River Otters (*Lontra canadensis*) in Southcentral Alaska: Distribution, Relative Abundance, and Minimum Population Size Based on Coastal Latrine Site Surveys

Final Report

July 30, 2009

Merav Ben-David
University of Wyoming
Department of Zoology and Physiology
1000 E. University Ave.
Laramie, WY 82071
BenDavid@uwyo.edu

Howard Golden
Alaska Department of Fish and Game
Division of Wildlife Conservation
PO Box 25526
Juneau, Alaska 99802
howard.golden@alaska.gov

RM-CESU Cooperative Agreement Number: H1200040001

Funding & Support Provided by:
National Park Service, Inventory & Monitoring Program
National Science Foundation
US Forest Service, Chugach National Forest
Oil Spill Recovery Institute
University of Wyoming, NSF-EPSCoR program
Alaska Department of Fish and Game

File Name: BenDavidM_2009_SWAN_RiverOtter_FinalRpt.doc

Recommended Citation:
Ben-David M. and H. N. Golden. 2009. River Otters (*Lontra canadensis*) in Southcentral Alaska: Distribution, Relative Abundance, and Minimum Population Size Based on Coastal Latrine Site Surveys. SWAN I&M program report, National Park Service. Anchorage, AK. 43 pg.

Topic(s):
Inventory and Monitoring, Biological

Theme Keywords:
Lontra Canadensis, river otter, density estimation, mark-recapture, latrine, feces, DNA analysis, genetic monitoring.

Placename Keywords:
Alaska, Southcentral Alaska, Kenai Peninsula, Prince William Sound, Kenai Fjords National Park, Katmai National Park and Preserve, Lake Clark National Park and Preserve, Kodiak Island Archipelago, Upper Cook Inlet.

Acronyms:
EVOS Exxon Valdez Oil Spill
GIS Geographic Information System
I&M Inventory & Monitoring Program
KATM Katmai National Park
KEFJ Kenai Fjords National Park and Preserve
KOD Kodiak Island Archipelago
LACL Lake Clark Nationa Park and Preserve
NPS National Park Service
PWS Prince William Sound
SWAN Southwest Alaska Inventory & Monitoring Network

Initial Distribution:
Kenai Fjords National Park– 1 hardcopy, 1 electronic
Southwest Alaska Network – 1 hardcopy, 1 electronic

Abstract

In the aftermath of the Exxon Valdez oil spill (*EVOS*), studies of coastal river otters (*Lontra canadensis*) in PWS indicated they are a keystone species for the land-margin ecosystem and a sentinel species for monitoring levels of environmental contamination. In 2004-2007, we surveyed latrine sites and used DNA fingerprinting to establish baseline information on the distribution, relative abundance, and minimum number of river otters alive in KEFJ, KATM, LACL, PWS, and KOD, Alaska. We also assessed connectivity and geneflow among these otter populations and identified parameters that may contribute to differences in abundance and distribution of river otters in these areas. We surveyed approximately 2,000 km of shoreline and sampled fresh feces from

641 active otter latrines. Each site was visited between 1 and 9 times during the sampling periods. Fecal DNA analyses revealed that all populations were genetically distinct as expected from isolation by distance models, although otters inhabiting KOD were isolated from their mainland conspecifics in KATM despite the relatively short distance between these 2 shorelines. Latrine density (sites/km) varied from 0.20 to 1.90 and fecal deposition rate (feces/site/day) ranged from 0.82 to 4.77. The naïve density ranged from 0.07 known individuals per km to 0.68 otters per km. Finally, we found no relation between latrine density and fecal deposition rate and the minimum number of otters known alive.

Executive Summary

This report summarizes the activities in calendar years 2004 – 2009 of a multi-agency collaborative project. Surveys in Kenai Fjords National Park (KEFJ), Katmai National Park and Preserve (KATM), Lake Clark National Park and Preserve (LACL), Prince William Sound (PWS) and the Kodiak Island Archipelago (KOD) were conducted under separate study plans and funding, but utilize the same methodologies to meet similar goals. All projects and datasets are presented here for comparative purposes and to better illustrate the regional scale of this effort.

The goals of this study were: : (1) to establish baseline information on the distribution, relative abundance, and minimum number known alive of river otters in KEFJ, KATM, LACL, PWS, and KOD using latrine site surveys and DNA fingerprinting of fecal samples; (2) to conduct formal mark recapture analysis for estimating numbers of river otters in KEFJ and PWS from DNA fingerprinting of fecal samples; (3) compare patterns of abundance and distribution of river otters in KEFJ, KATM, LACL, PWS and KOD; (4) assess the relation between formal population estimates and indices of abundance (distribution and use of latrine sites and the deposition rates of scats); and (5) identify parameters that may contribute to differences in abundance and distribution of river otters among the 5 areas.

Survey of the coastline for latrine sites in KEFJ was conducted in July 5-10, 2004 and June 25-29, 2005. In 2006, a survey of random sites was conducted from June 20-24 in that location. In KATM, latrine site survey was conducted in July 2-10, 2005, and in LACL in July 25-29, 2006. In PWS, a sound-wide survey was conducted in August 9-21, 2004 and a more localized survey in Herring Bay, Lower Passage, and Eleanor Island from May 25 – August 15, 2006. In KOD survey was conducted 13–25 June, 2007. The length of shoreline surveyed at each location ranged from 60-945 km and the number of occasions varied from 1 in KEFJ in 2004, KATM in 2005, and KOD in 2007, to 9 in PWS in 2006. In PWS 2004 a second occasion occurred 48 hours after the first; in KEFJ 2005 a second occasion occurred 24 hours after the first. In PWS 2006 the interval between occasions was 5-7 days.

Latrine density (sites/km) varied from 0.35 to 1.90 and fecal deposition rate (feces/site/day) ranged from 0.82 to 4.77. Between 22 – 65% of collected samples yielded DNA. Higher yield occurred in PWS 2006 and KOD 2007. Our analyses indicate that to reduce time, effort, and cost associated with amplifying poor-quality samples,

observers should preferentially collect samples that contain anal gland secretions. Also, any sample should be discarded if it does not amplify after three PCRs with the most reliable primers, and one or more of the following conditions apply: 1) it contains parasites, 2) it contains remains of Cottidae or Pholidae, or 3) it was collected when ambient temperatures exceeded 16˚C. There was no observer bias in the identification of samples that would yield DNA. Also, none of the habitat features that we measured could explain genotyping success or failure. Thus, it will be possible to train novice observers to identify otter latrines based on habitat features as well as to collect high quality samples.

Of the 2,553 fresh otter feces we collected, 603 yielded consensus genotypes at 7 or more loci (KEFJ – 120 samples; PWS – 374; KATM – 15; KOD – 94). These samples represented 422 unique individuals (KEFJ – 103 samples; PWS – 237; KATM – 12; KOD – 82). The probability of obtaining an incorrect multilocus genotype after replication at all eight microsatellite loci ranged from 0.0004 to 0.009 across all populations. Allelic richness and heterozygosity were high in all areas except KOD. The probability that two individuals drawn at random from a given population share identical genotypes at all loci was low (KEFJ – 1 in 15,948,963; PWS – 1 in 7,575,758; KATM – 1 in 333,333; KOD – 1 in 38,760). Except for PWS 2006, recapture rate of individuals was low and most recaptured individuals were detected on the same day, precluding the calculation of formal abundance estimates. In PWS in 2006 we identified 58 individuals that were encountered between two and eight times and additional 73 individuals that were observed only once yielding an estimate of 123 (± 29) river otters (or 1 otters per 1.18km of shoreline). Our analyses revealed that a minimum of 6 occasions will be required for obtaining unbiased and precise population estimates for coastal river otters. Finally, an analysis combining the data from all subpopulations of otters in this study and a companion study in British Columbia indicated no relation between latrine density and fecal deposition rate and the minimum number of otters known alive. Thus, the status of river otter populations could not be assessed accurately with indices such as latrine density and/or fecal deposition rate.

