A NON-INVASIVE APPROACH EXAMINING NORTH AMERICAN RIVER OTTER ABUNDANCE AND SOCIALITY

by

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ABSTRACT

A Non-invasive Approach Examining North American River Otter Abundance and Sociality

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An understanding of population demographics is a key component for developing successful wildlife conservation and management plans. However, elusive and secretive carnivores such as river otters can be difficult to trap and observe, making investigations of population number and social structure extremely challenging. Advances in molecular genetics techniques have facilitated the use of non-invasive methods to examine abundance and genetic structure of wild populations when traditional wildlife survey methods may not be appropriate. I applied non-invasive methods to estimate abundance, sociality and kinship of a population of North American river otters (*Lontra canadensis*) inhabiting the Humboldt Bay area, California, USA. Through microsatellite multi-locus genotyping and closed population mark/recapture modeling, I estimated abundance as 41-51 river otters in the Humboldt Bay region. Coastal river otters associated in family groups, with evidence for the temporary formation of male bachelor groups. There was fine scale population structuring that was most likely a function of social family groups and isolation by distance gene flow. With these results, we can better understand population demographics of coastal-living river otters and thus inform future research and conservation decisions in the Humboldt Bay region.

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TABLE OF CONTENTS

Page

ABSTRACT....iii

ACKNOWLDGMENTS....iv

TABLE OF CONTENTS....v

LIST OF TABLES....vi

LIST OF FIGURES....viii

LIST OF APPENDICES....x

INTRODUCTION....1

METHODS....5

Study Area....5

Non-invasive field collection....8

Molecular methods....8

Test of assumptions....10

Abundance estimates....13

Genetic population structure and relatedness....15

RESULTS....18

Field sampling and molecular methods....18

Test of assumptions....18

Abundance estimates....25

Genetic population structure and relatedness....35

DISCUSSION....45

LITERATURE CITED....55

LIST OF TABLES

Table Page

1 Total number of river otter scat, jelly, and mixed scat with jelly samples collected throughout the Humboldt Bay region, California, USA from 18 May-31 October, 2008. Samples from north to south were Little Rive000r, Mad River, Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Woodley Island, Elk River, and Humboldt Bay National Wildlife Refuge complex (HBNWR).....19

2 Genotyping success of river otter DNA extracted from scat samples in the Humboldt Bay region, California, USA from 18 May-31 October, 2008. Sampling sites from north to south were Little River, Mad River, Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Woodley Island, Elk River, and Humboldt Bay National Wildlife Refuge complex (HBNWR).....20

3 Diet and amplification success of river otter DNA extracted from scat samples collected in the Humboldt Bay region, California, USA from 18 May-31 October, 2008.....22

4 Sample type, [scat, jelly or mixed samples (scat and jelly)], and genotyping success of river otter DNA extracted from samples collected in the Humboldt Bay region, California, USA from 18 May-31 October, 2008….....23

5 The number of alleles, observed heterozygosity (H_O), expected heterozygosity(H_E), tests for conformance to Hardy-Weinberg equilibrium, and allele sizes and frequencies of six microsatellite loci, among 41 river otters in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008.....24

6 Repeat motifs, Probability of Identity (P_{ID}), sibling Probability of Identity ($P_{(ID)sibs}$), allelic dropout rates (ADO), and false allele rates (FA) at six loci, among 41 river otters in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008.....26

7 Home site (location most often detected), sex, sites detected at, and total linear distance traveled (km; sometimes on multiple trips) for all river otters detected at multiple sites in the Humboldt Bay region, California, USA from 18.....28

LIST OF TABLES (CONTINUED)

8 Pairs of roaming river otters detected at the same site on the same date and pairwise relatedness values (Queller and Goodnight's *R* values from GenAlEx) between dyads in the Humboldt Bay region, California, USA from 18 May-31 October 2008.....29

9 Total number of male, female and unknown sex river otters (and maximum number including roaming otter visitations) detected using non-invasive genetic samples from the Humboldt Bay region, California, USA, from 18 May-31 October 2008.....32

10 Closed population model rankings from program MARK for river otter captured in August-September 2008 and only September 2008, in the Humboldt Bay region, California, USA. Corrected Akaike Information Criterion (AIC_c) was used to rank models.....33

11 Population estimates and corresponding standard error (SE) and 95% confidence intervals (CI), from top ranked closed population models for river otters sampled from August-September, 2008, in the Humboldt Bay region, California, USA. Estimates were evaluated for August-September combined capture histories and September alone.....34

12 The number of correct (assigned same location as sampled) and incorrect (assigned different location than sampled) assignments based on log-likelihood values for 41 river otter genotypes in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008.....38

13 Relatedness coefficients (*R*) between study locations (within locations on diagonal) in the Humboldt Bay region, California, USA, calculated from river otters non-invasively sampled 18 May-31 October 2008.....40

14 Mean coefficient of relatedness (Queller and Goodnight's *R* values from GenAlEx) within and between study areas for river otter genotypes detected in the Humboldt Bay region, California, USA, from 18 May-31 October 2008.....41

LIST OF FIGURES

Figure Page

1 Focal latrine sites sampled non-invasively for river otter scat from 18 May-31 October, 2008, Humboldt Bay, California, USA.....7

2 Agarose gel image showing results of restriction enzyme *Taq*$^{\alpha}$I digestion of PCR product from ZFX/ZFY primer pairs P1-5EZ/P2-3EZ (far left) and ZFKF 203L/AFKF 195H (far right). Primer pair 203L/AFKF 195H yielded one X band of 153 bp and one Y band of 203 bp; molecular weight scale on right in base pair size.....11

3 Total number of river otter scat samples collected, total number of individuals successfully genotyped, and newly detected genotypes in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008. *May only sampled for 2 weeks …….....…….....….....21

4 Probabilities of identity (P_{ID}) from river otter genotypes sampled non-invasively from the Humboldt Bay region, California, USA, from 18 May-31 October 2008. Probabilities were calculated for six microsatellite loci and arranged in order of increasing P_{ID} value.....27

5 Movement patterns for the only 8 river otters detected at multiple sites in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008. Circles denote sampling site and lines and arrows indicate paths of movement.....30

6 Bayesian Information Criterion (BIC) values for models with increasing clusters to determine the number of genetically differentiated groups derived from multi-locus genetic data of 41 river otters in the Humboldt Bay region, California, USA, from 18 May-31 October 2008….....36

7 Discriminate Analysis of Principal Components constructed from 41 river otter genotypes determining genetic differentiations between locations in the Humboldt Bay region, California, USA, from 18 May-31 October 2008. The locations from north to south were Little River, Mad River, Arcata Marsh Wildlife Sanctuary (Marsh), Mad River Slough (MRS), Elk River, and Humboldt Bay National Wildlife Refuge complex (HBNWR). Eigenvalues (insert) show the majority of variance was captured within the first and second principal component (PC)….....37

LIST OF FIGURES (CONTINUED)

8 Mean relatedness values (Queller and Goodnight's *R* values from GenAlEx) within sampling locations for (a) all river otters sampled and (b) for only river otters that exhibited no movement between sites, in the Humboldt Bay region, California, USA, from 18 May-31 October 2008. Sites from north to south were Little River (Little) Mad River (Mad), Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Elk River (Elk), and Humboldt Bay National Wildlife Refuge (HBNWR). Upper and lower whiskers denote 95% confidence limits determined by 999 bootstrap resampling. Female (♀), male (♂), and unknown sex (?) sample sizes displayed above sampling sites.....42

9 Mean relatedness values (Queller and Goodnight's *R* values from GenAlEx) for male and female river otters sampled in the Humboldt Bay region, California, USA, from 18 May-31 October 2008. Upper and lower whiskers denote 95% confidence interval determined by 999 bootstrap resampling.....43

10 A test of isolation by distance for river otters detected at all sampling locations (a) and all locations but Little River (b) in the Humboldt Bay region, California, USA from 18 May-31 October 2008. Genetic distance was measured by *R* values and geographic distance was measured in linear distance (km).....44

LIST OF APPENDICES

Appendix		Page
A	Universal Transverse Mercator (UTM) coordinates of river otter latrine sites sampled 18 May-31 October 2008 in Humboldt Bay, California, USA	64
B	Multi-locus microsatellite genotypes (N=41) for 6 loci and sex type derived from DNA extracted from non-invasive scat sampling of river otters in Humboldt Bay, California, USA, from 18 May-31 October 2008	65
C	River otters found at more than one sampling location, sex type, and dates detected at all sites in Humboldt Bay, California, USA, from 18 May-31 October 2008. No roaming river otters were detected at Little River	66
D	Capture probability estimates (*p*), standard error (SE), and 95% confidence limits (CI) estimated from closed population models as determined by program MARK for river otters sampled in the Humboldt Bay region, California, USA, from 1 August-30 September, 2008. The top section is for capture histories combining August and September (9 capture occasions); the bottom section is only for September (5 capture occasions)	67

INTRODUCTION

North American river otters (*Lontra canadensis*) are sensitive to anthropogenic changes in the landscape and are therefore key indicators of intact wetland ecosystems (Lariviere and Walton 1998, Bowyer et al. 2003). They suffer more from environmental degradation than other mammals due to movement between terrestrial and aquatic landscapes, inherently increasing their exposure to water pollutants and environmental contaminants (Foster-Turley 1990, Ben-David et al. 1998, Melquist et al. 2003). The species must contend with persistent and damaging chemical pollutants shown to decrease fecundity and survival (Elliott et al. 2008). Future habitat alterations due to global climate change are predicted to alter wetland hydrology potentially severely enough to be beyond the limits of adaptation and tolerance for carnivores such as river otters (Burkett and Kusler 2000). Monitoring river otter populations is essential for establishing baseline data for future comparison and creating management plans to mitigate population disturbance due to past threats.

Despite their importance as indicators of wetland health, relatively little is understood about river otter ecology (Melquist and Dronkert 1987, Kruuk 2006*)*. Understanding the natural history of an animal, particularly social structure, spatial organization, and movement, which influence gene flow, is essential to appropriate conservation and management (Kruuk 2006, Manel et al. 2003). In general, female river otters exhibit small, exclusive core-areas of use with overlapping home ranges outside this core area, while males exhibit larger home ranges that overlap with both other males and females (Kruuk 2006). However, social patterns tend to be complex and variable

among and even within contiguous river otter populations (Melquist and Hornocker 1983, Reid et al. 1994, Blundell et al. 2002a, Spinola 2003, Gorman et al. 2006). For example, sociality ranges from completely solitary males and females, to social family groups, to unrelated large-male bachelor associations (Melquist and Hornocker 1983, Spinola 2003, Gorman et al. 2006). Group sizes vary depending on type of social group. Female family groups may have 1-3 adult females, usually a mother with reproductive daughters and all of their associated pups, while reported male bachelor groups can be as large as 18, though generally average 6-8 river otters (Shannon 1989, Rock et al. 1994, Blundell et al. 2002a).

River otters adjust their spatial distribution in response to environmental conditions and seasonal resource availability (Mason and Macdonald 1986, Blundell et al. 2000). Accordingly, river otter gene flow should also vary by habitat conditions and characteristics. Genetic diversity, partially maintained through dispersal and movement between populations, is important for a species to adapt and persist in a changing environment (Manel et al. 2003). Several comprehensive studies employing both behavioral and genetic data for Alaskan coastal river otters found low levels of dispersal and gene flow, as well as significant isolation by distance for male otters (Testa et al. 1994, Blundell et al. 2002a, Blundell et al. 2002b, Blundell et al. 2004). Understanding metapopulation dynamics is necessary for population monitoring since the low dispersal rate of river otters may make natural population growth slow and increase genetic structuring of subpopulations (Blundell et al. 2002b). Study of coastal river otter

demographics and genetic structure has been mostly limited to Alaska. Parallel assessments from California would elucidate the generality of the findings.

