.149$ | 0.56 |
| Shrub | Chi-square | $\chi^2 = 0.094$ | 0.95 |
| Grass | Chi-square | $\chi^2 = 1.7406$ | 0.63 |
| Coarse Woody Debris | Chi-square | $\chi^2 = 1.5137$ | 0.45 |

*Bolted variables indicate statistically significant difference

Table 3.3. Results of the habitat logistic regression model.

| Variable | β -coefficients | SE | <i>z</i> -value | <i>p</i> -value |
|-------------------------------------|-----------------------|----------------|-----------------|-----------------|
| (Intercept) | -4.55372 | 1.14127 | -3.99 | 6.61E-05 |
| Sinuosity | 0.23548 | 0.54743 | 0.43 | 0.66708 |
| Beaver | 0.14523 | 0.09216 | 1.576 | 0.11506 |
| Bank.Hgt | 0.79299 | 0.39423 | 2.012 | 0.04427 |
| as.factor(Understory.Cover)2 | 1.3833 | 0.50707 | 2.728 | 0.00637 |
| as.factor(Understory.Cover)3 | 0.8063 | 0.86456 | 0.933 | 0.35102 |
| as.factor(Understory.Cover)4 | 0.39816 | 1.13443 | 0.351 | 0.7256 |
| as.factor(Canopy.Cover)2 | -0.15706 | 0.54314 | -0.289 | 0.77246 |
| as.factor(Canopy.Cover)3 | 0.22958 | 0.77017 | 0.298 | 0.76564 |
| as.factor(CWD)2 | 0.09006 | 0.59672 | 0.151 | 0.88004 |
| as.factor(CWD)3 | -13.97676 | 958.5808 | -0.015 | 0.98837 |
| as.factor(Shrub)2 | -0.72667 | 0.49465 | -1.469 | 0.14182 |
| as.factor(Shrub)3 | -0.20217 | 0.75818 | -0.267 | 0.78973 |
| as.factor(Grass)2 | 0.44624 | 0.94817 | 0.471 | 0.6379 |
| as.factor(Grass)3 | 0.77745 | 0.8433 | 0.922 | 0.35658 |
| as.factor(Grass)4 | 0.60182 | 0.86365 | 0.697 | 0.48591 |