Our results also indicate that otters in southcentral and southwest Alaska belong to genetically distinct populations with isolation by distance as the main mechanism leading to differentiation. Nonetheless, despite the relatively short distance between the Kodiak Island Archipelago and the Alaska Peninsula (approximately 50 km away), KOD animals appear to be as isolated genetically from their mainland conspecifics as otters inhabiting PWS are from those from British Columbia. Our results also indicate that KATM and KOD otters likely differentiated from one ancestral stock that inhabited the Pleistocene southwestern shores of Alaska, and was isolated from other more easterly populations by distance. In addition, although the straight line distance between KEFJ and KATM is shorter (160 km) than that between PWS and KATM (325 km), animals from the latter appeared to genetically cluster with those from PWS. Also, estimated migration rates between these 2 populations were slightly higher and the pairwise F_{ST} value slightly lower than those between KEFJ and KATM. Although geneflow between KEFJ and PWS was higher than among other populations, none of the KEFJ individuals was mis-assigned to the PWS-KATM cluster with probability greater than 0.5 and only 16 individuals from PWS (or 6.7%) were mis-assigned to the KEFJ cluster with

probability greater than 0.7. Finally, our analyses indicate that otters in KEFJ belong to two sub-populations, with one cluster assigned to the coast between Resurrection Bay and Harris Bay and the second found along the shores of McCarthy Fjord and Nuka Bay. Thus, the high levels of population structuring among and within southwestern and southcentral Alaska should be incorporated into future management decisions of this species and its habitat.

Our inventory surveys of the coastline in KEFJ, KATM, and LACL, highlighted the differences in habitat availability for river otters. Much of the coastline in LACL consists of muddy tidal flats that are selected against by river otters. In our survey of the shoreline we found few latrines and none of the samples we collected yielded viable DNA. Nonetheless, our results suggest that this area may be an important corridor for geneflow among otter populations in southcentral and southwest Alaska, and thus should be afforded high levels of protection.

Introduction

In the aftermath of the Exxon Valdez oil spill (EVOS), studies of coastal river otters (*Lontra canadensis*) in Prince William Sound (PWS) indicated they are a keystone species for the land-margin ecosystem and a sentinel species for monitoring levels of environmental contamination (Bowyer et al. 2003). River otters in coastal environments of Alaska tend to select old-growth forest habitats close to the shore, where their chief food items are marine bottom-dwelling fishes (Larsen 1983; Bowyer et al. 1994). The effects of oil contamination and logging on habitat use, movements, and food habits of river otters indicate these animals are sensitive to disturbance by humans (Bowyer et al. 2003). Because of their role as keystone species for the land-margin, responses of river otter to human disturbances, such as oil contamination, logging, harvest, construction of dwellings, and heavy recreational use, should be addressed. Also, river otters in coastal Alaska rely on nearshore resources similar to sea otters (*Enhydra lutris*) and seals (*Phoca vitulina*) and thus represent a reliable model for the responses of nearshore predators to human disturbance in coastal areas of Alaska. River otters have been identified as a vital sign by the Kenai Fjords National Park (KEFJ), Katmai National Park and Preserve (KATM), and Lake Clark National Park and Preserve (LACL) monitoring programs. The effects of human disturbances, such as logging, harvest, and construction of dwellings and other disturbances such as oil contamination and heavy recreational use on river otters within these park units should be evaluated. No information on the abundance and distribution of otters in the 3 areas was available before the initiation of this effort.

The goals of this study were:

1. To establish baseline information on the distribution, relative abundance, and minimum number known alive of river otters in KEFJ, KATM and LACL using latrine site surveys and DNA fingerprinting of fecal samples.
2. To conduct formal mark-recapture analysis for estimating numbers of river otters in KEFJ and PWS from DNA fingerprinting of fecal samples.
3. Compare patterns of abundance and distribution of river otters in KEFJ, KATM, LACL, PWS, and KOD.

4. Assess the relation between formal population estimates and indices of abundance (distribution and use of latrine sites and the deposition rates of scats).
5. Identify parameters that may contribute to differences in abundance and distribution of river otters among the 5 areas.

River otters inhabiting marine environments deposit marine nutrients onto the land by marking specific locations along the shoreline with feces, urine, and anal gland excretions (Bowyer et al. 1995). Known as latrine sites, these areas are typically 10–50 m in radius and 25–700 m apart (ca. 160 latrines/ 100 km of shoreline). The location of latrine sites along the coast is dependent on several habitat variables related mainly to features of the intertidal zone, such as tidal slope, rock size, and extent of *Laminaria* sp. beds (Ben-David et al. 1996; 2005; Bowyer et al. 1995).

Nutrient transports by river otters to terrestrial landscapes can be significant. With densities ranging from 1 otter per 2.7 km (Melquist and Hornocker 1983) to 1 otter per 1.3 km of shoreline (Testa et al. 1994), 100 km of shoreline may receive from 536 to 1,112 kg of N per year. Assuming latrines are distributed every 700 m and are 50 m^2 in area (Swimley et al. 1998), N deposition will range between 0.075 and 0.16 kg $m^{-2}\,y^{-1}$. In comparison, atmospheric wet N deposition in Alaska ranges between 0.00001 and 0.0002 kg $m^{-2}\,y^{-1}$ (Lilleskov et al. 2001, http://nadp.sws.uiuc.edu). Similarly, river otter latrines may receive between 5 and 22 g $m^{-2}\,y^{-1}$ of phosphorus. For comparison, heath soils in northern Alaska contain 0.007–0.01 g m^{-2} P (Giblin et al. 1991). Our recent studies suggest that otter fertilization significantly alters plant community composition (Roe et al. *In prep*) and increases photosynthetic capacity of the overstory layer of coastal forests in the region (Roe et al. *In review*).

Bowyer et al. (1995) studied river otter habitat selection and home ranges in the marine environment of PWS following the oiling of portions of the sound from *EVOS*. Their habitat model showed that otters strongly selected areas of old-growth forest in both the oiled and nonoiled areas and preferred large rocks in oiled areas and shallower tidal slopes in the nonoiled area. Otters in the nonoiled area seemed to avoid commercially logged habitats, yet home ranges were about twice the size for otters in oiled areas. Bowyer et al. (1994) found significant declines in species richness and diversity of otter food items in oiled versus nonoiled areas. These data suggest that oil contamination can be detrimental to otters and cause a reduction in their population (Bowyer et al. 2003). Such reduction in otter numbers will likely reduce N and P fertilization of coastal forests. Monitoring population status of river otters in coastal environments may be crucial for evaluating the carbon sequestration capacity of Alaskan coastal forests.