The elusive nature of river otters makes direct observations challenging (Swimley et al. 1998). River otters are difficult to trap and live trapping can lead to severe animal injury (Kruuk 1995, Serfass et al. 1996, Blundell et al. 1999). Molecular genetics allow utilization of non-invasive techniques as an alternative to traditional wildlife field methods (Bellemain and Taberlet 2004), providing a new tool to census populations and examine social structure and spatial interactions (Hughes 1998, Piggott and Taylor 2003, Hung et al. 2004). Non-invasive sampling of scat, hair, mucus or any tissue left behind by an animal can be used to establish individual identification via DNA 'finger-printing' methods (Gerloff et al. 1999, Taberlet and Luikart 1999). These techniques facilitate monitoring of elusive species and eliminate the risk of animal injury (Taberlet et al. 2001, Anderson et al. 2006, Solberg et al. 2006).

Northern California has an entirely native river otter population and, consistent with nationwide trends, there have been relatively few river otter monitoring efforts conducted within the state (Gould 1977, Raesly 2007, Black 2009). The Humboldt Bay region in northern California has a rich fish and avian community, the predominant components of river otter diets (Chamberlain and Barnhart 1993, Colwell 1994, Penland and Black 2009). These features make it conducive to supporting stable river otter populations. This coastal system is ideal for examining social grouping and gene flow of river otters within a discrete wetland system and along a coastline. A citizen scientist database and local observations have provided anecdotal evidence of female family and

male bachelor group formation in the Humboldt Bay region (Shannon 1989, 1998, Black 2009). Utilizing non-invasive molecular techniques, I identified individual animals to meet the following objectives:

1. Estimate abundance of male and female river otters in the Humboldt Bay region.
2. Evaluate genetic population structure and relatedness to elucidate social groups and the presence of family groups among river otters in the Humboldt Bay region.

METHODS

Study area

Humboldt Bay is located in northern, California (Humboldt County), approximately 145 km south of the Oregon-California border. The Bay is California's second largest bay (approximately 66 km^2). The diversity and abundance of estuarine organisms is greater only in San Francisco Bay (Chamberlain and Barnhart 1993). Humboldt Bay supports a vast number and variety of fish and avian species, preferred diet of Humboldt County river otters (Modafferi and Yocom 1980, Reeves 1988, Penland and Black 2009). Over 30 fish species and two crab species have been detected in the Bay (Chamberlain and Barnhart 1993), and the coast hosts naturally-spawning Pacific salmon (*Oncorhynchu spp.*) and steelhead (*Oncorhynchus mykiss*) stocks (Nehlsen et al. 1991). The region is also a major winter and stopover site for migratory shorebirds along the Pacific flyway (Cowell 1994). More than 200 bird species regularly feed, rest, or nest around the Bay throughout the year.

The study area covered approximately 45 km of coastal habitat. Linear home ranges of coastal river otters have a large degree of spatial variation, reported to range from approximately 5-73 km in length (Mason and Macdonald 1986, Bowyer et al. 1995). Average home ranges for female and male coastal river otters range 8-20 km and 21-45 km, respectively (Bowyer et al. 1995, Blundell et al. 2001). The Humboldt Bay study area was long enough to detect numerous individual river otter home ranges. Although river otters have been reported to occur at a density of one adult otter per linear km within resource rich environments (Kruuk 1995), a more common range in coastal

regions is 26-60 animals/100 km (Testa et al. 1994, Blundell et al. 2004). The unique attributes and resource richness of the Humboldt Bay area could support a high density of river otters.

River otters deposit scat and mucus produced in the lower intestine (referred to as jellies) as scent marks at terrestrial latrines sites usually within their home ranges (Melquist and Hornocker 1983, Kruuk 1992, Ben-David et al. 1998, Lariviere and Walton 1998, Oldham and Black 2009). Latrine sites are persistent over time, prominent in their environment, and distinguishable from other species, easing detection and collection of river otter feces (Modafferi and Yocom 1980, Newman and Griffin 1994, Steven and Serfass 2008). Latrine collection sites were chosen to encompass all major watersheds with consistent river otter activity around Humboldt Bay (Penland and Black 2009). Each site had records of river otter groups ranging in size from 1-9 individuals and breeding activity with litter sizes ranging from 1-4 pups (Black 2009). Sites included, from north to south: Little River, Mad River, Arcata Marsh and Wildlife Sanctuary, Mad River Slough, Woodley Island, Elk River, and the Humboldt Bay National Wildlife Refuge complex (Figure 1). These seven sites encompassed a combination of fresh, brackish and salt water river otter activity areas (habitat with known river otter detections as determined from multiple observations from the citizen science database) and each had 4-6 river otter latrines (Appendix A).

Figure 1. Focal latrine sites sampled non-invasively for river otter scat from 18 May-31 October, 2008, Humboldt Bay, California, USA.

Non-invasive field collection

Scat and jelly samples were collected from all latrine sites two consecutive mornings each week from 18 May to 31 October 2008. This sampling period was established in order to collect enough samples to encompass the entire population and also ensure recapture of individuals. Only fresh scat and jellies (determined visually by moistness and odor) were collected to assure samples were deposited within the previous 24 hours. The glossy, mucus sections of scat were targeted in order to reduce prey material and enhance quality of DNA extractions (Hajkova et al. 2006). In cases of heavily used or layered latrines, only the top layer of feces was collected to prevent any contamination between samples. Collected scat and jellies were placed into sterile 50 ml centrifuge tubes with sterile tongue depressors and stored at -20˚C until DNA extraction (Arrendal et al. 2007, Lampa et al. 2008). Scat sampling was approved by the Humboldt State University Animal Care and Use Committee (#07/08.W.40.A) and was consistent with the American Society of Mammalogist guidelines (Gannon et al. 2007).

Molecular methods

DNA extractions were conducted in an isolation room free of concentrated DNA. Aerosol-barrier pipette tips were used and work areas and equipment were cleansed with 10% bleach and radiated with ultra-violet (UV) light to reduce contamination. DNA was extracted from scat and jelly samples using QIAmp® DNA Stool Mini Kit (Qiagen, Inc., Valencia, California) following manufacturer's instructions or with a standard phenol/chloroform extraction protocol (Ausubel et al. 2003). Negative controls were executed in all extractions and polymerase chain reactions (PCR) to monitor

contamination. PCR set-up was conducted within a UV radiated hood. River otters were genotyped at six microsatellite loci (Lut453, Lut733, Rio08, Lut701, Rio18, Lut604; Dallas and Piertney 1998, Beheler et al. 2004, Beheler et al. 2005). Samples were run in 10 microliter (µL) reaction volumes: 2 µL genomic DNA, 10x PCR Gold Buffer, 1 unit Amplitaq Gold, 1.5 mM MgCl2, 400 µM dNTPs, 0.15 µM reverse and m-13 labeled primers, 0.30 µM forward primer, 0.7 µM licor m-13 labeled primer, and 0.3 µM bovine serum albumin (BSA). Thermal cycling temperatures and times followed published conditions except for Rio08 and Rio18, which were modified to 40 cycles. Products were read using a LI-COR DNA 4300 Analyzer Gene Readir (LI-COR Biosciences, Lincoln, Nebraska). GENE PROFILER imaging software was used to estimate allele sizes. To address potential factors that may have influenced genotyping success, I ran chi-squared tests to evaluate the impact of diet and sample type (scat, jelly, or mixed scat/jelly) on PCR success, hereafter referred to as amplification.

Gender was determined using PCR/RLFP (restriction length fragment polymorphism) analysis of the zink-finger protein gene (ZFX/ZFY; Shaw et al. 2003). Because this method has not been applied to North American river otters, I validated the technique using otter tissue samples of known sex (2 males, 2 females). A 447 basepair (bp) fragment of the ZFX/ZFY gene was amplified with primers P1-5EZ and P2-3EZ (Aasen and Medrano 1990). PCR product was purified with the QIA-quick PCR purification kit and sequenced at the San Diego State CSUPERB MicroChemical Core Facility. Results were analyzed with Code-on-Code software and I identified a unique restriction enzyme site for *Taq*$^{\alpha}$I. This site was identical to the site identified in the

Eurasian otter (*Lutra lutra)*, where females yielded one band and males yielded two (Mucci and Randi 2007, Statham et al. 2007). Since the P1-5EZ/P2-3EZ primer set was long at 447 bp an internal set of primers which were only 195 bp in length (ZFKF 203L and AFKF 195H) were tested (Ortega et. al 2004). The latter primer pair amplified more consistently with degraded fecal DNA obtained in this study. *Taq*$^{\alpha}$I digest yielded one 153 bp fragment in females (X band) and two fragments in males (one X band of 153 bp and one Y band of 203 bp; Figure 2).

PCR was conducted in 10 µL reaction volumes: 2 µL genomic DNA, 10x PCR Gold Buffer, 1 unit Amplitaq Gold, 1.5 mM MgCl2, 400 µM dNTP, 0.15 forward and reverse primers, and 0.3 µM BSA. PCR conditions followed Ortega et al. (2004) except for an increase to 40 cycles. PCR product was digested by restriction enzyme *Taq*$^{\alpha}$I at the following recommended volumes: 10 µL PCR product, 5 units *Taq*$^{\alpha}$I, 2 µL Buffer E, 0.2 µL BSA, and 7.3 µL ddH_2O (New England Biolabs, Ipswich, MA). The digestion was incubated at 65°C for 3 hours followed by 80°C for 20 minutes. Products were visualized on a 2% agarose gel, run at 85 volts for 3 hours, stained with ethidium bromide, and photographed.

Test of assumptions

A comparative multi-tube PCR approach was followed to create consensus microsatellite genotypes (Frantz et al. 2003). The comparative multi-tube method reduces total number of amplifications compared to other approaches (Taberlet et al. 1996), yet still produces reliable genotypes and decreases both cost and time (Hansen et al. 2007). Genotypes were determined through two positive PCR reactions for

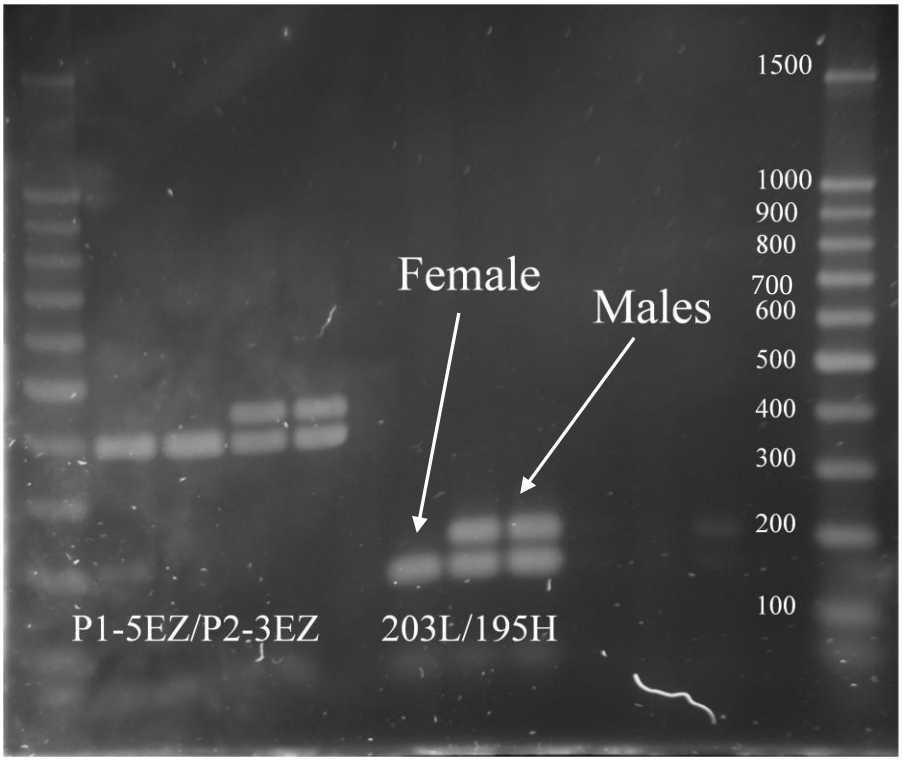


Figure 2. Agarose gel showing results of restriction enzyme *Taq*$^{\alpha}$I digestion of PCR product from ZFX/ZFY primer pairs P1-5EZ/P2-3EZ (far left) and ZFKF 203L/AFKF 195H (far right). Primer pair 203L/AFKF 195H yielded one X band of 153 bp and one Y band of 203 bp; size standard on right in base pair size.

heterozygotes and three for homozygotes (Frantz et al. 2003, Hansen et al. 2007). Identical multilocus genotypes were identified using GIMLET (Valière 2002). All scat samples that produced at least one validated genotype at one microsatellite locus were sexed using the multi-tube approach. Incomplete multilocus genotypes (less than 5 confirmed loci) were manually grouped based on the most informative loci genotyped, sex and collection location (Frantz et al. 2003). I used GenAlEx, Genetic Analysis in Excel (Version 6.3, Peakall and Smouse 2006), to test divergence from Hardy-Weinberg Equilibrium and to calculate allele frequencies for each locus.