*Bolted variables indicate statistically significant difference

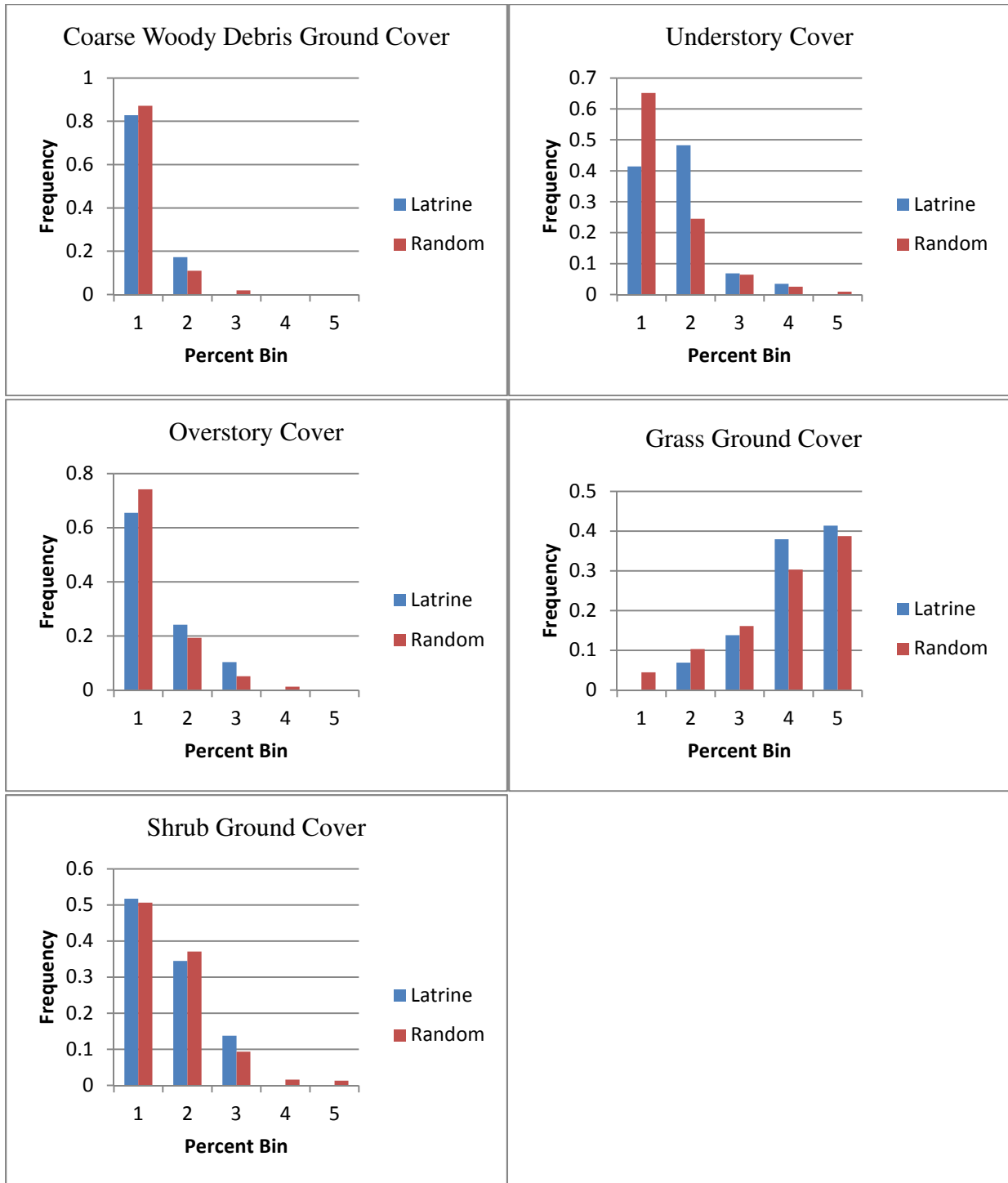


Figure 3.6. Frequency of both latrine and random sites for 5 habitat variables. Percent bins are as follows: 1= 1-5%, 2= 6-25%, 3= 26-50%, 4= 51-75%, 5= 76-100%.