In previous studies, density estimates of river otters have relied on home range calculations derived from radio telemetry data (Larsen 1983, Melquist and Dronkert 1987). Following *EVOS*, Testa et al. (1994) estimated otter densities in western PWS using a mark-recapture technique with scats containing radioisotopes (from implants placed in captured animals) supplemented with movement data from radiomarked animals. Their estimates were 36 to 42 otters/100 km of coastline for the oiled site at Herring Bay on Knight Island and 32 to 44 otters/100 km in the nonoiled site at Esther

Passage. However, the 95% confidence intervals for those estimates overlapped. The inaccuracy of using radio telemetry data alone and the restrictions of using radioisotopes in wild animals make these techniques unsuitable for current estimates of otter numbers.

Monitoring the status of river otter populations is challenging because their life history patterns make them difficult to survey using traditional mark-recapture methods (Williams et al. 2002). Current methods for monitoring relative abundance involve estimating the distribution and use of latrine sites (Bowyer et al. 2003) and the deposition rates of scats (i.e., scats deposited/site/day). Using these measures it could be possible to monitor population levels and trends among different areas of coastline as long as these indirect measures are correlated with formal population estimates. In addition, because the distribution and use of latrines depends on available habitat, habitat use and availability (as established by Bowyer et al. 1995) need to be measured concurrently with monitoring fecal deposition rates.

Modern techniques for extracting and analyzing DNA from river otter scats were recently developed (Hansen et al. 2008). These procedures use microsatellite DNA extracted from freshly deposited scats to identify individual otters based on DNA contained in cells sloughed from their intestinal lining (Hansen et al. 2008). Microsatellites are hypervariable, noncoding regions of short repeats within DNA that vary in size. They can serve as genetic markers because the regions may be amplified with specific microsatellite primers and their sizes compared among individuals with the aid of polymerase chain reaction products (Foran et al. 1997). Such methods provide identification of individual animals (Blundell et al. 2002; Hansen et al. 2008).

An increasing number of studies have successfully employed fecal DNA analyses to obtain formal population estimates for Eurasian otters (*Lutra lutra*; Dallas et al. 2003, Janssens et al. 2007, Prigioni et al. 2006), although few have been completed for North American river otters (Guertin et al. *In review*). Nonetheless, because fecal DNA analyses are expensive (estimated at $100 per sample after collection), developing protocols that will provide reliable indices of abundance is crucial for a relatively inexpensive monitoring program.

This report summarizes the activities in calendar years 2004 – 2009 of a multi-agency collaborative project. Surveys in Kenai Fjords National Park (KEFJ), Katmai National Park and Preserve (KATM), Lake Clark National Park and Preserve (LACL), Prince William Sound (PWS) and the Kodiak Island Archipelago (KOD) were conducted under separate study plans and funding, but utilize the same methodologies to meet similar goals. All projects and datasets are presented here for comparative purposes and to better illustrate the regional scale of this effort.

Methods

Latrine site surveys

We surveyed the KEFJ coastline for latrine sites during July 5-10, 2004 and June 25-29, 2005 (Figure 1). In 2006, we also conducted a survey of random sites in that location to assess habitat availability during June 20-24 (Figure 2). In KATM, a latrine site survey was conducted during July 2-10, 2005 (Figure 3), and in LACL during July 25-29, 2006 (Figure 4). In PWS, we conducted a sound-wide survey August 9-21, 2004 (Figure 5) and a more localized survey in Herring Bay, Lower Passage, and Eleanor Island between May 25 and August 15, 2006 (Figure 6). We surveyed latrines in KOD during 13–25 June, 2007 (Figure 7). The length of shoreline surveyed at each location ranged from 60-945 km and the number of occasions varied from 1 in KEFJ in 2004, KATM in 2005, and KOD in 2007, to 9 in PWS in 2006. We conducted a second sampling of latrine sites to measure scat deposition rates and collect fresh feces 48 hours after the first sampling occasion in PWS in 2004, 24 hours afterward in KEFJ in 2005, and 5-7 days afterward in PWS in 2006.

In all but the LACL and PWS 2006 surveys, a large vessel (M/V Serac in KEFJ and KATM, and the M/V Babkin in PWS and KOD) served as a mobile camp. The surveys in LACL and in PWS 2006 conducted from shore-based camps. Surveys were conducted with 2-3 skiffs and a crew of 7-10 people.

In all surveys, every effort was made to locate and visit all potential latrine sites. We recorded the location of each positive site (i.e., containing at least 10 total scats or new scats) with a handheld GPS unit. In PWS in 2004, in areas where surveys were conducted during previous efforts (northern Knight Island: Herring Bay and Lower Passage), Dangerous Passage (Jackpot, Ewan, and Paddy Bays and the western coast of Chenega Island), Esther Passage, Port Gravina, and Orca Bay (Olsen, Parshas, Sheep, Simpson, and Windy Bays), known latrines were re-visited and otter activity was evaluated.

Habitat features of latrines and random sites

At each new site in each area, habitat features within a 10-m radius of the main entrance from the water were evaluated and recorded following methods described by Bowyer et al. (1995; 2003). These included aspect, exposure to wave action, slope of the vegetated and tidal areas (in degrees), composition of intertidal substrate ranked based on percent cover of sand, gravel, small and large rock and bedrock, composition of vegetation cover based on percent overstory and understory vegetation and old growth trees, and potential burrow sites.

To estimate habitat availability in KEFJ, we sampled approximately 660 km of the Park coastline from Bear Glacier to Yalik Point excluding islands (Figure 2). To ensure that data collection on random sites was equivalent to that on latrines, we divided the coastline into 20-m segments or potential random sites. This resulted in 32,993 sites along the 660-km coastline. We estimated that we had sufficient funds and time to

sample approximately 400 of those sites. Because we lacked covariates to describe anticipated habitat variability along the coastline, we established a systematic array of evenly spaced groups of potential random sites. Each group contained 82 sites except the last group, which contained 29 sites. This resulted in the establishment of 403 groups out of the 32,993 potential random sites for an overall sampling effort of 1.2%. We used stratified-random sampling to select a single random site from within each group. To maintain consistent sampling probability, we added 53 ghost sites to the last group of 29 sites and randomly sampled from its new total of 82 sites. Although this created the potential for missing real sites from this segment, we did sample a site from this group. For each of these random sites, we measured the same habitat features estimated at latrines within a 10-m radius of the GPS location.