Genotyping errors due to allelic drop-out and false allele rates were calculated following Broquet and Petit (2004). Allelic drop-out occurred when one allele of a heterozygous individual did not amplify during a PCR that resulted in a recordable allele. A false allele was defined as a PCR-generated allele artifact that was a result of replication slippage. Since allelic drop-out can only occur in heterozygous individuals (homozygous allelic drop-out simply results in a failed PCR), this error rate is not representative of all genotypes, but gives a more unbiased estimation of genotyping error than including homozygous samples in allelic drop-out calculations (Broquet and Petit 2004). Allelic drop-out and false allele weighted averages of all loci were calculated as an indication of overall data quality.

To evaluate the power of the marker set for individual identification, I calculated Probability of Identity (P_{ID}), the probability that two individuals drawn at random from a population will have the same genotype at multiple loci (Waits et al. 2001, Valière 2002). GIMLET was used to calculate $P_{IDunbiased}$ (P_{ID} corrected for small sample size) and $P_{(ID)sib}$

(P_{ID} among a population of siblings). P_{ID} and $P_{(ID)sib}$ provided upper and lower bounds for identifying the most efficient and error-free set of loci to discriminate individuals. More loci improve P_{ID} but introduce genotyping error especially with poor quality DNA. Current literature recommends a P_{ID} or $P_{(ID)sibs}$ of 0.01 but due to increased genotyping error higher values are acceptable (Mills et al. 2000, Waits et al. 2001). The extraneous addition of a false individual is particularly problematic for mark/recapture studies because it inflates population estimates (Waits and Leberg 2000). Consequently, a $P_{(ID)sibs}$ between 0.01-0.05 was accepted here.

Abundance estimates

To estimate population size, I ran closed population models using MARK. Heterogeneity in capture probability among individuals can be difficult to evaluate in non-invasive sampling and can bias population estimates (Otis et al. 1978, White et al. 1982). To model heterogeneity and time variation in capture probabilities, five models were built: 1) varying encounter probability by time, 2) allowing encounter probability and recapture probabilities to differ (behavioral response), 3) holding encounter probability constant, 4) including heterogeneity among individuals and holding encounter probability constant, and 5) including heterogeneity among individuals and varying encounter probability by time. Sexes were combined in all models because sample sizes were too small to include sex as a group variable. Models were ranked using corrected Akaike Information Criterion (AIC_c; Lukacs and Burnham 2005). Closed capture-recapture model notation in the literature sometimes still follows Otis et al. (1978), but

with the extensive capabilities available in MARK, I chose to use extended model notation to describe the closed-population models (Lukas and Burnham 2005).

Capture histories for individual river otters were constructed using repeated detections of the same genotype. Sampling occasions were broken into week sessions; if multiple captures of an individual occurred during two consecutive days, they were pooled and recorded as a single detection for the week. To ensure population closure and to evaluate different sampling period durations, two separate capture histories were built. For the first modeling regime I used detections from August and September; for the second I used detections only from September. Both periods were after early summer months of high pup mortality and before fall dispersal events (Melquist and Hornocker 1983). River otters only occur near water bodies with sufficient food resources, thus to meet the assumption that every individual had a reasonable probability of detection, I attempted to survey all major watersheds around Humboldt Bay. Although there was a possibility that additional river otters occurred in small, un-surveyed areas, I focused on areas with consistent river otter activity over the past 10 years (Black 2009). I determined overall detection probability by taking each sessions estimated capture probability (p), subtracting it from 1, then multiplying those values together and finally subtracting that overall product from one ($1-[(1-p_1)(1-p_2)...(1-p_i)]$); personal communication, T. L. George 2010. HSU, 1 Harpst Street, Arcata, CA 95521). This value provided an estimated probability of capture.

Genetic population structure and relatedness

To determine the number of genetically divergent groups (K) in the Humboldt Bay river otter population I performed a cluster analysis and a Discriminant Analysis of Principal Components using ADEGENET 1.2-3 for Program R (Jombart 2008, Jombart et al. submitted). The cluster function was designed to infer K from multi-locus genetic data. The analysis transforms the data using a principal component analysis and then runs successive models with an increasing number of clusters. For each model, a statistical measure of goodness of fit (by default, Bayesian Information Criterion) was computed, and the number of clusters in the data was identified when subsequent K values no longer led to an appreciable improvement in fit. I graphically investigated population differentiation using the Discriminant Analysis of Principal Components. The Discriminant Analysis of Principal Components aims to maximize between-group variability and achieve the best discrimination of genotypes into predefined clusters. Sampling site locations were used as prior population information. If an individual genotype was detected at multiple sampling locations, the site with the most visits was used to define the individual's prior population assignment. This multivariate approach has been shown to reliably cluster individuals based upon genotypes and does not make assumptions regarding Hardy–Weinberg or linkage equilibrium (Jombart et al. 2008).

An assignment test using the frequency-based Paetkau et al. (1995) criterion was run in GeneClass2 (Version 2.0, Piry et al. 2004). Assignment tests provide an indication of population structuring based on whether samples can be reliably assigned to their location of origin. To compute the probability individuals belonged to each sampling

location, 10,000 Monte-Carlo simulations were run with a leave-one-out resampling algorithm. Samples not assigned correctly were examined to see if sex or location was an indication of poor assignment using a Fisher's exact test.

Pairwise relatedness (*R*) values (Queller and Goodnight's 1989) were calculated with GenAlEx. Relatedness coefficients range from -1 to 1, negative values denote no relation and positive values denote relatedness. *R* coefficients of approximately 0.25 and 0.50 suggest half-sibling and full-sibling levels of relatedness, respectively (Konovalov and Heg 2008). *R* values were evaluated to see if mean relatedness within sampling locations was greater than mean relatedness of all other pair-wise comparisons. I repeated the same analysis without genotypes of individuals detected at multiple sites to see if roaming individuals were not part of potential resident family groups utilizing a site. I also calculated relatedness between and among sexes. A total of 999 permutations were completed to create mean *R* values, 95% confidence intervals, and to evaluate significance of *R* among and between sampling locations.

A Mantel test (Isolation by Distance Web Service version 3.61; Jensen et al. 2005) was used to evaluate a genetic isolation by distance model for all river otters in the study area as a whole and after removing the Little River site. Little River was the furthest northern site, and not technically within Humboldt Bay proper, thus removing the site allowed evaluation of isolation by distance within the Bay. Mantel's test is specifically designed for pair-wise values such as genetic and geographic distances that are not independent of each other. I used *R* values as the measure of genetic distance. Methods similar to Dallas et al. (1999) and Blundell et al. (2002b) were used to calculate

geographic ‘otter distance’ defined as the route most likely taken by a river otter via waterways rather than determining the straight line distance over land. This ‘otter distance’ was measured as the most direct route between midpoints of each sampling location following a linear course parallel to the shoreline and the shortest distance possible overland when necessary.

RESULTS

Field sampling and molecular methods

From 18 May to 31 October 2008, I collected 357 river otter scats, 82 jellies and 44 mixed scat/jelly samples (Table 1). Among the 483 collections 124 (25.7%) were successfully genotyped (Table 2). The largest number of collections (n=110) and new genotypes detected (first time captures; n=17) were in June, while the most samples genotyped (first time captures and recaptures) was in September (n=24, Figure 3). There was a significant difference in sampling location and number of genotypes detected at each location (χ^2_6=16.40, P=0.01). When Elk River and Humboldt Bay National Wildlife Refuge were removed from the analysis, there was not a significant difference among sites (χ^2_5=8.87, P=0.11 and χ^2_5=5.75, P=0.33, respectively). Thus either Elk River otters had more samples successfully genotyped, or Humboldt Bay National Wildlife Refuge had fewer samples successfully genotyped. Prey type found in scat did not influence amplification success of scat (χ^2_3=1.15, P=0.76, Table 3). Jellies amplified significantly better than scat (χ^2_2=43.86, P<0.001, Table 4). When scat samples were removed from the analysis, there was a marginal but non-significant difference in amplification success between jelly and mixed scat/jelly samples (χ^2_2=4.94, P=0.08).

Test of assumptions

All six microsatellite loci were polymorphic, the number of alleles ranged from 2-4 (Table 5). A global test calculated over all sampling locations showed all loci met Hardy-Weinberg equilibrium assumptions; observed heterozygosity (H_O) ranged 0.361-

Table 1. Total number of river otter scat, jelly, and mixed scat with jelly samples collected throughout the Humboldt Bay region, California, USA from 18 May-31 October, 2008. Samples from north to south were Little River, Mad River, Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Woodley Island, Elk River, and Humboldt Bay National Wildlife Refuge complex (HBNWR).

	Little	Mad	AMWS	MRS	Woodley	Elk	HBNWR	Total
Scat	23	40	68	36	14	103	73	357
Jelly	8	8	17	10	3	25	11	82
Scat and jelly	4	0	4	2	6	18	10	44
Total	35	48	89	48	23	146	94	483

Table 2. Genotyping success of river otter DNA extracted from scat samples in the Humboldt Bay region, California, USA from 18 May-31 October, 2008. Sampling sites from north to south were Little River, Mad River, Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Woodley Island, Elk River, and Humboldt Bay National Wildlife Refuge complex (HBNWR).