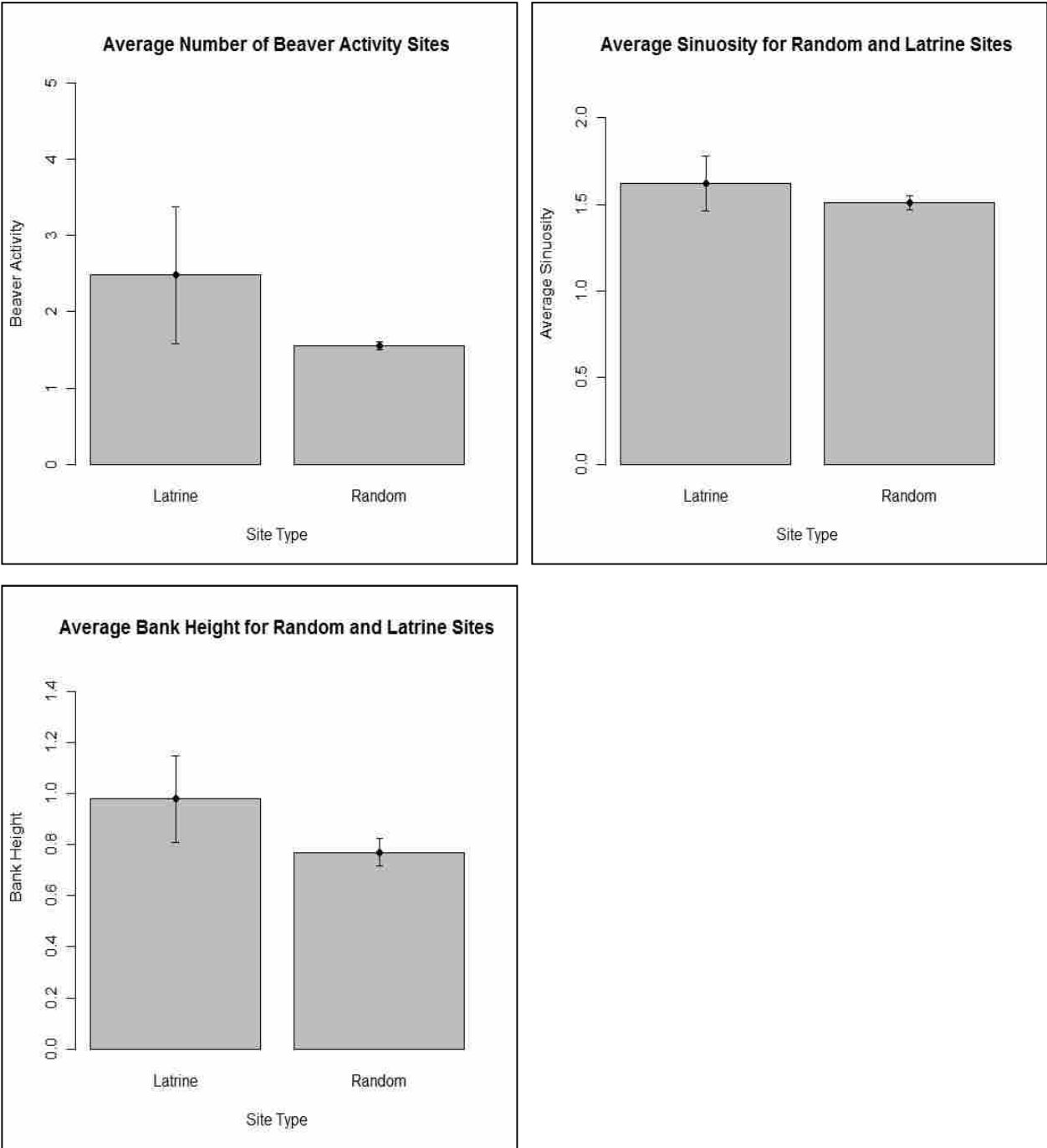


Figure 3.7: Average number of beaver activity sites, sinuosity, and bank height at latrine and random sites. Graphs show mean and 95% CIs.

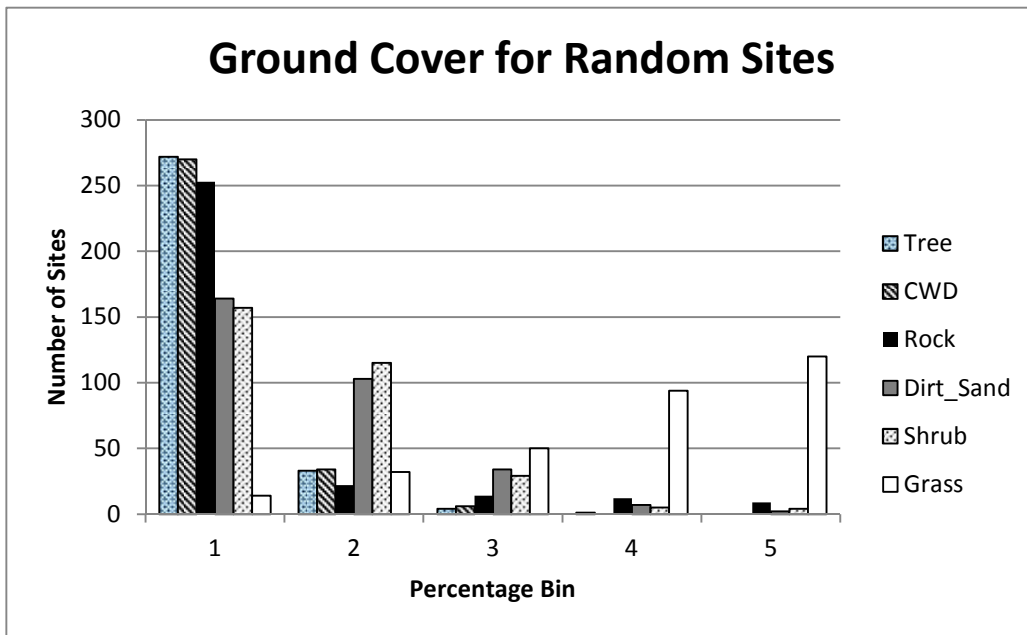
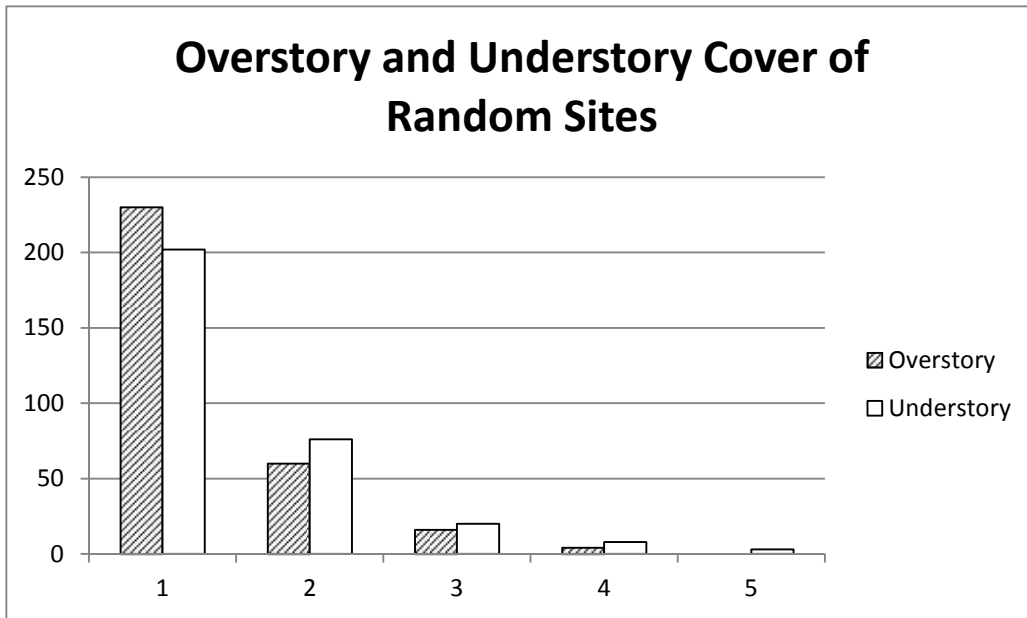