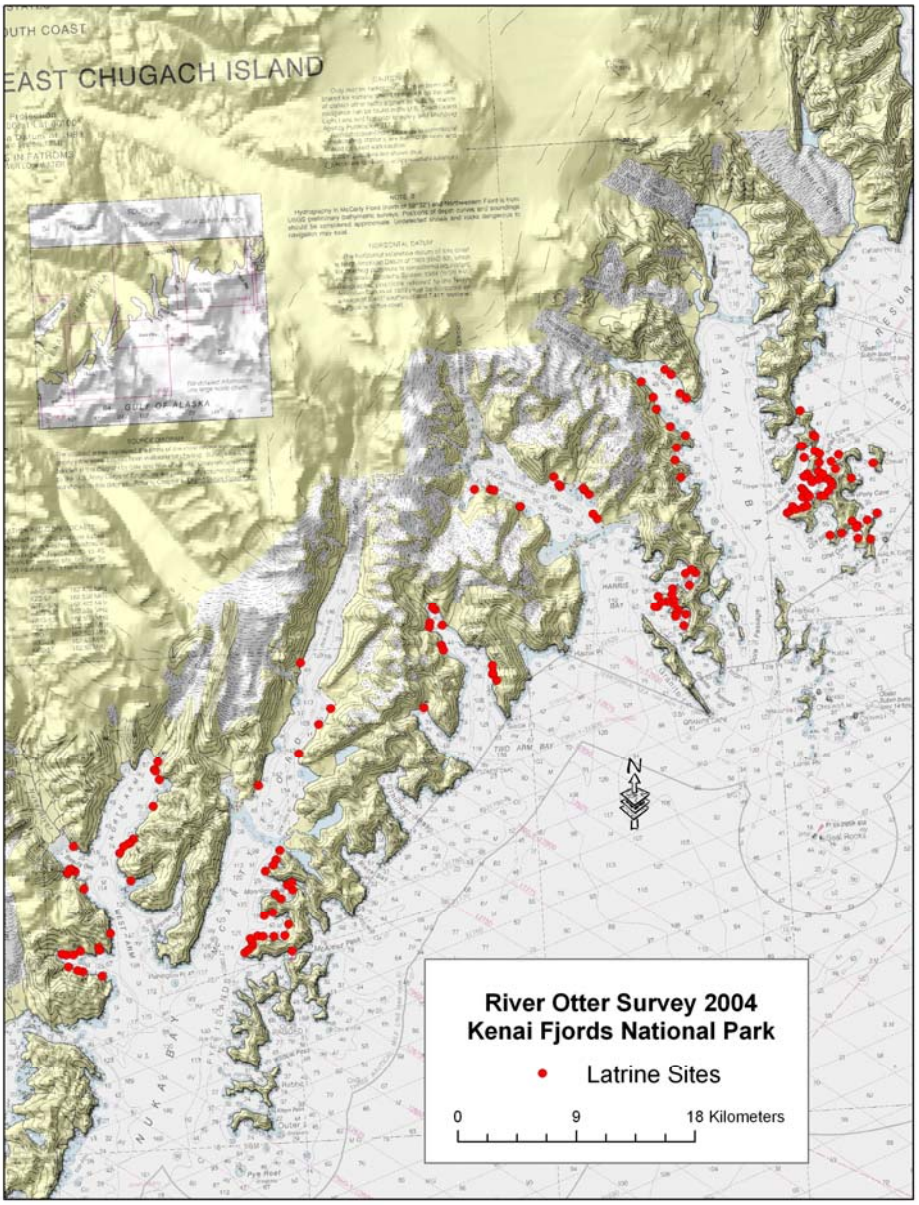


Figure 1. Distribution of river otter latrines along the coast in KEFJ. Survey was conducted July 5-10, 2004 and encompassed 354 km of shoreline. Ninety four of these sites were re-visited in 2005 from June 25-29. Each site was visited twice representing one occasion of "mark" and one of "recapture".

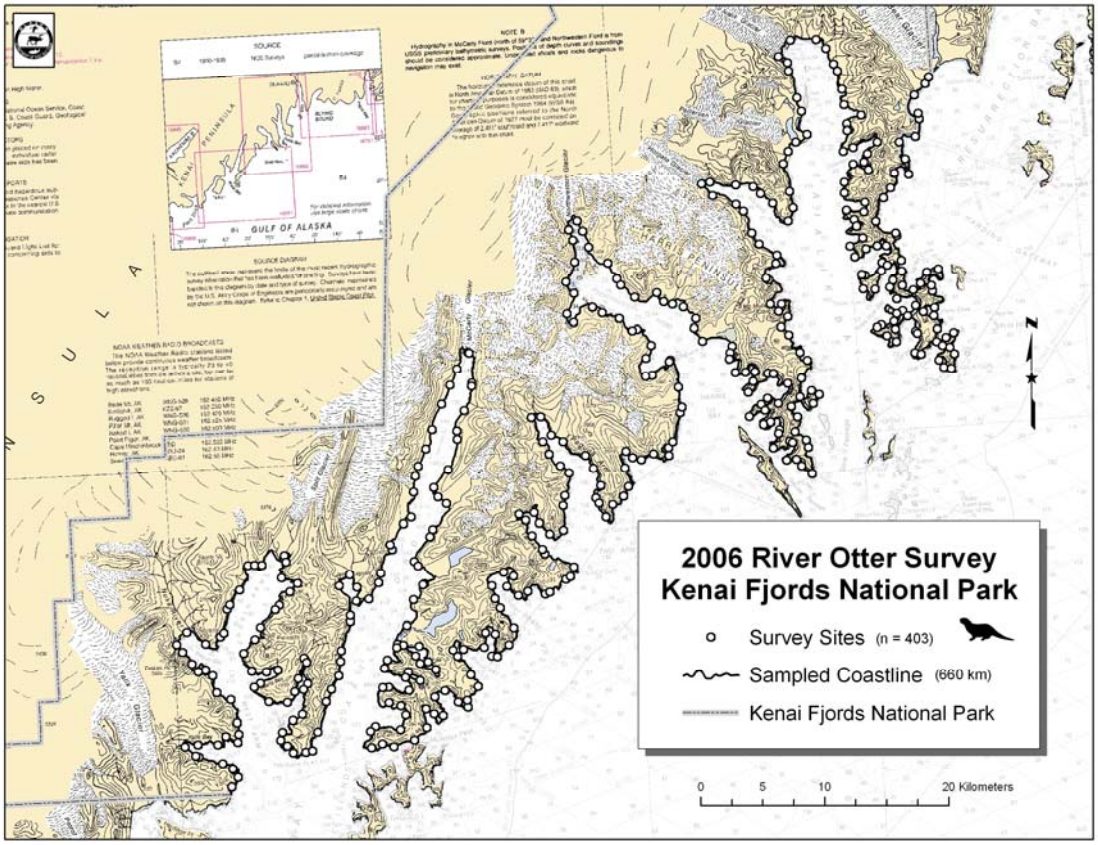


Figure 2. Location of random sites sampled for habitat features in KEFJ in June 2006.

Scat monitoring and collection

At each site, the number of feces was counted and all fresh feces, characterized by their distinct glossy appearance and strong smell, were collected and preserved in 100% ethanol (EtOH). Each sample was collected in a separate a 50-ml sterile tube. All samples were labeled and stored in coolers packed with glacial ice.

In those surveys where we conducted 2 or more sampling occasions, all scats found on the initial survey day were marked with colored glitter and any new unlabeled scats were counted in the following occasion. Relative abundance was measured by the distribution and number of active latrine sites and fecal deposition rate (Table 1). The latter was calculated based on the number of unmarked scats found on each site divided by the number of elapsed days between inspections (i.e., scats/ latrine site/ day).

DNA analyses

Prior to DNA extraction, we sieved each fecal sample through fine-mesh stainless steel, autoclavable sieves to ensure the removal of all hard parts of prey material. This is an important step for improving the quality and quantity of extractable DNA through reducing the extraction and amplification of non-target DNA. Sieving also helps avoid

the problem of the uneven distribution of cells shed through the intestinal lining as documented by Kohn et al. (1995). Excess EtOH was evaporated from each sample after sieving in a closed hood (Hansen 2004). Following the sieving, we used a 200 µl sub-sample to extract DNA with a QIAamp DNA Stool Mini Kit (Hansen et al. 2008).