	Little	Mad	AMWS	MRS	Woodley	Elk	HBNWR	Total
Genotyped	9	13	24	8	7	51	12	124
Failed	26	35	65	40	16	95	82	359
Total Collected	35	48	89	48	23	146	94	483
% Success	25.7	27.1	27.0	16.7	30.4	34.9	12.8	25.7

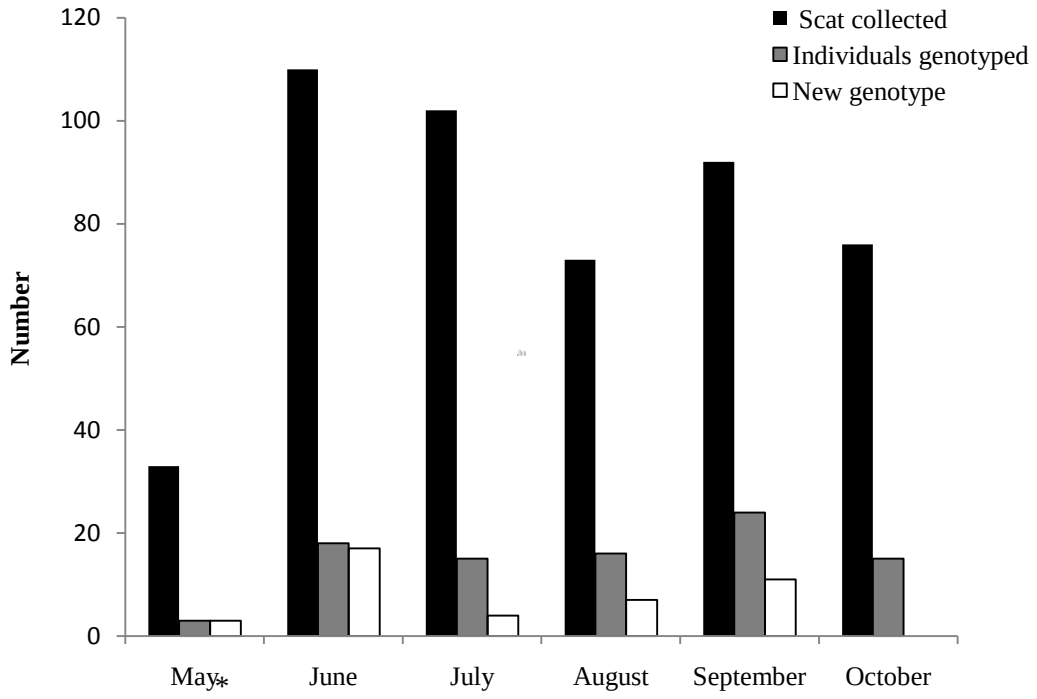


Figure 3. Total number of river otter scat samples collected, total number of individuals successfully genotyped, and newly detected genotypes in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008. *May only sampled for 2 weeks.

Table 3. Diet and amplification success of river otter DNA extracted from scat samples collected in the Humboldt Bay region, California, USA from 18 May-31 October, 2008.

	Scat Prey Content					
	Fish	Crab	Invert	Bird	Mixed	Unknown
Genotyped	47	6	0	2	9	1
Failed	183	38	6	9	34	22
Total	230	44	6	11	43	23
% success	20.4	13.0	0	18.0	20.9	4.3

Table 4. Sample type, [scat, jelly or mixed samples (scat and jelly)], and genotyping success of river otter DNA extracted from samples collected in the Humboldt Bay region, California, USA from 18 May-31 October, 2008.

	Scat	Jelly	Scat and Jelly
Genotyped	65	43	16
Failed	292	39	28
Total	357	82	44
% success	18.0	52.4	36.0

Table 5. The number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), tests for conformance to Hardy-Weinberg equilibrium, and allele sizes and frequencies of six microsatellite loci, among 41 river otters in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008.

Locus	No. Alleles	H_O	H_E	P	Allele size(bp)/frequency			
Lut453	4	0.692	0.651	0.675	142/0.18	144/0.04	146/0.32	150/0.46
Lut733	3	0.650	0.587	0.683	171/0.23	174/0.56	179/0.20	
Rio08	3	0.485	0.606	0.150	217/0.53	219/0.20	223/0.27	
Lut701	4	0.541	0.537	0.480	194/0.64	198/0.11	202/0.22	206/0.04
Rio18	3	0.487	0.444	0.565	156/0.72	162/0.15	172/0.13	
Lut604	2	0.361	0.453	0.222	133/0.35	139/0.65		
Mean	*3.27*	*0.536*	*0.546*	*0.462*				

0.692 and expected heterozygosity (H_E) ranged 0.444-0.651 (Table 5). When broken into sampling locations, the only location not in Hardy-Weinberg equilibrium was Elk River at locus Rio08 (P=0.01). Allelic dropout rates ranged from 22.6-32.5%, with a weighted average over all six loci of 28.6% (Table 6). False alleles occurred in 22 of 2,200 PCR reactions and ranged from 0.6-1.5% among loci (Table 6). When evaluated for all six microsatellites, P_{ID} and $P_{(ID)sib}$ were 0.00037 and 0.026, respectively (Figure 4).

Abundance estimates

Among the 124 scat samples genotyped, 40 unique microsatellite genotypes were identified (Appendix B). However, two individuals were identical at all six loci but different in sex typing, providing a conservative minimum count of 41 river otters. Of these 41 individuals, 22 were males, 16 females, and 3 were unknown. The number of recaptures ranged from 0 to 12 (mean=3.0 ± 0.39 (SE)). There was no significant difference in number of recaptures between males (mean=3.3 ± 0.65) and females (mean=2.7 ± 0.38; t_{37}=0.83, P=0.42). Only eight individuals were detected at more than one sampling location, of which seven were males and one was female (Table 7, Appendix C). There were five instances of pairs of individuals detected at the same site on the same date (Table 8). The largest distance moved was by a male river otter (Ott20). Ott20 was detected moving from Woodley Island (7 July) to Elk River (19 Aug) to Arcata Marsh and Wildlife Sanctuary (25 Aug) back to Woodley Island (23 Sept) then Elk River again (28 Sept) then Humboldt Bay National Wildlife Refuge (29 Sept) and back to Elk River (5-26 Oct), a total distance of approximately 60 km over 4 months (Table 7, Figure 5). This was a linear path from north to south of approximately 26 km.

Table 6. Repeat motifs, Probability of Identity (P_{ID}), sibling Probability of Identity ($P_{(ID)sibs}$), allelic dropout rates (ADO), and false allele rates (FA) at six loci, among 41 river otters in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008.

Locus	Repeat motif	P_{ID}	$P_{(ID)sibs}$	ADO rate%	FA%
Lut453	$(CA)_{26}$	0.19	0.47	30.9	1.0
Lut733	$(GATA)_4GAT(GATA)_{12}$	0.24	0.52	28.2	1.2
Rio08	$(TG)_{15}$	0.22	0.50	32.5	1.5
Lut701	$(GATA)_{11}GAA(GATA)_2GAA(GATA)_4$	0.26	0.55	29.8	1.1
Rio18	$(CT)_6(CTAT)_{14}$	0.35	0.62	22.6	0.6
Lut604	$(CA)_{26}$	0.40	0.62	24.1	0.7
Over all loci		*0.0004**	*0.026**	*28.6***	*1.0***

*Multiplied together to get over all loci values
**Weighted average

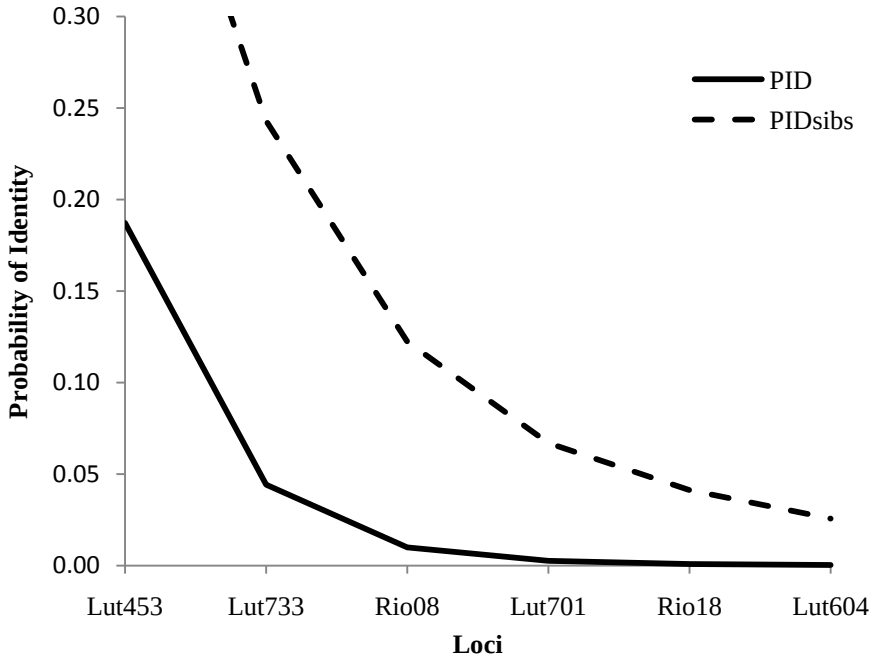


Figure 4. Probabilities of identity (P_{ID}) from river otter genotypes sampled non-invasively from the Humboldt Bay region, California, USA, from 18 May-31 October 2008. Probabilities were calculated for six microsatellite loci and arranged in order of increasing P_{ID} value.

Table 7. Home site (location most often detected), sex, sites detected at, and total linear distance traveled (km; sometimes on multiple trips) for all river otters detected at multiple sites in the Humboldt Bay region, California, USA from 18 May-31 October, 2008.

Home site*	Animal code	Sex	Sites visited	Total distance traveled (km)
AMWS	Ott16	M	MRS, AMWS	4
AMWS	Ott27	M	AMWS, Elk	17
AMWS	Ott33	M	AMWS, MRS, Mad	11
MRS	Ott18	F	HBNWR, MRS	21
Elk	Ott1	M	Elk, HBNWR	26
Elk	Ott8	M	Woodley, Elk	21
Elk	Ott20	M	Woodley, Elk, AMWS, HBNWR	60
Elk	Ott40	M	Elk, HBNWR	9

*Sites from north to south were Mad River (Mad), Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Woodley Island (Woodley), Elk River (Elk), and Humboldt Bay National Wildlife Refuge (HBNWR).

Table 8. Pairs of roaming river otters detected at the same site on the same date and pairwise relatedness values (Queller and Goodnight's *R* values from GenAlEx) between dyads in the Humboldt Bay region, California, USA from 18 May-31 October, 2008.

Pairs	Site*	Date	*R* value
Ott8/Ott20	Woodley	7/7	-0.5777
Ott8/Ott20	Elk	8/19	-0.5777
Ott8/Ott20	Elk	10/13	-0.5777
Ott20/Ott27	AMWS	8/25	0.8492
Ott8/Ott40	Elk	9/8	-0.4465

* Sites were Arcata Marsh and Wildlife Sanctuary (AMWS), Woodley Island (Woodley), and Elk River (Elk).