Figure 3.8: Overstory, understory, and ground cover for random sites along the Upper Clark Fork River. A total of 310 locations were measured.

Both Bridge Distance and Mine Distance were significantly different between latrine sites and random locations for the human-landscape variables using Welch's *t*-tests (Table 3.4, Figure 3.9). The logistic regression model showed that otter latrine sites tended to be farther away from abandoned mines than random locations, and closer to bridges (Table 3.5).

Table 3.4. Results of Welch's *t*-tests for the human-landscape variables.

| Variable | Test | Test Statistic | <i>p</i> -value |
|------------------------|------------------------------|-------------------------|-----------------|
| Bridge Distance | Welch's <i>t</i>-test | <i>t</i> = -2.04 | 0.048 |
| Mine Distance | Welch's <i>t</i>-test | <i>t</i> = 2.15 | 0.039 |
| Cropland Distance | Welch's <i>t</i> -test | <i>t</i> = -0.98 | 0.33 |
| Rangeland Distance | Welch's <i>t</i> -test | <i>t</i> = 0.57 | 0.57 |

*Bolted variables indicate statistically significant difference

Table 3.5. Results of the logistic regression model of human-landscape variables

| Variable | β -coefficients | SE | <i>z</i> -value | <i>p</i> -value |
|----------------------|-----------------------|-----------------|-----------------|-----------------|
| (Intercept) | -2.752000 | 5.94E-01 | -4.631 | 3.65E-06 |
| Bridge Distance | -0.000231 | 1.61E-04 | -1.434 | 0.1517 |
| Mine Distance | 0.000298 | 1.46E-04 | 2.045 | 0.0408 |
| Cropland Distance | -0.000098 | 1.83E-04 | -0.536 | 0.5919 |
| Rangeland Distance | 0.000066 | 1.98E-04 | 0.334 | 0.7385 |