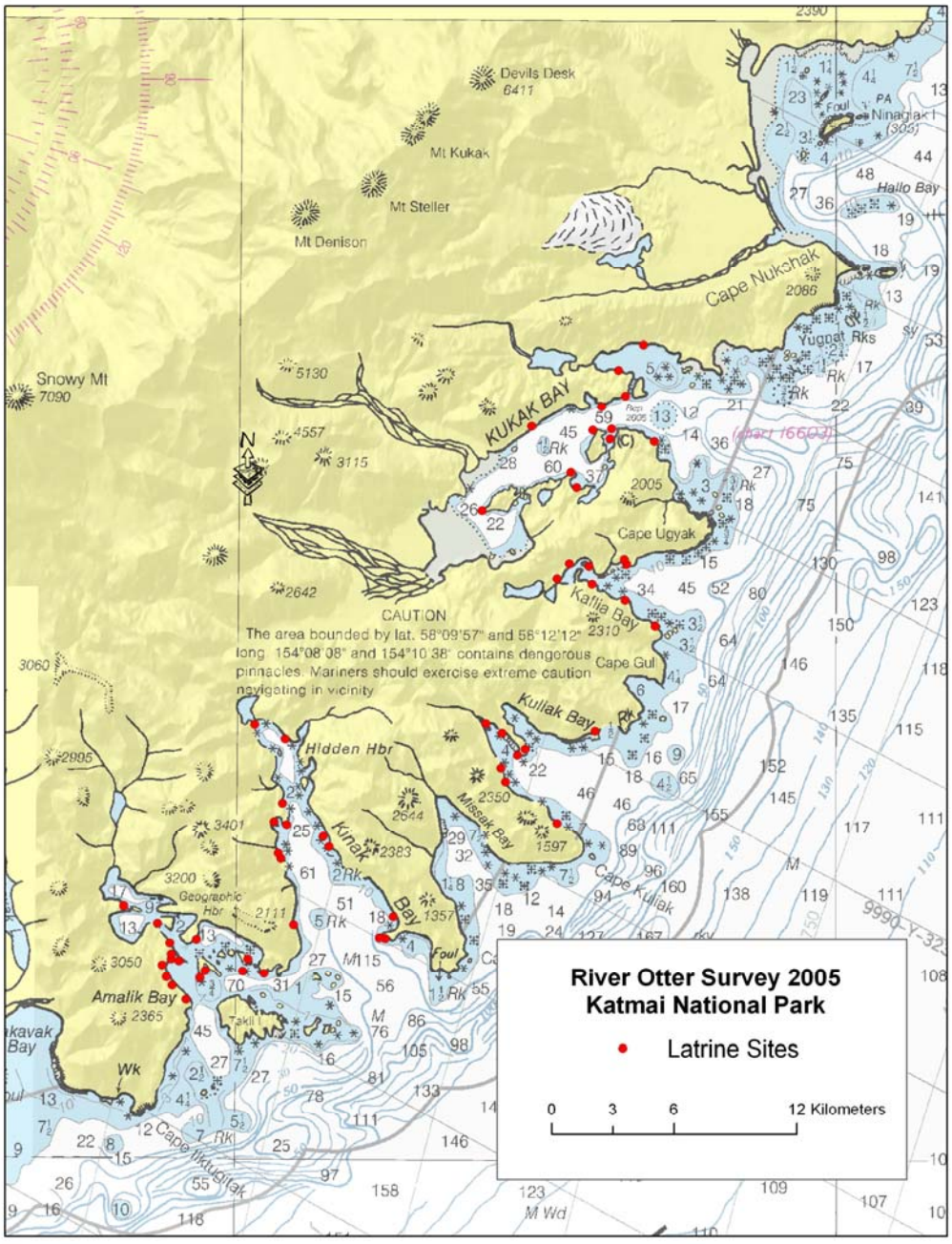


Figure 3. Distribution of river otter latrines along the coast in KATM. Survey was conducted July 2-10, 2005 and encompassed 168 km of shoreline.

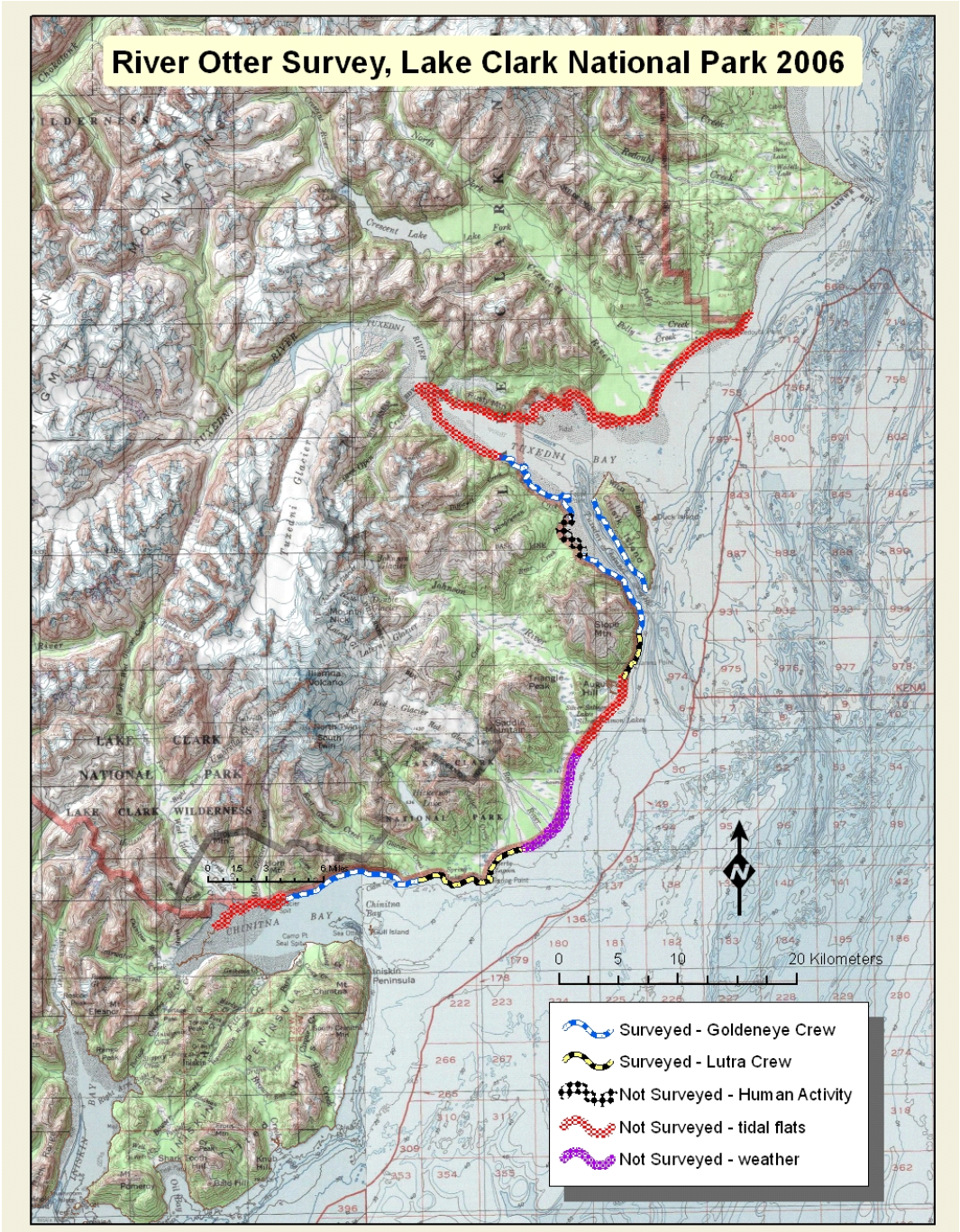


Figure 4. Coastline surveyed for river otter latrine sites in Lake Clark National Park in July 2006. Of the official coastline measurement of 198 km for LACL, we surveyed approximately 50 km (25%) within park boundaries between Difficult Creek in Tuxedni Bay and Glacier Spit in Chinitna Bay. We also surveyed an additional 10 km along the southwest shore of Chisik Island, which is contained within the Alaska Maritime National Wildlife Refuge.