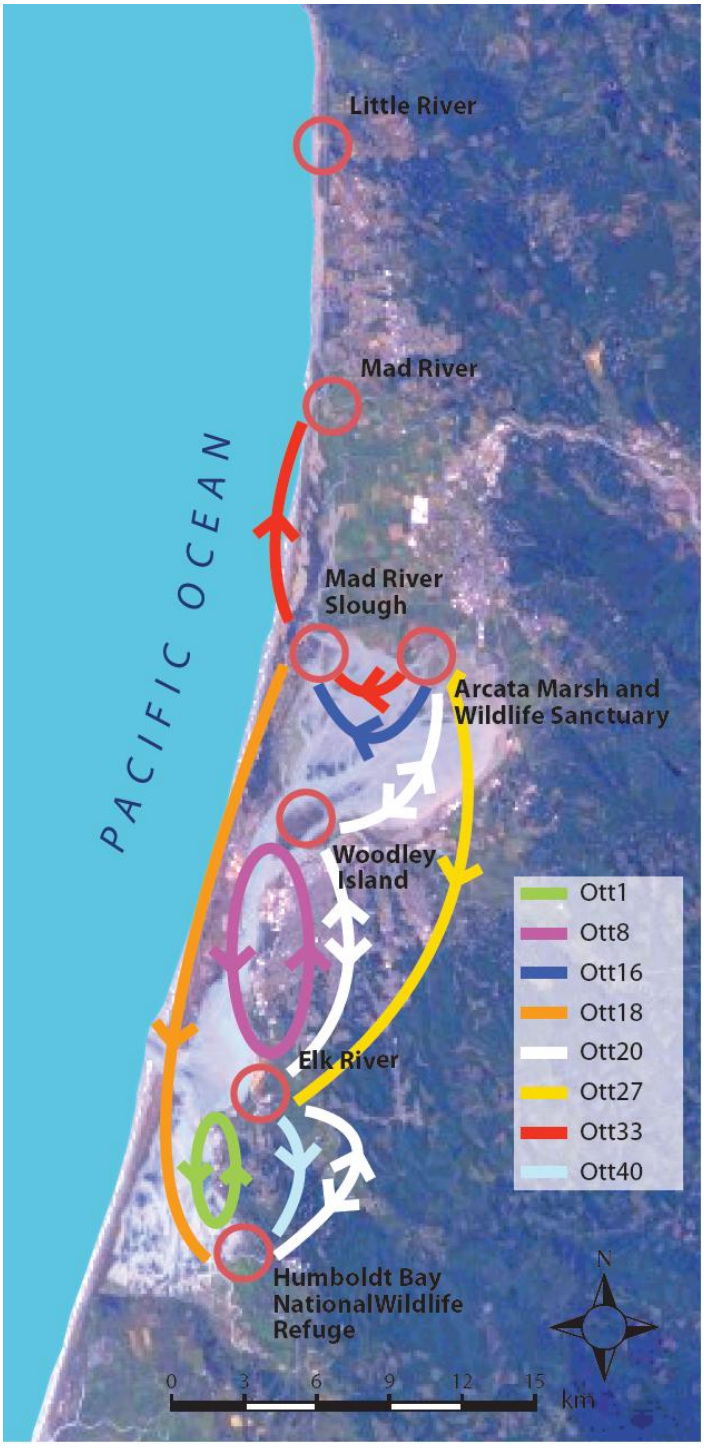


Figure 5. Movement patterns for the only 8 river otters detected at multiple sites in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008. Circles denote sampling site and the lines and arrows indicate paths of movement.

Roaming river otters increased the number of individuals detected at sites. Each sampling site had multiple individual detections, but individuals sampled at Woodley Island were all more often detected at Elk River, and were therefore grouped with Elk River animals (Table 9). Overall density, using minimum genotypes detected (41) over 45 km of linear coast line, was 0.9 river otters/ km.

Mark/recapture models designed to estimate population size were compared to the total number of genotypes detected and used to evaluate the proportion of the population sampled. The first model set based on samples collected during August and September yielded 29 unique genotypes and the second model set based on September alone had 24 unique genotypes. Both modeling efforts yielded the same top closed population model which held encounter probability (*p)* and recapture probabilities (*c*) equal but varied by time (Table 10). Encounter probability estimates and associated standard errors were reasonably small and varied widely between capture occasions (Appendix D). Overall capture probabilities were 85.6% and 73.0%, respectively, for the 2-month and single-month capture histories. The 95% confidence intervals around abundance estimates ranged 30-44 individuals and 26-50 individuals for the 2-month and single-month capture histories, respectively. Both modeling regimes encompassed the minimum number of genotypes (41) detected (Table 11). Given these ranges and the high overall capture probabilities, the majority of Humboldt Bay river otters were sampled.

Table 9. Total number of male, female and unknown sex river otters (and maximum number including roaming otter visitations) detected at each site using non-invasive genetic samples from the Humboldt Bay region, California, USA, from 18 May-31 October 2008.

	Little	Mad	AMWS	MRS	Elk	HBNWR	Total
Males	2	4 (5)	3 (4)	0 (2)	11 (12)	2 (5)	22
Females	1	3	5	3	3	1 (2)	16
Unknown	1	1	1	0	0	0	3
Total	4	8 (9)	9 (10)	3 (5)	14 (15)	3 (7)	41

*Sites from north to south were Little River (Little) Mad River (Mad), Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Elk River (Elk), and Humboldt Bay National Wildlife Refuge (HBNWR).

Table 10. Closed population model rankings from program MARK for river otters captured in August-September 2008 and only September 2008, in the Humboldt Bay region, California, USA. Corrected Akaike Information Criterion (AIC_c) was used to rank models.

Model*	Description	August - September			September		
		Δ AICc	AICc Weights	K**	Δ AICc	AICc Weights	K**
{(N,p(t)=c(t))}	Time varying *p*	0.00	0.85	10	0.00	0.75	6
{(N,p(.),c(.))}	Behavioral response	4.37	0.10	3	3.17	0.15	2
{(N,p(.)=c(.))}	Constant *p*	6.36	0.04	2	5.26	0.05	3
{(N,π,p_a(.)=c_a(.),p_b(.)=c_b(.))}	Heterogeneous *p*	8.40	0.01	3	6.73	0.03	4
{(N,π,p_a(t)=c_a(t),p_b(t)=c_b(t))}	Heterogeneous time varying *p*	10.85	0.00	20	8.71	0.01	12

*N=abundance estimate parameter, p=capture probability, c=recapture probability, (t) denotes temporal variation, (.) denotes being held constant, π denotes heterogeneity model, a and b are heterogeneity periods.
**K=number of parameters.

Table 11. Population estimates and corresponding standard error (SE) and 95% confidence intervals (CI), from top ranked closed population models for river otters sampled from August-September, 2008, in the Humboldt Bay region, California, USA. Estimates were evaluated for August-September combined capture histories and September alone.

Model*	N	SE	Lower 95% CI	Upper 95% CI
{(N,p(t)=c(t))} Aug-Sept	33.38	3.06	30.27	44.07
{(N,p(t)=c(t))} Sept	32.36	5.44	26.61	50.77

*N=abundance estimate parameter, p=capture probability, c=recapture probability, (t) denotes temporal variation.

Genetic population structure and relatedness

Based on the cluster analysis, there were 6-9 distinct genetic clusters (Figure 6). Bayesian Information Criterion (BIC) value leveled off between 6-7 clusters (BIC=15.96 and 15.91 respectively), was lowest at 8 genetic clusters (BIC=15.50) and began to increase again at 9 (BIC=15.96). This indicated that 6-9 clusters was the optimum number of genetically differentiated groups. The Discriminant Analysis of Principal Components output visually expressed the differentiated groups (Figure 7). The central sampling locations (Mad River, Arcata Marsh and Wildlife Sanctuary, Mad River Slough, and Elk River), overlapped in allelic contribution, while Little River to the north and Humboldt Bay National Wildlife Refuge complex to the south were the most genetically divergent from the main group. Despite overlap among central locations, they were still grouped as separate clusters. Most of the variance was explained by the first and second principle components as shown by Eigenvalues (Figure 7). Overall assignment probability for the Discriminant Analysis of Principal Components, that is, the percent of individuals correctly grouped to their true sampling location during the initial steps of the Discriminant Analysis of Principal Components analysis to minimize variance, was 85.7%.

The assignment test correctly assigned individuals to their location of origin 73% (30/41) of cases (Table 12). The number of incorrectly assigned samples was significantly different among sampling sites (Fisher's Exact test, P=0.001), with most incorrectly assigned individuals originating from Elk River. Five out of six individuals incorrectly assigned from Elk River were males, but overall there was no difference in

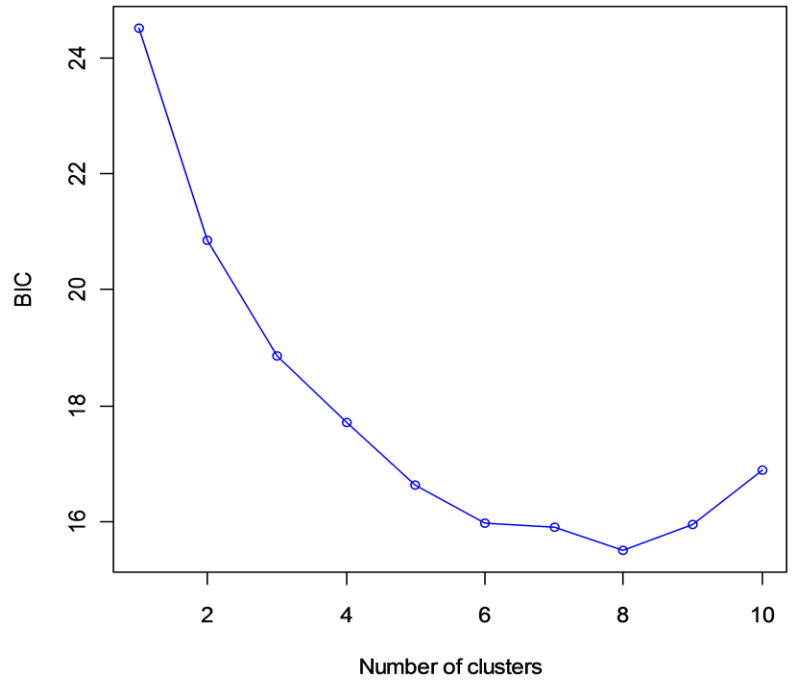


Figure 6. Bayesian Information Criterion (BIC) values for models with increasing clusters to determine the number of genetically differentiated groups derived from multi-locus genetic data of 41 river otters in the Humboldt Bay region, California, USA, from 18 May-31 October 2008.

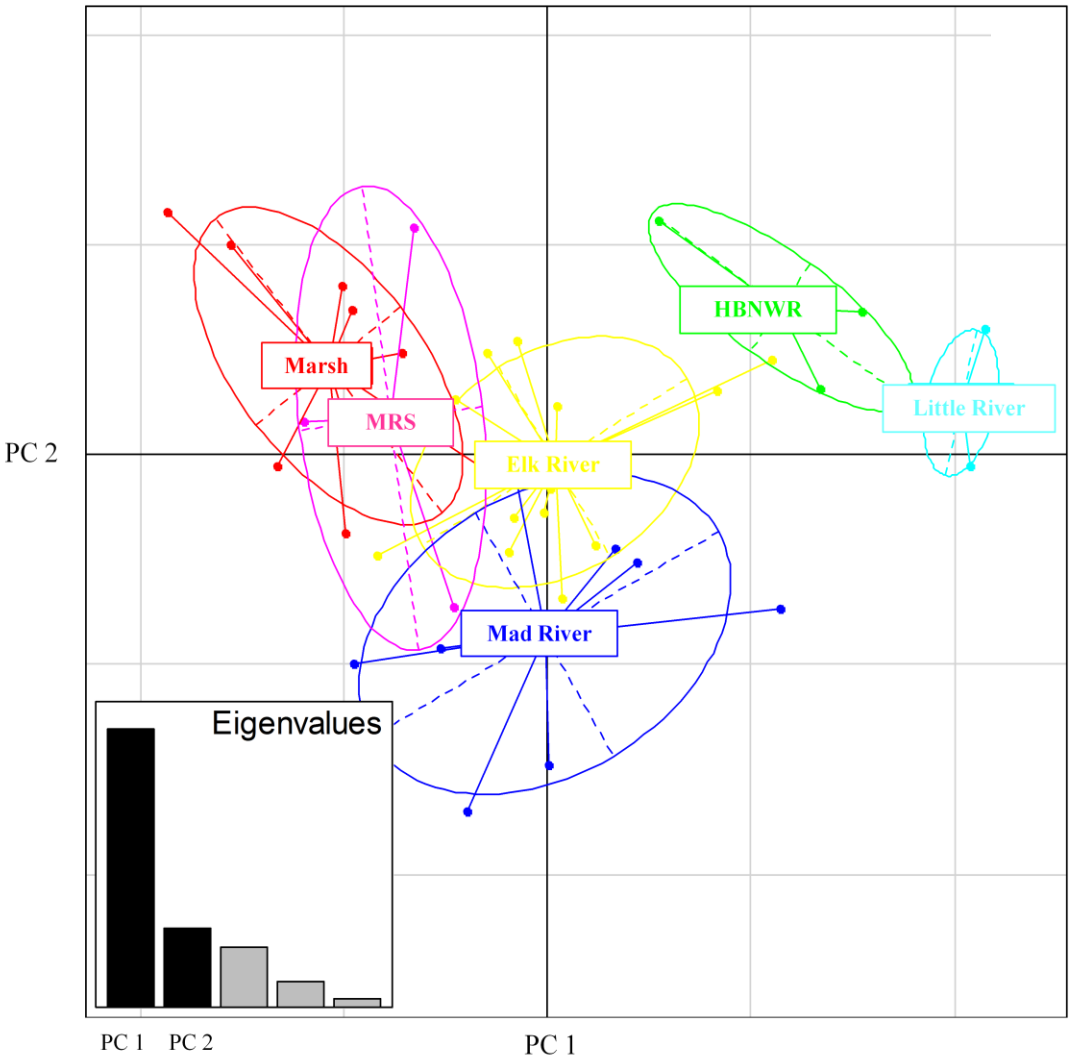


Figure 7. Discriminate Analysis of Principal Components constructed from 41 river otter genotypes determining genetic differentiations between locations in the Humboldt Bay region, California, USA, from 18 May-31 October 2008. The locations from north to south were Little River, Mad River, Arcata Marsh Wildlife Sanctuary (Marsh), Mad River Slough (MRS), Elk River, and Humboldt Bay National Wildlife Refuge complex (HBNWR). Eigenvalues (insert) show the majority of variance was captured within the first and second principal component (PC).