*Bolted variables indicate statistically significant difference

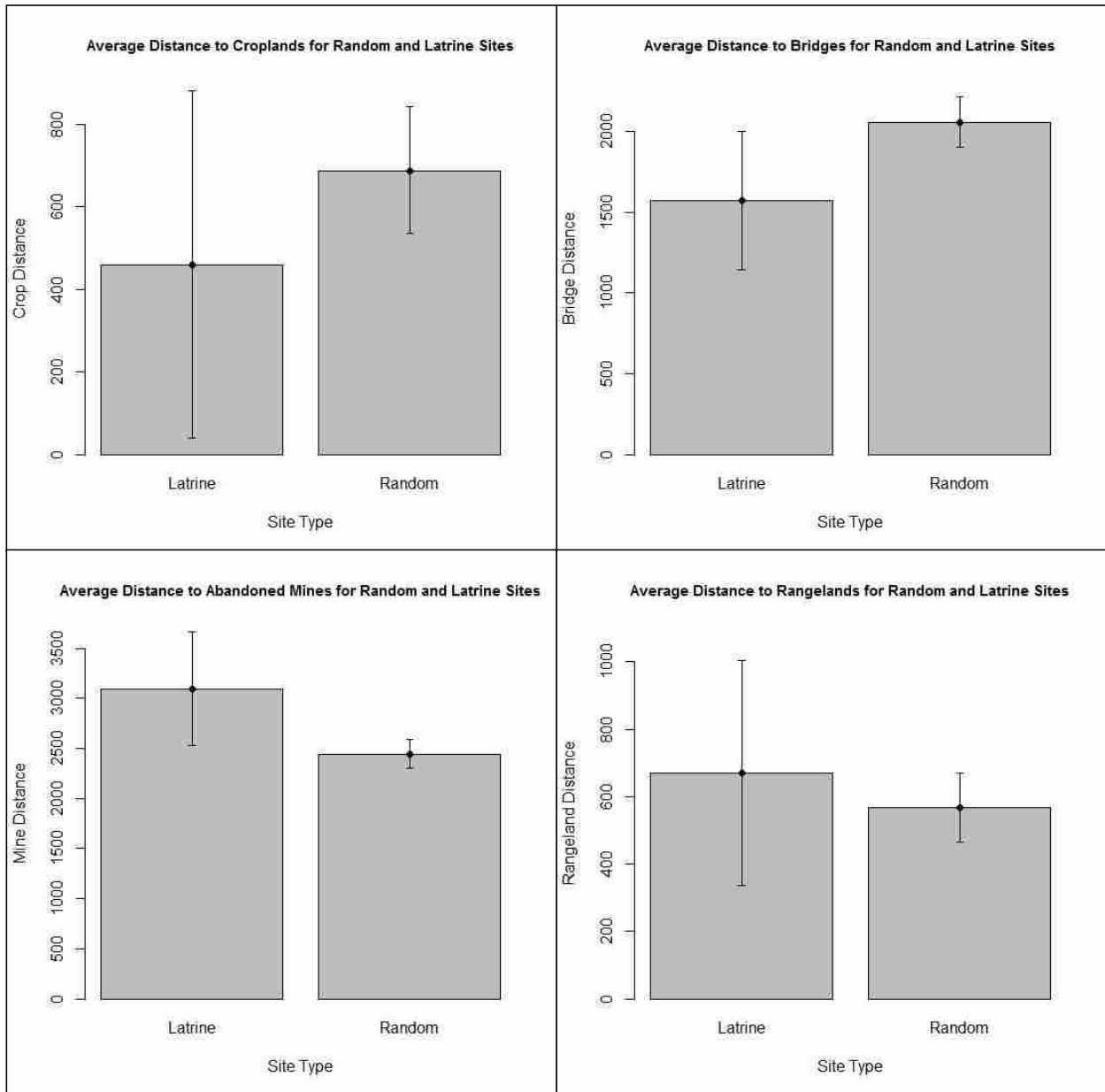


Figure 3.9. Average distance of latrine and random sites to 4 human variables. Graphs show means and 95% CIs.

Genetic Population Estimation

I collected 37 scat samples in 2009 and 49 in 2010, and collected 17 hair samples in 2010. Five of the 86 scat samples provided sufficient DNA for amplification (5.8% success), while 6 of the 17 hair samples successfully amplified (35%). I identified 8 individuals out of the 11 successful genotyped samples, with 3 individuals being recaptured. I was unable to use capture-mark-recapture techniques to estimate the otter population due to low sample size. The loci and number of alleles per loci used for the samples appear to be sufficient to differentiate individuals ($P_{ID} = 2.0 \times 10^{-4}$, $P_{SIB} = 0.015$).

Discussion

Habitat

When comparing vegetation characteristics in the Upper Clark Fork River with those found to be important for otters in other studies, it is difficult to determine whether the UCFR provides quality habitat for otters. Understory cover was found to be higher in sites occupied by otters compared to unoccupied sites on the Flathead River, MT (Dronkert-Egnew 1991, Waller 1992), and otters avoided areas with <25% understory cover (Dronkert-Egnew 1991). Around 90% of the random sites along the UCFR had understory cover <25%. This may be an indication that bank vegetation, specifically vegetation between 1-2 m, may be limiting otters in the UCFR. Occupied sites on the Flathead River also had more bank holes and overhanging vegetation than unoccupied sites (Waller 1992). However, I was unable to measure these variables along the UCFR.