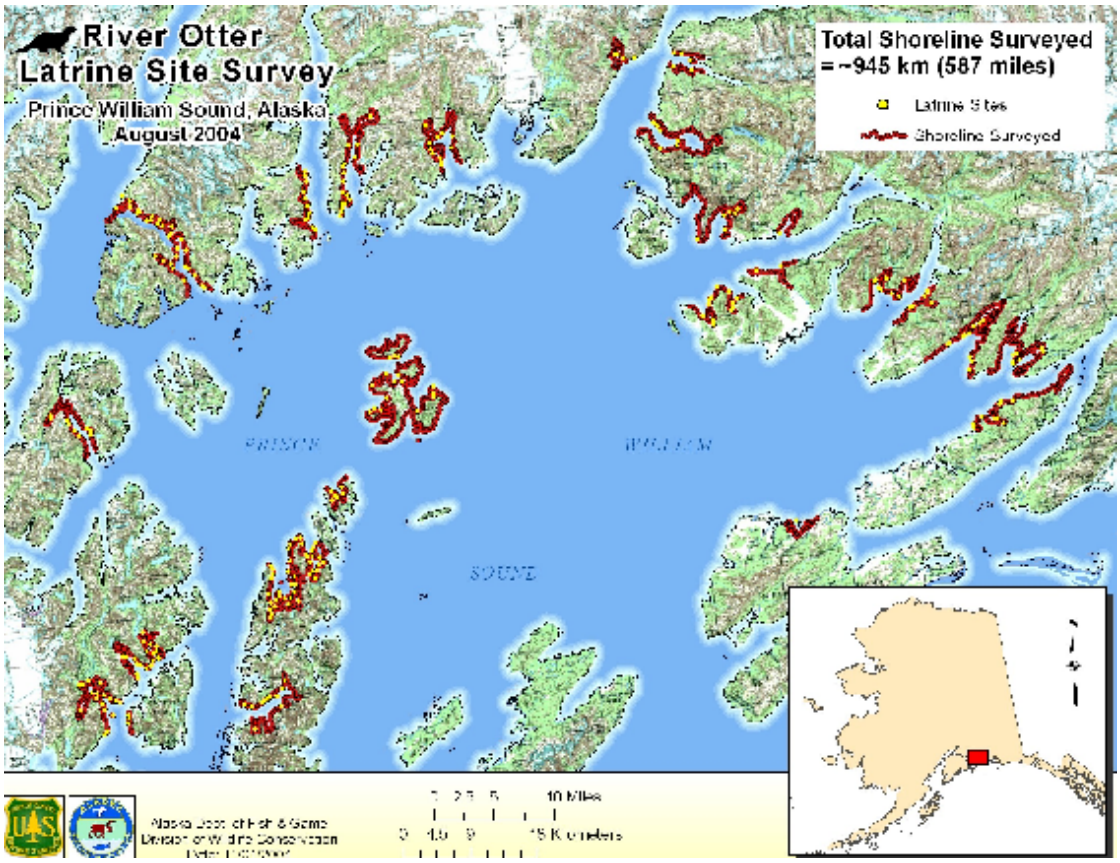


Figure 5. Length of surveyed coastline in Prince William Sound (in red), and distribution of otter latrines in the surveyed area (yellow). Survey was conducted August 9-21, 2004 and encompassed 945 km of shoreline.

We performed DNA amplifications (PCR) using a PTC-0200 DNA Engine Peltier Thermal Cycler (MJ Research, Inc., Waltham, MA). Primers RIO-01, RIO-05, RIO-17, RIO-19, and RIO-20 developed for river otters (Beheler et al. 2004; 2005), and LUT-701, LUT-733, LUT-801 and LUT-829 developed for Eurasian otters (*Lutra lutra*; Dallas and Piertney 1998) were used in PCR reactions. Positive (blood samples from river otters with known genotypes) and negative (PCR blank) controls were included with each PCR run to ensure the reliability of PCRs and monitor contamination (Hansen et al. 2008). Successful PCR reactions were resolved on an ABI 3130xl Automated Sequencer (Applied Biosystems Foster City, CA; ABI) with formamide-Liz ladder as an internal size standard for each lane. Products were analyzed using ABI analysis software GeneMapper v4. Successful amplification was determined by the presence of PCR product of the expected size (Hansen et al. 2008).

We obtained a consensus genotype from positive PCRs with identifiable alleles that had sufficient replication (Goossens et al. 2000). To reduce genotyping error and time spent trying to amplify poor quality samples, each sample that did not amplify after three PCR runs with the three most reliable markers (RIO-19, LUT-733 and LUT829) was discarded (Morin et al. 2001, Paetkau 2003). We evaluated genotypes after two initial

reactions (Frantz et al. 2003, Hansen et al. 2008). Loci that amplified the same heterozygous individual twice were recorded, and homozygote genotypes were accepted on a provisional basis after a stepwise amplification approach of up to seven PCRs. In the case that an allele amplified only once to yield one heterozygote genotype in seven runs with the other six runs resulting in the same homozygous genotype, we designated the allele as constituting a half-genotype (Frantz et al. 2003).

Effects of habitat, environmental conditions, diet, and parasite load on genotyping success

To optimize field collection of samples, we explored which variables affected genotyping success of feces in KEFJ and PWS. First, we used logistic regression with genotyping success as the dependent variable (successful coded as 1 and unsuccessful coded as 0) and habitat features as the independent ones to determine if any measures of shading or exposure are correlated with DNA degradation in feces. Because we collected multiple samples at several of the latrines, we called a site successful if at least one sample from that site yielded usable DNA. We also evaluated whether environmental conditions such as temperature and humidity were correlated with amplification success. Nsubuga et al. (2004) reported a negative relationship between the amount of DNA obtained per sample and the ambient temperature at the time of fecal collection for wild mountain gorillas (*Gorilla beringei beringei*). Lucchini et al. (2002) found that amplification success of wolf (*Canis lupus*) scats was higher for samples collected in the winter than those collected in the summer. Farrell et al (2000) found that carnivore feces collected in the rainy season had a much lower amplification success than those collected in the dry season. Thus, establishing a relation between genotyping success and environmental conditions or habitat features will allow early screening of samples and will result in significant savings in effort and costs. We obtained data on average daily temperature and relative humidity from the weather stations in Cordova, Seward, Valdez, and Whittier. To explore the effects of environmental conditions on genotyping success we used regression analyses with either temperature, or humidity, or their multiplication as the independent variable and percent of samples that were successfully genotyped on each day as the dependent variable.

Similarly, because other studies noted the effects of diet on genotyping success of feces (Hansen 2004; Murphy et al. 2002), we evaluated the effects of diet on genotyping success by submitting prey remains sieved from successful and unsuccessful fecal samples (n = 100) to Pacific Identification Lab (University of Victoria, Victoria, British Columbia). Samples that contained parasites and samples that were free of parasites were included in this analysis because our preliminary observations indicated lower genotyping success in infected feces. We compared the effects of prey remains on genotyping success with the binomial test (Zar 1999).

Observer bias

Because it is likely that future monitoring surveys will be conducted by different park personnel, we evaluated whether genotyping success varied among samples collected by different observers. Because the decision to collect a sample or not is subjective based on the smell and appearance, differences between observers are likely. We calculated the proportion of successful samples for each observer and used a Z-test for multiple proportions (Zar 1999) to identify observer bias.