Table 12. The number of correct (assigned same location as sampled) and incorrect (assigned different location than sampled) assignments based on log-likelihood values for 41 river otter genotypes in the Humboldt Bay region, California, USA, from 18 May-31 October, 2008.

Location*	Correctly assigned	Incorrectly assigned	Location incorrectly assigned (# samples)
Little	4	0	
Mad	7	1	MRS (1)
AMWS	7	2	MRS (2)
MRS	1	2	AMWS (1), Elk (1)
Elk	8	5	Little (1), Mad (1), MRS (2), AMWS (1)
HBNWR	3	0	

*Sites from north to south were Little River (Little) Mad River (Mad), Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Elk River (Elk), and Humboldt Bay National Wildlife Refuge (HBNWR).

sex of incorrectly and correctly assigned individuals (Fisher's Exact test, P=0.45).

River otters were more related to individuals within sampling locations compared with individuals among all sampling sites (Tables 13, 14). Differences were significant for all sites except for Mad River Slough and Elk River (Table 14). When roaming individuals, i.e., genotypes detected at more than one site, were removed from the analysis, there was little change in R-values, except for the Elk River site (Figure 8). At Elk River, within site relatedness became significantly greater compared with individuals among all sites (P=0.043) and the mean R value, although still low, increased (from 0.03 to 0.10). After removing roaming individuals, Mad River Slough only had two genotypes left making bootstrapping impossible, thus it was removed from the analysis. Relatedness among males and among females (analyzed separately) was low and there was no difference within sex compared to between sex R values (Figure 9). I was unable to compare R values between males and females within sites due to small sample sizes. Isolation by distance regression with R values was not significant (Mantel Test, correlation statistic=0.02, P=0.439, R^2=0.35%, Figure 10a). When comparisons involving Little River were removed from the analysis, there was a significant positive correlation between geographic and genetic distance (Mantel test, correlation statistic=-0.64, P=0.035, R^2=41.10%; Figure 10b).

Table 13. Relatedness coefficients (*R*) between study locations (within locations on diagonal) in the Humboldt Bay region, California, USA, calculated from river otters non-invasively sampled 18 May-31 October 2008.

Sampling location*	Little	Mad	AMWS	MRS	Elk	HBNWR
Little	0.2944	--	--	--	--	--
Mad	0.0478	0.3088	--	--	--	--
AMWS	-0.2942	-0.0552	0.1563	--	--	--
MRS	-0.3153	0.0728	0.1905	0.0698	--	--
Elk	-0.0943	0.0128	-0.0949	-0.0783	0.0319	--
HBNWR	0.2626	-0.0774	-0.2314	-0.2988	-0.1332	0.5860

*Sites from north to south were Little River (Little) Mad River (Mad), Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Elk River (Elk), and Humboldt Bay National Wildlife Refuge (HBNWR).

Table 14. Mean coefficient of relatedness (Queller and Goodnight's *R*-values from GenAlEx) within and between study areas for river otter genotypes detected in the Humboldt Bay region, California, USA, from 18 May-31 October 2008.

Location*	Mean within	Mean between	*P*
Little	0.294	-0.079	0.044
Mad	0.310	0.000	0.001
AMWS	0.156	-0.097	0.022
MRS	0.070	-0.086	0.330
Elk	0.032	-0.078	0.136
HBNWR	0.586	-0.096	0.009
Mean	*0.241*	*-0.072*	

*Sites from north to south were Little River (Little) Mad River (Mad), Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Elk River (Elk), and Humboldt Bay National Wildlife Refuge (HBNWR).

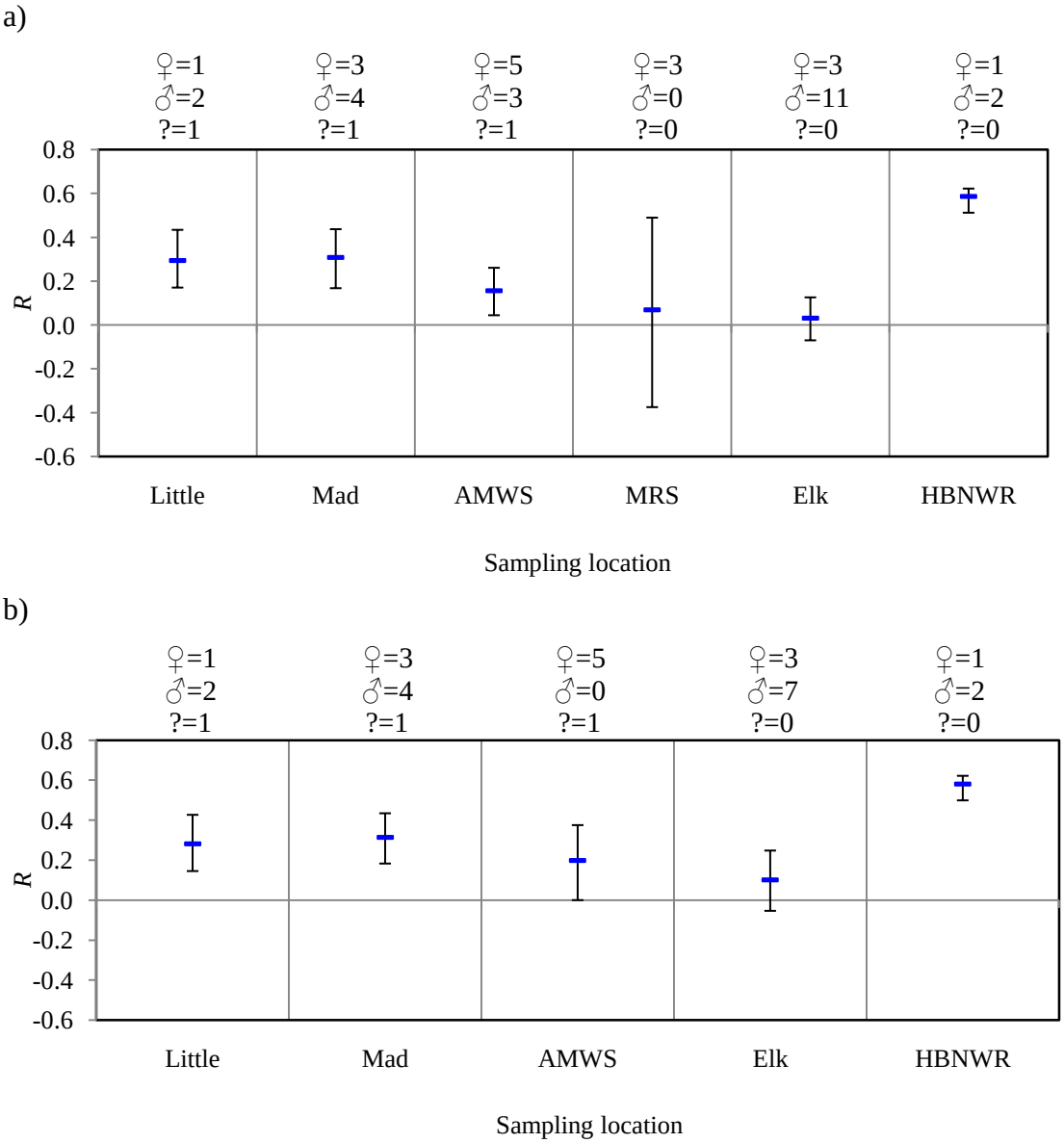


Figure 8. Mean relatedness values (Queller and Goodnight's *R* values from GenAlEx) within sampling locations for (a) all river otters sampled and (b) for only river otters that exhibited no movement between sites, in the Humboldt Bay region, California, USA, from 18 May-31 October 2008. Sites from north to south were Little River (Little) Mad River (Mad), Arcata Marsh and Wildlife Sanctuary (AMWS), Mad River Slough (MRS), Elk River (Elk), and Humboldt Bay National Wildlife Refuge (HBNWR). Upper and lower whiskers denote 95% confidence limits determined by 999 bootstrap resampling. Female (♀), male (♂), and unknown sex (?) sample sizes displayed above sampling sites.

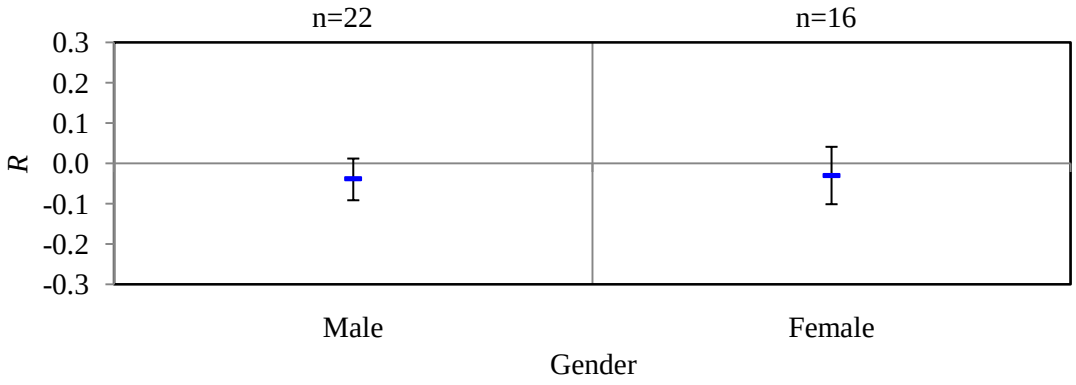
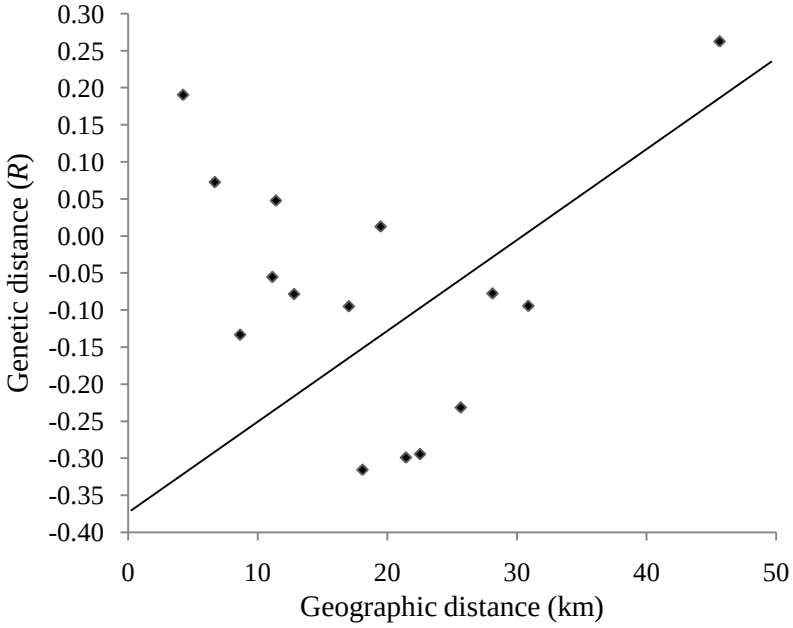