Otters seem to prefer areas with beaver lodges or other waterway obstructions (Melquist and Hornocker 1983, Dronkert-Egnew 1991, Waller 1992, Reid et al. 1994). Based on the presence of beaver activity throughout the UCFR (Figure 3.10), most of the UCFR could be

considered good habitat for otters, although the exact reasoning why otters tend to be associated with beavers is unknown. Otters may seek out slow moving waters and deeper pools created by beaver activity for fishing, and beaver lodges for den sites.

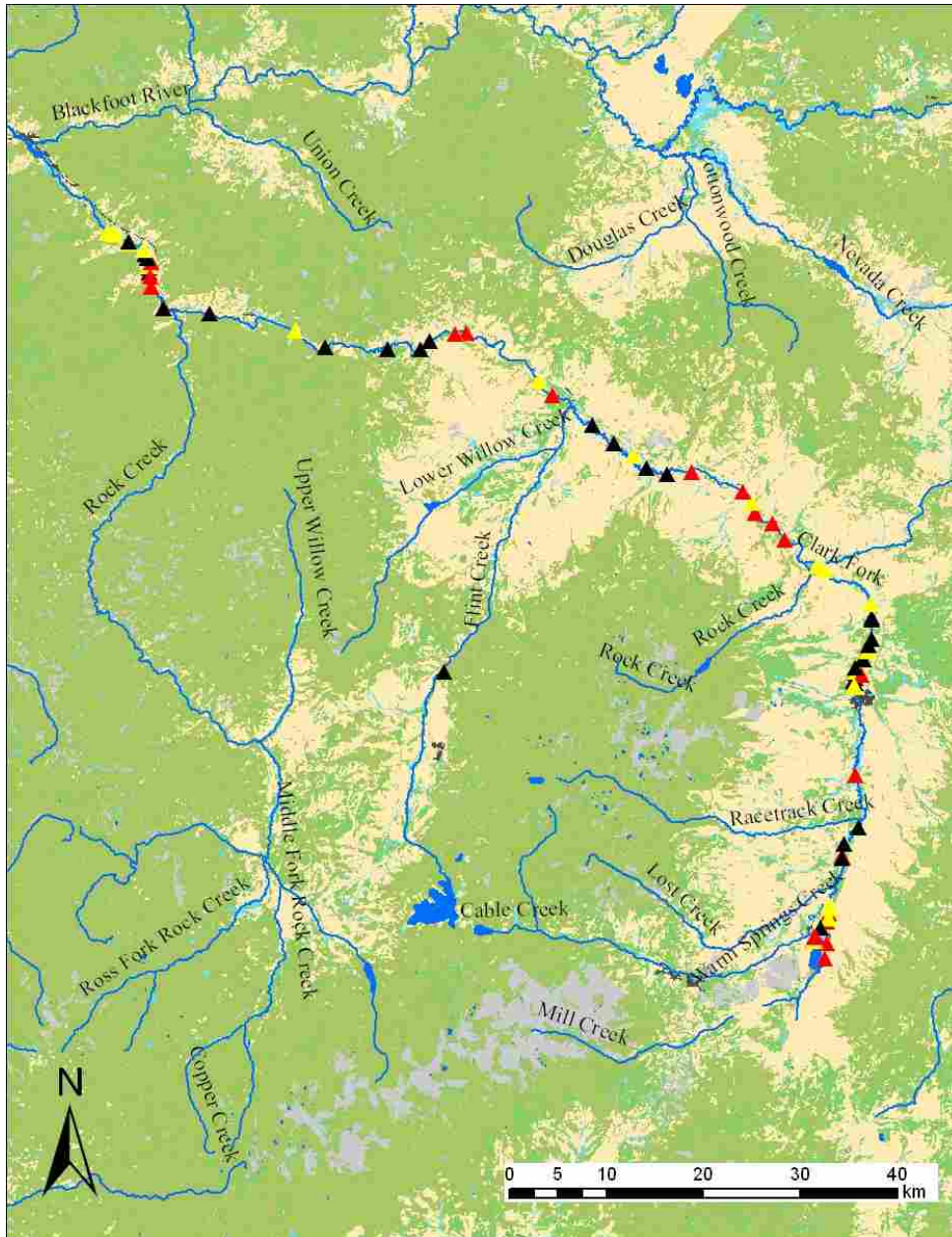


Figure 3.10. Map showing beaver lodges detected during summer 2010. Red triangles = active lodges, yellow triangles = inactive, and black triangles = remnant.