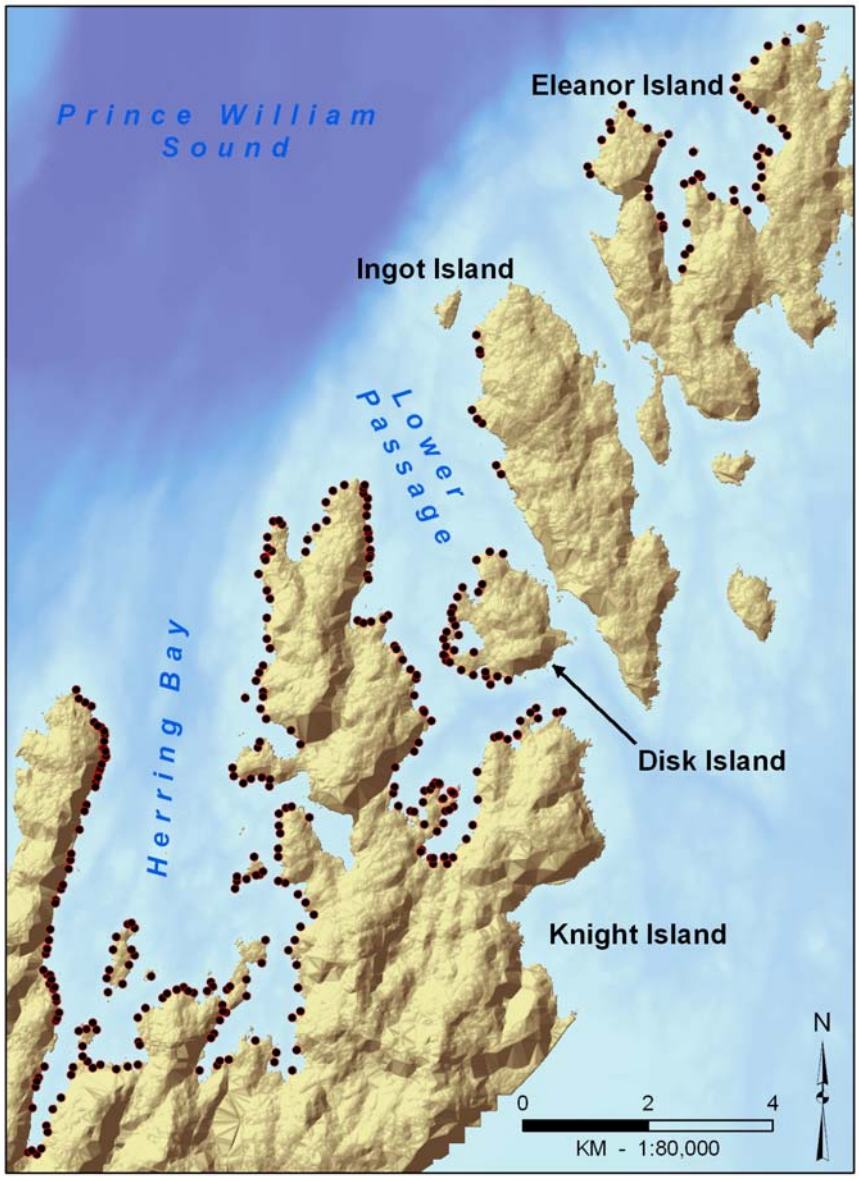


Figure 6. Distribution of river otter latrine sites in Herring Bay, Lower Passage, and Eleanor Island in Prince William Sound. Survey was conducted May 25 – August 15, 2006 and encompassed 145 km of shoreline.

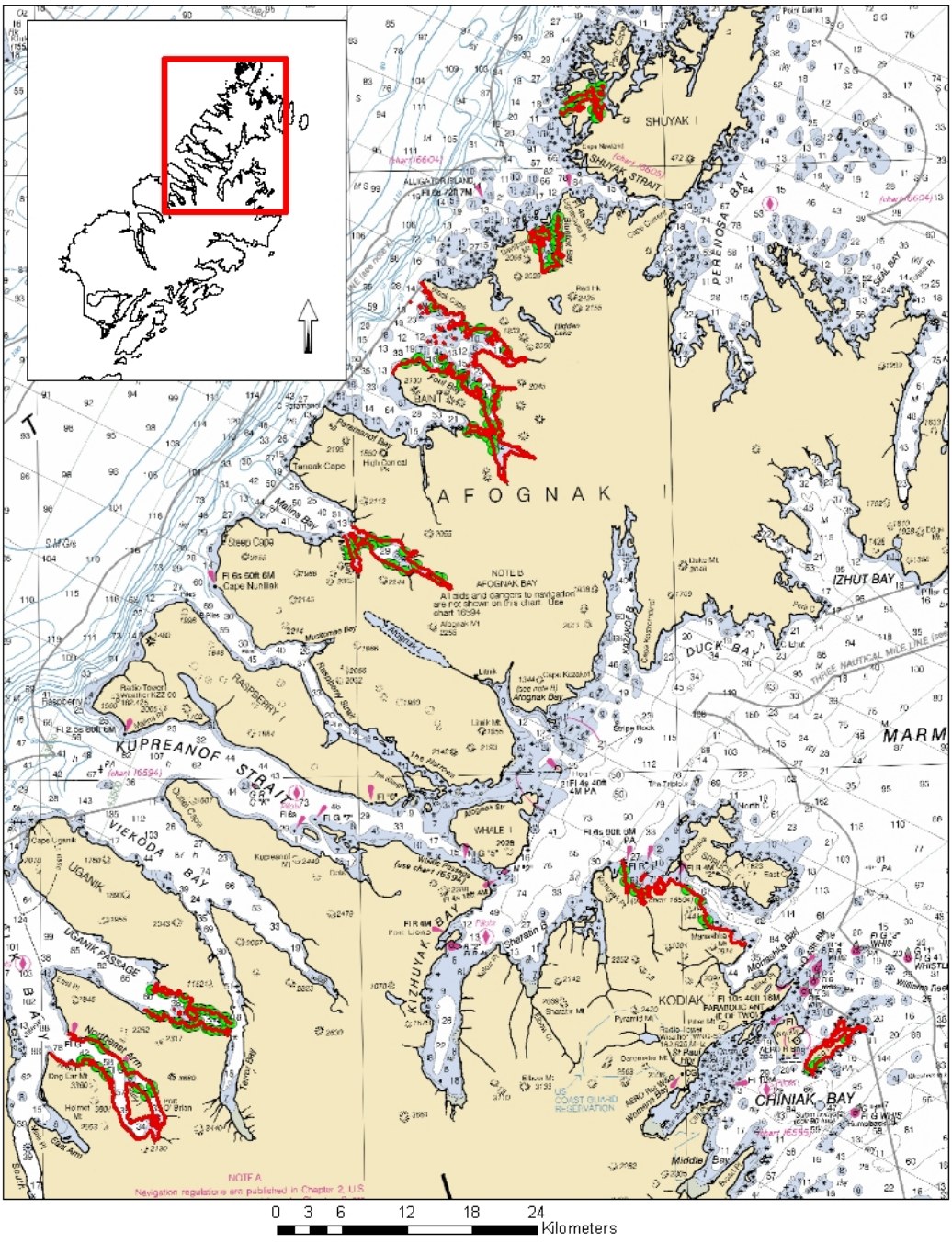


Figure 7. Distribution of river otter latrine sites in Big Bay (Shuyak Island), Blue Fox Paramanof, Foul, and Malina Bays (Afognak Island), Uganik Bay and Chiniak Bay/Ouzinkie Narrows (Kodiak Island). Survey was conducted 13–25 June, 2007 and encompassed 376 km of shoreline.