Figure 9. Mean relatedness values (Queller and Goodnight's *R* values from GenAlEx) for male and female river otters sampled in the Humboldt Bay region, California, USA, from 18 May-31 October 2008. Upper and lower whiskers denote 95% confidence limits determined by 999 bootstrap resampling.

a) All sampling locations



b) Little River removed

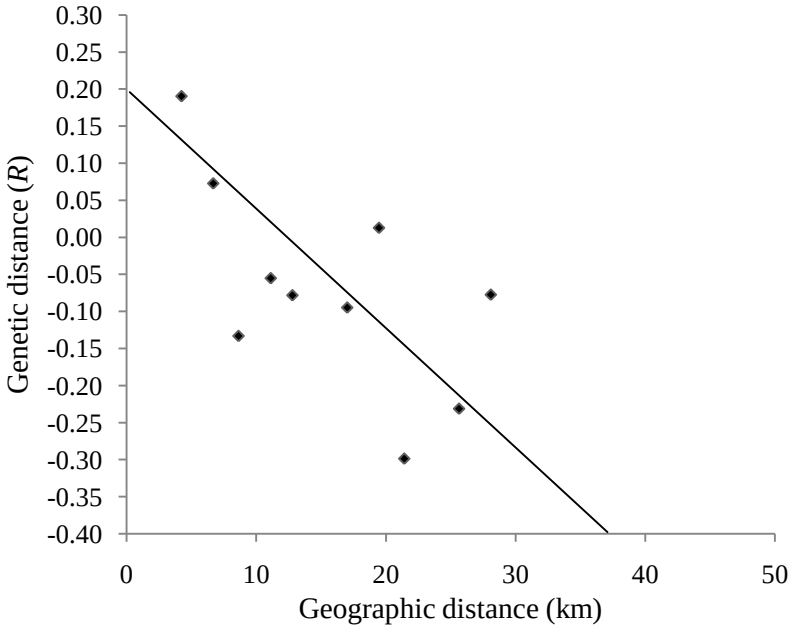


Figure 10. A test of isolation by distance for river otters detected at all sampling locations (a) and all locations but Little River (b) in the Humboldt Bay region, California, USA from 18 May-31 October 2008. Genetic distance was measured by *R* values and geographic distance was measured in linear distance (km).

DISCUSSION

Non-invasive genetic surveys are an effective way to monitor elusive carnivores (Banks et al. 2003, Ruell et al. 2009, Beja-Pereira et al. 2009). Even with relatively low genotyping success, by employing an extensive sampling regime I was able to census almost all Humboldt Bay area river otters. This conclusion was supported by the two modeling efforts as the confidence intervals encompassed the total number of unique genotypes and by the lack of new genotype detections in October suggesting most individuals were sampled before the end of the study (Table 11, Figure 3). The ability to capture a majority of individuals non-invasively was possible because of the ease of sampling latrines, which serve as a communicative tool integral in social interactions of river otters and are therefore frequently used (Hornocker et al. 1983, Kruuk 1992, Melquist et al. 2003, Rostain et al. 2004, Oldham and Black 2009). Based on these results, non-invasive sampling during summer months can provide sufficient data for population estimates applicable for monitoring coastal California river otters, as well as other similar populations.

The 25.7% genotyping success for all samples herein was similar to published river otter fecal DNA studies (20% Dallas et al. 2003, 24% Kalz et al. 2006, 40% Prigioni et al. 2006), and as expected, was lower than other non-invasive genetic studies (Palomares et al. 2002, Oretga et al. 2004, Solberg et al. 2006). Overall genotyping error rates (average allelic drop out=28.63%, average false allele=1.00%) were also comparable to other non-invasive genetic studies that defined and calculated unbiased allelic drop out and false allele rates (Broquet and Petit 2004). Thus, my genotyping

error rates were within current literature trends acceptable for individual identification. With slightly higher $P_{(ID)sibs}$, there was not differentiation between at least two closely related individuals, but this was considered a less costly mistake than adding ghost individuals due to genotyping error (Mills et al. 2000, Waits and Leberg 2000). In mark/recapture studies built from genotypes, a shadow effect (when two individuals are lumped as one) may result in a lowered *N* but this bias tends to be small, unlike extraneous ghost genotypes which quickly inflate *N* (Mills et al. 2000, Waits and Leberg 2000). A conservative population estimate was more desirable for the purpose of evaluating Humboldt Bay river otters than grossly overestimating *N* with ghost individuals.

Unequal capture probability is another potential cause of underestimating abundance and is a long-standing issue for population studies (Otis et al. 1978). To account for unequal detection probabilities, I modeled five different scenarios of capture variation. Both capture history regimes' top models included temporal variation of capture probability between sampling sessions while holding equal capture and recapture probabilities. For the August-September capture history, the time varying model had overwhelming support with 85% of AIC_c weight. With relatively narrow confidence intervals encompassing the minimum number of genotypes detected (Table 11), this model performed well estimating abundance. The capture history from September alone produced very similar results, receiving 75% of AIC_c weight. Confidence intervals were larger, but overall this model also performed well with only one month of data (Table 11). Heterogeneity models (models that correct for initial capture probabilities differing

among individuals of a population) have been shown to limit error in other non-invasive studies (Mills et al. 2000, Solberg et al. 2006, Ruell et al. 2009), but were the lowest ranking models in my analyses. This was due to strong temporal differences in capture probabilities, shadowing any other individual heterogeneity in capture histories.

The large temporal variation in capture probability could have been caused by two factors: either there was a difference in defecation rate among sampling occasions or a difference in genotyping success among sampling occasions. I suspect both of these factors played a role. Changes in latrine usage would indicate behavioral variation in scent marking by individuals over time. Several studies documented male and females river otters scent mark at the same rate (Dallas et al. 2003, Janssens et al. 2008). Similarly, I detected no difference between sexes in detection rates. Variation in genotyping success probably contributed to temporal differences in capture occasion considerably more than changes in defecation rates since genotyping success rates were low. Temporal variation could be due to a number of uncontrollable environmental factors. Non-invasive fecal studies have found that diet, time of collection, and weather conditions can affect amplification success and therefore encounter rates (Murphy et al. 2003, Nsubuga et al. 2004, Hajkova et al. 2006). These factors may have been negligible here since diet did not influence amplification rates. Samples were only collected in morning hours and the Pacific Northwest is a temperate region with comparatively little temporal variation in temperature. Laboratory methods could also account for some genotyping variation, but every measure was taken to treat samples identically and I was the only individual processing samples, which maintained consistency. Despite trying to

control these factors, a relatively extended mark/recapture sampling regime for a closed population model (i.e., longer than a few days or weeks), was necessary to capture the variation and accurately model detection probability, thereby providing unbiased *N* estimates.

Determining the length of the sampling period is critical for meaningful closed population modeling. It is important to create a sampling regime long enough to capture sufficient numbers of individuals in a population without violating geographic and demographic closure assumptions (White et al. 1982). Due to low genotyping success with river otters it can be difficult to achieve both of these at the same time. Given reasonable model estimates, strong support for top models, and considering both models had almost identical results, it appears population closure was maintained and an adequate number of individuals were sampled. Since the models performed so similarly, but the single month had wider confidence limits, I would suggest a 2-month sampling regime if time and money allow. Future non-invasive river otter studies focused on abundance estimates could sample intensively August and September, encompassing temporal changes in capture probabilities and producing river otter population estimates comparable to these results.

Based on minimum genotypes detected and the top confidence interval range from mark/recapture model estimates, there were between 41-51 river otters throughout the Humboldt Bay region. Initially, this appeared to be a large number of river otters in one bay as compared to other studies (Testa et al. 1994, Bowyer et al. 2003). Among several bays in Alaska, the highest density range was 64 river otters per 138.6 km of

linear coast line (0.46 river otter/km; Bowyer et al. 2003). In Humboldt Bay, I found a minimum of 41 river otters per 45 km of linear coast line (0.93 river otter/km). However, the inability to discriminate juveniles from adults inflated overall resident population estimates since some juveniles will disperse out of the area and would not become resident breeding adults. Considering this, the density of river otters in the Humboldt Bay area was likely similar to other coastal systems.

Evaluation of genetic structure was representative of a majority of Humboldt Bay river otters because a high proportion of the population was detected. Sample sizes were small but this was not due to a lack of data or poor sampling design. Rather, it was simply the number of animals in the study area. By utilizing several methods to assess genetic differentiation I could be confident in congruent results even with small sample sizes. Overall there was faint but detectable population structuring of river otters sampled at different geographic locations indicating the presence of social groups. The cluster analysis found 6-9 distinct groups, approximately matching the number of sampling locations. This was not a result of *a priori* population assignment because the cluster function does not incorporate location data. The assignment test also supported genetically differentiated groups with 73% of individuals correctly assigned to their location of origin. The Discriminate Analysis of Principal Components (Figure 7) showed some genetic overlap among the central locations. Most notably Mad Rive Slough overlapped with Mad River, Arcata March and Wildlife Sanctuary, and Elk River. Given the few individuals detected at Mad River Slough (n=3), large genetic overlap with neighboring sites, and low *R* values, it was not a distinct group. Remaining sites

displayed visible clustering, that based on relatedness values were likely related family groups.

Mad River, Arcata March and Wildlife Sanctuary, and Elk River had between 8-14 unique individuals (Table 8), a higher number than one solitary female and her pups (3-5; Melquist and Hornocker 1983). Give this, it is likely more than one family group used the same areas. Female-based family groups have been observed where mature female river otters will remain with or join a group composed of her mother and pups, even at times helping rear the young (Shannon 1989, Rock et al. 1994). *R* values at Mad River (*R*=0.3) and AMWS (*R*=0.2) were close to half-sibling values (*R*=0.25), suggesting social family groups were utilizing the same space and tolerant of spatial overlap with other potentially unrelated mature individuals. Little River and Humboldt Bay National Wildlife Refuge had fewer individuals detected, 4 and 3 respectively. Little River *R* values (*R*=0.3) were also close to half-sibling values and Humboldt Bay National Wildlife Refuge *R* values (*R*=0.6) were as high as full-sibling values (*R*=0.50). Based on the few genotypes detected and the high relatedness coefficients, these two sites most likely consisted of a single female and her pups. Solitary females have been found to be prevalent at the same time as more social river otters (Melquist and Hornocker 1983, Blundell et al. 2002a), so it is possible to have different social strategies observed within one region. I was unable to detect differences in relatedness between males and females due to small sample size and because of an inability to distinguish juveniles from adults. As a result, any potential difference between the sexes was masked by relatedness between parent-offspring dyads.