It is challenging to characterize the habitat available to otters in the Upper Clark Fork River. This section of the river is extremely varied, transitioning from a windy, narrow river at its headwaters to a wide, straight and braided river towards the confluence with the Blackfoot River near Bonner, MT. Otter sign and otter sightings clustered together in certain stretches of the river, but these stretches were spread evenly throughout the UCFR. Therefore, the river characteristics (width, sinuosity, etc.) themselves probably have little to do with otter presence. Also, otters seem to be habitat type generalists, and throughout their range different habitat variables seem to be correlated with otter presence (see Introduction chapter). One possible variable that could explain otter presence, which has never been incorporated into an otter habitat selection study, is prey availability/abundance. Otters are top predators in aquatic systems (Melquist and Hornocker 1983, Kruuk 2006), and are rarely preyed upon by larger carnivores (Melquist and Hornocker 1983, Melquist and Dronkert 1987). Therefore, especially in areas where otter numbers are low and competition is minimal, prey should be a limiting factor for river otters (Schoener 1989). Anecdotally, otter occurrence along the UCFR seems to coincide with higher fish abundance (Jason Lindstrom, MFWP, verbal comm.).

Higher banks, more beaver activity, and understory cover 6-25% tended to occur more often at otter latrine sites than at random locations along the UCFR. Beaver activity appears often as a predictor of otter latrine sites in other studies. Beaver activity was consistently in top models for predicting otter latrine site selection in arid Colorado (Depue and Ben-David 2010). Latrine sites were also found significantly more often near beaver activity than random locations in Pennsylvania (Swimly et al. 1998). This relationship between otter latrine sites and beaver activity most likely is due to the fact that otters, in general, tend to be found near beaver activity. Otters may be selecting higher banks for latrine sites to avoid getting washed out, or because

otters are using the deeper pools usually associated with higher banks. Other variables found to predict otter latrine sites are prominent rocks, deep water, steep banks, and backwater (Swimly et al. 1998, Depue and Ben-David 2010).

Based on latrine sites, otters tend to be farther from abandoned mines in the UCFR than random sites. This could be the result of historic or current pollution from these mines. Without a study of water chemistry at random and latrine sites, no conclusions can be made. However, otters can be found in pockets throughout the UCFR. One of the areas with the most otters (near Warm Springs, MT) also happens to be the one of the most polluted and closest to the largest historic mining and smelting activities. Therefore, it is doubtful that otters are avoiding areas near abandoned mines. It does not appear that otters are avoiding areas with cropland or rangelands.

Genetic Population Estimation

In 2010 I documented 35 latrine sites, or 0.19 latrines per km (35 latrines/187 km). Latrine density in southwest Montana was 0.98 latrines per km (Zackheim 1982) and 2.5 latrines per km in Pennsylvania (Swimley et al. 1998). Thus, using latrines/km as a simple index of otter density, it appears the otter population in the UCFR is at a relatively low density.

Otter scat yielded very low quality DNA and the genotyping success was only 5.8%. Other studies have had better success with otter scats, but success rates only ranged from 15% – 60% (Jansman et al. 2001, Hajkova et al. 2006, Koelewijn et al. 2010, Mowry et al. 2011). Because of the low success and cost of running samples in a lab, using otter scat for genetic population estimates does not seem to be a cost-effective method for monitoring otters. Because otter scat is relatively easy to collect, it may be an effective tool for population estimates if single nucleotide polymorphisms (SNPS) are developed for otters, since SNPS can be amplified from

lower quality DNA samples (Allendorf and Luikart 2007). Although hair samples had better amplification rates they are difficult to collect in the field, especially in an area with low otter density. I had snares out for roughly 5000 trap nights and only collected 17 hair samples. The hair snares have been used with more success in areas with higher otter densities (Depue and Ben-David 2007, Sager-Fradkin 2009).

Conclusions and Management Implications

Although otters are recovering throughout North America and in Montana, they still face population specific threats, typically from pollution and habitat degradation (Toweill and Tabor 1982, Raesly 2001). The otter population in the UCFR is no exception. This study showed that certain habitat variables found along the UCFR could be indicative of good otter habitat, although additional variables found to be predictive of otter occurrence in other studies were missing. Three habitat variables were shown as being more common at otter latrine sites than random sites along the river. However, 2 of those have little restoration potential. Most of the river is already inhabited by beavers, and bank heights are often a function of how the river is naturally shaped. Providing moderate understory cover (25-50%; vegetation 1-2 m tall) along the banks may be beneficial to otters. Although otters in the Upper Clark Fork River do not appear to be at high densities, they do appear to be doing well in certain stretches of the river. These areas seem to, anecdotally, correspond to areas where the fisheries have recovered the most. The fact that otters are present in the river indicates that restoration efforts thus far have been successful.