Melquist and Hornocker (1983) reported variation in daily movements of river otters, with the mean daily distance traveled being approximately 5 km or less. The largest daily movement detected in this study was individual Ott20, a male, traveling about 8.5 km over a 24-hr period as evidence by being detected at Elk River on 28 Sept and then Humboldt Bay National Wildlife Refuge on 29 Sept. Although there were other individuals detected at multiple sites (Table 7, Figure 5), Ott20 moved the most. All but one of the roaming river otters were males. Among otters, the formation of bachelor groups (large gregarious groups of unrelated males and occasionally non-reproductive females) is unique to North American river otters (Shannon 1989, Kruuk 1995, Blundell et al. 2002a, Hansen et al. 2009). Roaming river otters could be an indication of bachelor group formation, as there were five instances of two roaming males being detected at the same site on the same date. This suggests some degree of gregarious behavior and space sharing. Relatedness values varied between these pairs and several were found together repeatedly over a 4-month period (Table 8). Also, when roaming individuals were removed from relatedness analyses, the mean Elk River *R* value became greater, suggesting that roaming animals were not part of the Elk River family group. The spatio-temporal distribution of scat for a few roaming individuals supports the existence of at least short-term bachelor groups alongside larger related family groups.

All sites, except for Little River and Humboldt Bay National Wildlife Refuge, exhibited significant isolation by distance gene flow. The isolation by distance analysis showed relatedness values decreased as geographic distance between sites became larger. The likely presence of family groups could create significant isolation by distance gene

flow. Detected trends also explain river otter dispersal patterns, indicating that low rates of natal dispersal within Humboldt Bay may cause gene flow to be spatially restricted, as documented in other river otter populations (Melquist and Hornocker 1983, Blundell et al. 2002b). The presence of limited dispersal and gene flow has important conservation and management implications applicable to river otter populations elsewhere. Low levels of dispersal may decrease or slow natural recolonization if local populations decline or are extirpated. Limited gene flow increases genetic differentiation within populations so management actions involving river otter translocation should be undertaken cautiously to avoid loss of local genetic diversity (Blundell et al. 2002b).

Little River and Humboldt Bay National Wildlife Refuge complex were the most geographically distant sites and yet were more genetically similar to each other than to central groups. This is contradictory to the isolation by distance gene flow to which the rest of the sites conformed. One potential explanation for this could be rare dispersal events of genetically similar females that led to established home ranges at geographically distant sites. River otters display low levels of male-biased natal dispersal (Kruuk 1995, Blundell et al. 2002b, Dallas et al. 2003, Janssens et al. 2008). Females are mostly philopatric, but when dispersal events do occur, females have been reported to disperse larger distances than males, traveling up to 60-90 km (Blundell et al. 2002b), more than the distance between Little River and Humboldt Bay National Wildlife Refuge (~45 km). These two locations currently appear to each host one distinct family group, but it is possible that the female founding members of these small groups were related and separated due to a female dispersal event.

In general, the Elk River otters stood out among those at all other sites. Elk River had the highest number of animals detected, these individuals were less related to one another than river otters at other sites were to one another, and there was a trend of greater movement among individuals detected most often at Elk River. Five of the eight roaming river otters were either from Elk River or were detected at least once at Elk River (Table 7). Based on these various factors, there appears to be multiple, unrelated social groups utilizing Elk River. Even when roaming individuals were removed and relatedness values within Elk River became significantly greater than between sites, the mean *R* value was still much lower than other sites (Figure 10). This could be possible if more than one unrelated family group utilized Elk River at the same time. The Elk River travels inland, providing more up-river habitat for river otters to utilize, which may contribute to the patterns detected at the site. It is also possible that the area served as a corridor between northern and southern Humboldt Bay.

Mark/recapture models using non-invasive sampling were an effective way to estimate river otter abundance in the Humboldt Bay area. A two-month long sampling regime during summer was sufficient for abundance estimates, although with exhaustive sampling over 4.5 months, I was able to capture and genotype the majority of the population and map movements of individual otters. Thus, I could evaluate structure and social organization of these coastal river otters. Overall there was evidence for fine-scale population structuring that was most likely a function of social family groups. There was also support for some degree of bachelor group formation. These data suggest that contemporary patterns of gene flow within Humboldt Bay may be geographically

restricted, creating genetic population structure. Understanding population social structure, gene flow, and local behavioral adaptations is crucial for improving ecological knowledge of river otters as well as properly managing this elusive species at a regional level. The Humboldt Bay system highlights the fine-scale structuring and complex social behavior river otters exhibit across their range. I would recommend these methods and models to others interested in examining populations of river otters for management of wetland ecosystems in a changing world.

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Appendix A. Universal Transverse Mercator (UTM) coordinates of river otter latrine sites sampled 18 May-31 October 2008 in Humboldt Bay, California, USA.

Sampling Site	Latrine	Easting	Northing
Little River	flatrock	406709	4542435
Little River	rock1	406717	4542432
Little River	rock2	406613	4542504
Little River	rock3	406706	4542435
Mad River	pumpinglog	411905	4528818
Mad River	pumpingden	411215	4529121
Mad River	tyeecitylog1	404977	4530885
Mad River	tyeecityslide	404979	4530877
Mad River	tyeecitylog2	404937	4530954
Mad River	boatramp	404876	4531475
MRS	slide1	403271	4524579
MRS	slide2	403351	4524666
MRS	slide3	403366	4524735
MRS	lamphere	403705	4512036
AMWS	kloppslide	407536	4523432
AMWS	kloppculvert	407971	4523263
AMWS	grassyknoll	407297	4523914
AMWS	hauserroad	407614	4523739
AMWS	gallenrr1	407957	4523616
AMWS	gallenrr2	407893	4523814
Woodley Island	WIdock1	402196	4518122
Elk River	tressel	399282	4512340
Elk River	parkinglot1	399340	4512697
Elk River	parkinglot2	399347	4512722
Elk River	elkriver101	399448	4512328
HBNWR	barn1	397800	4504677
HBNWR	bridge1	397246	4504528
HBNWR	bridge2	396868	4504640
HBNWR	birdblind	397883	4504537
HBNWR	HSdock	396739	4503672
HBNWR	HSculvert	396479	4503993

Appendix B. Multi-locus microsatellite genotypes (N=41) for 6 loci and sex type derived from DNA extracted from non-invasive sampling of river otters in Humboldt Bay, California, USA, from 18 May-31 October 2008.

Sample	Site	Lut453		Lut733		Rio08		Lut701		Rio18		Lut604		Sex
ott3	Little	150	150	171	174	217	223	194	194	172	172	139	139	M
ott12	Little	146	150	171	174	217	217	194	198	156	172	139	139	F
ott15	Little	146	-	179	179	217	217	-	-	156	172	139	139	U
ott35	Little	146	150	171	179	217	223	194	202	156	172	139	139	M
ott2	Mad	150	150	174	174	217	-	194	194	162	172	-	-	F
ott4	Mad	146	150	174	179	217	219	194	198	156	156	139	139	F
ott7	Mad	146	150	174	174	217	217	194	202	156	162	-	139	F
ott9	Mad	150	150	174	174	217	217	194	-	156	162	133	139	M
ott28	Mad	146	150	171	174	217	223	202	206	156	156	133	139	M
ott29	Mad	146	150	174	-	-	-	194	194	156	156	133	133	U
ott31	Mad	146	150	174	174	217	217	202	206	156	156	133	133	M
ott32	Mad	146	150	171	174	217	217	194	202	156	172	133	133	M
ott5	AMWS	142	150	171	174	217	219	194	194	156	162	133	139	F
ott10	AMWS	142	142	174	179	219	223	194	202	156	156	133	139	F
ott16	AMWS	146	150	171	179	219	219	194	194	156	156	133	139	M
ott19	AMWS	142	146	174	179	217	219	194	194	156	162	133	133	F
ott21	AMWS	150	150	171	179	219	219	194	194	156	156	133	139	F
ott27	AMWS	144	150	174	174	217	217	194	198	156	156	139	139	M
ott33	AMWS	142	150	174	179	217	219	194	202	156	162	133	139	M
ott36	AMWS	142	142	174	174	-	-	194	-	156	-	133	139	U
ott37	AMWS	142	150	174	174	217	223	202	202	156	156	133	139	F
ott6	MRS	142	144	174	179	217	219	198	202	156	156	139	139	F
ott11	MRS	142	150	174	179	217	219	194	202	156	156	133	139	F
ott18	MRS	146	150	174	174	217	223	194	194	156	156	139	139	F
ott1	Elk	146	150	174	179	217	223	194	194	156	162	139	139	M
ott8	Elk	142	146	171	174	217	223	194	194	156	162	133	139	M
ott13	Elk	142	146	174	179	-	-	194	202	172	-	133	139	F
ott14	Elk	146	150	171	174	223	223	194	194	156	172	139	139	M
ott17	Elk	146	150	171	174	223	223	194	194	156	156	139	139	M
ott20	Elk	144	150	174	174	217	217	198	202	156	156	139	139	M
ott23	Elk	142	146	174	179	-	-	194	202	156	156	133	-	M
ott24	Elk	146	146	171	174	223	223	194	194	156	156	139	139	M
ott26	Elk	146	150	171	174	217	223	194	194	156	162	133	133	M
ott30	Elk	146	146	171	174	217	-	194	194	156	162	133	133	F
ott34	Elk	144	-	174	174	217	217	198	202	156	162	-	-	M
ott39	Elk	142	150	174	174	217	217	194	198	156	162	139	139	M
ott40*	Elk	146	150	171	179	219	219	194	202	156	156	139	139	M
ott41*	Elk	146	150	171	179	219	219	194	202	156	156	139	169	F
ott22	HBNWR	150	150	171	179	217	-	194	198	156	172	133	139	M
ott25	HBNWR	150	150	171	174	217	223	194	206	156	172	139	139	M
ott38	HBNWR	150	150	171	171	223	223	194	194	156	156	139	139	F

*Dyad with same multi-locus genotype, but different sex typing. "-" indicates a non-confirmed allele.

Appendix C. River otters found at more than one sampling location, sex type, and dates detected at all sites in Humboldt Bay, California, USA, from 18 May-31 October 2008. No roaming river otters were detected at Little River.

Animal code	Sex	Mad	AMWS	MRS	Woodley	Elk River	HBNWR
Ott16	M		10/27	7/1			
Ott27	M		8/25			10/19	
Ott33	M	10/20	8/6	9/29			
Ott18	F			7/28			7/1
Ott1	M					5/27, 8/28	7/27, 9/15
Ott8	M				7/6, 7/7, 8/27	8/19, 9/8, 10/13	
Ott20	M		8/25, 10/26		7/7, 9/23	8/19, 9/28, 10/5, 10/13	9/29
Ott40	M					9/8	10/26

Appendix D. Capture probability estimates (*p*), standard error (SE), and 95% confidence limits (CI) estimated from closed population models as determined by program MARK for river otters sampled in the Humboldt Bay region, California, USA, from 1 August-30 September, 2008. The top section is for capture histories combining August and September (9 capture occasions); the bottom section is only for September (5 capture occasions).

Capture occasion	*p*	SE	Lower CI	Upper CI
August and September				
1	0.06	0.04	0.01	0.21
2	0.06	0.04	0.01	0.21
3	0.27	0.08	0.14	0.45
4	0.21	0.07	0.10	0.39
5	0.06	0.04	0.01	0.21
6	0.39	0.09	0.23	0.58
7	0.21	0.07	0.10	0.39
8	0.18	0.07	0.08	0.35
9	0.24	0.08	0.12	0.42
September only				
1	0.06	0.04	0.01	0.22
2	0.40	0.11	0.22	0.62
3	0.22	0.08	0.10	0.41
4	0.19	0.08	0.08	0.38
5	0.25	0.09	0.12	0.45