Otters can act as indicator species in aquatic systems. Therefore, managers should continue to monitor the otter population in the UCFR. Continuing to monitor otter latrine sites can be a time- and cost-effective way to track ecological responses to restoration. Another

technique that could be useful is to continue public sightings and sightings from fishing guides. This will especially help in determining new areas along the UCFR where otters may be residing. It is not recommended to use non-invasive sampling measures to estimate population until different types of genetic markers (e.g. SNPS) are developed for otters that can utilize low-quality DNA from otter scat.

Because few habitat variables modeled otter locations well, it is recommended that other aspects of the ecology of the Upper Clark Fork River, such as the fisheries, should guide restoration efforts. Considering the connection to other river systems and current low otter densities, I believe that otter numbers will continue to increase within the river as the fisheries recovers. Restoration efforts aimed at the fisheries will ultimately benefit otters.

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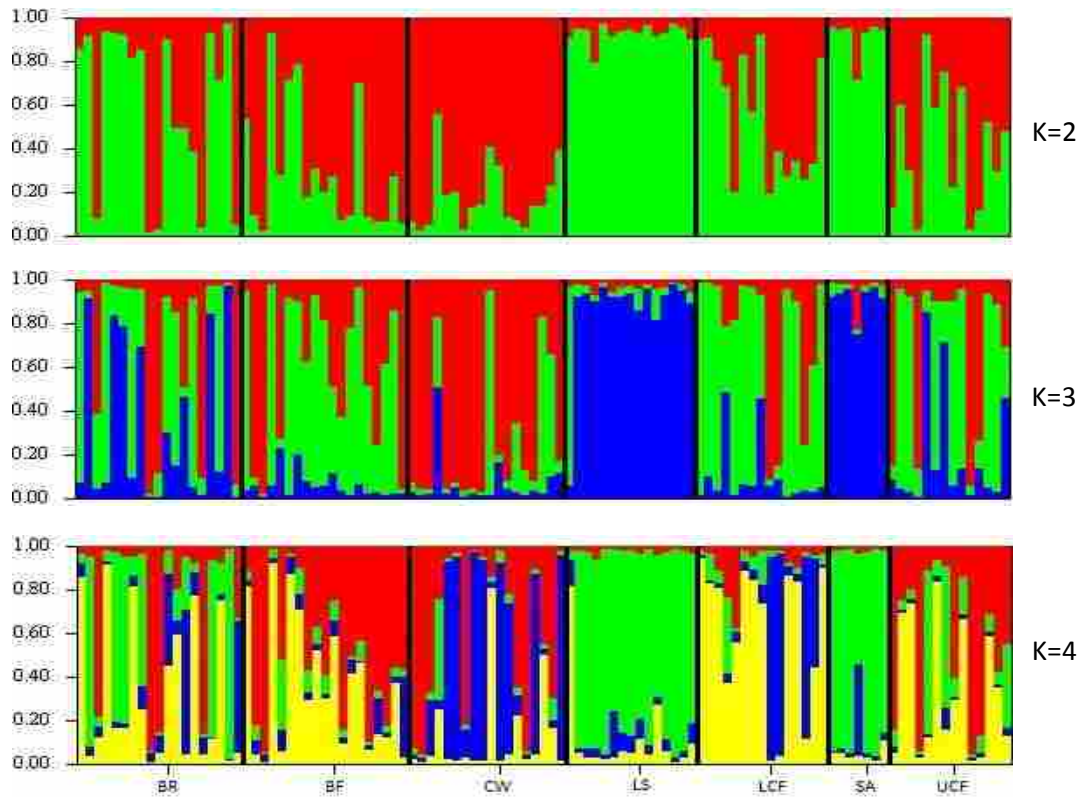
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Appendix



Appendix 1. Outputs from program STRUCTURE showing how individuals in each population separated into population clusters for K=2 – 4. Each vertical bar represents an individual, and each color represents a cluster. The more of a color a bar has, the greater percentage of the alleles STRUCTURE assigned to the corresponding cluster. BR = Bitterroot, BF = Blackfoot, CW = Clearwater, LS = Lochsa, LCF = Lower Clark Fork, SA = Salmon, UCF = Upper Clark Fork.