Reliability of genotyping results

We strictly adhered to the comparative multiple tubes approach for assigning consensus genotypes (Frantz et al. 2003, Hansen et al. 2008). We computed genotyping error rates

(false alleles and allelic drop-out) based on the final sample dataset with complete multilocus genotypes according to the formulae in Broquet and Petit (2004) and Prugh et al. (2005).

To ensure that we used a sufficient number of loci for individual identification, we calculated the probability of identity (P_{ID}; the probability that two individuals drawn at random from the population share identical genotypes at all typed loci) using GIMLET 1.3.2 (Valière 2002). We calculated the theoretical upper and lower limits of P_{ID} (Waits et al. 2001). The lower limit, $P_{ID\text{-unbiased}}$, assumes a randomly mating population of unrelated individuals in Hardy-Weinberg equilibrium (HWE). The upper limit, $P_{ID\text{-sib}}$, assumes the population to be composed only of siblings, and should be 0.01 or less if data are to be used for population estimation (Mills et al. 2000).

Population genetics

We assessed assumptions of random mating and tested for departures from HWE using the probability test by Guo and Thompson (1992) as implemented in GENEPOP 3.4 (Raymond and Rousset 1995). We used FSTAT 2.9.3.2 (Goudet 2000) to calculate Weir and Cockerham (1984) *F*-statistics and to test for linkage disequilibrium. Cavalli-Sforza chord distances were calculated for each pair of populations (Cavalli-Sforza and Edwards 1967) using the module GENDIST in PHLYIP 3.67 (Felsenstein 1989). These distances were then used to construct a tree diagram using Saitou and Nei's (1987) neighbor joining method with 1000 bootstrap replications implemented in the modules NEIGHBOR and CONSENSUS in PHYLIP.

Population differentiation in each of the sampling areas was further assessed using a Bayesian model implemented in STRUCTURE 2.2 (Pritchard et al. 2000). We assumed an admixture model with correlated allele frequencies (Falush et al. 2003). To estimate the number of subpopulations (K), we performed 20 independent runs of K = 1-6 with a burn-in period of 100,000 followed by 100,000 Markov chain Monte Carlo (MCMC) repetitions. We determined the most probable number of subpopulations based on the mean log-likelihood of K ($L(K)$), as well as ΔK, a measure of the second order rate of change in likelihood of K (Evanno et al. 2005). We performed a final run at the inferred K (100,000 burn-in and 500,000 MCMC repetitions) and assigned individuals to a subpopulation based upon their highest proportion of membership (q). We chose a threshold value of 0.70 to assign individuals to subpopulations (i.e., ≥ 70% of ancestry can be attributed to the respective subpopulation; Pritchard et al. 2000). Finally, to assess the degree of immigration and emigration among the sampled populations, we used the program MIGRATE 2.1.3 (Beerli and Felenstein 2001). We used the Brownian motion model following parameters suggested by the authors. We increased parameter stringency on consecutive analyses to include longer runs and more recorded trees. Using information on genetic differentiation, we delineated sub-populations and calculated latrine densities, fecal deposition rate (scats/site/day) and minimum number known alive (MNKA) for each sub-population.

Estimation of abundance

In this analysis only data from PWS 2006 were used because recapture rates in all other datasets were insufficient. Because male river otters are wide ranging (especially during the mating season; Blundell et al. 2002), a significant portion of our dataset may consist of transient males. Furthermore, because we sampled portions of the shoreline of 3 islands in the northern Knight Island complex (Figure 6), we likely introduced some degree of capture heterogeneity by detecting otters with home-ranges overlapping the edges of the study area. Finally, because the duration of our survey included natal dispersal of young otters from their dens we likely sampled new individuals recruited into the population (Crait et al. 2006). Consequently, the conditions of our study likely violate assumptions of geographic and demographic closure required for analysis with closed population CMR models (Amstrup et al. 2005). Therefore, we used the Cormack-Jolly-Seber (CJS) open population model in program MARK to obtain estimates of apparent survival (φ) and recapture probability (*p*; Lebreton et al. 1992, Pledger et al. 2003, Pollock 2000, Seber 1982, White and Burnham 1999). The assumptions of this model require that (1) animals have equal recapture probability, and (2) apparent survival probability is homogenous between all animals (Pledger et al. 2003, Pollock 2000, Seber 1982).

Because we lacked information on age and sex for otters in our dataset we could not explore models that incorporated these covariates. Instead, we compared models in which apparent survival (φ) and recapture probability (*p*) either varied through time or were constant. We evaluated model fit based on the Akaike Information Criterion (AIC) adjusted for small samples, which indicates the most likely model based on parsimony and optimal precision while attempting to minimize bias (Burnham and Anderson 2002). We then extrapolated river otter abundance in each occasion based on the formula:

$$N_j = \frac{n_j}{\hat{\theta}_j} \qquad (1)$$

where N_j is abundance and n_j is the number of animals marked (or captured) at the *j*th occasion, while $\hat{\theta}_j$ represents capture probability at time *j* of animals marked in the previous occasion (Amstrup et al. 2005).

In order to assess lack of geographic and demographic closure on population estimates, we first conducted the analyses using all individuals identified from their fecal genotypes. We then repeated the analyses but omitted any individuals that were encountered only once. We incorporated samples that were encountered at least twice (including individuals captured within the same occasion – i.e., an animal encountered once on June 7 on latrine HB06070, and again on June 8 at nearby LP06005), because individuals observed only once likely represented transients (introducing heterogeneity in *p;* Pradel et al. 1997).

For both datasets, we also calculated capture (*p*) and recapture (*c*) probabilities and abundance (*N*) with closed-population models in program MARK. We used AIC model selection procedures to select among competing models as described by Burnham and

Anderson (2002). We then estimated river otter density by accounting for the length of the shoreline surveyed.

Assessing the effect of sampling occasions on abundance estimates

To assess the effects of sampling occasions on the bias and precision of abundance estimates we calculated estimates of apparent survival (φ) and recapture probability with Cormack-Jolly-Seber (CJS) open population model for the full and residents only datasets from PWS 2006. In addition, we calculated capture (p) and recapture (c) probabilities and abundance (N) with closed-population models for these datasets. We repeated these analyses and sequentially truncated occasions from both datasets. For example, we obtained φ and p for 8 occasions and then for 7 occasions, until only data collected in the first 4 occasions were introduced into the models. We then plotted the population estimates with 95% confidence intervals to determine the number of occasions required to obtain the least biased and most accurate abundance estimate.

Relating latrine density and fecal deposition rate to minimum number known alive

To quantify the relation between otter abundance and measures of relative abundance, we used multiple regression procedures with latrine density and fecal deposition rate as the independent variables and MNKA as the dependent one (Zar 1999). We employed this measure of abundance rather than formal population estimates because we were unable to generate the latter for most of our sampling areas. For this analysis we used data from all sub-populations in KEFJ, PWS, and KOD as well as data gathered for KATM. In this analysis we also included data collected in a companion study on Vancouver Island, British Columbia (Guertin et al. *In review*).

Results

Latrine site surveys

In KEFJ, we identified 162 latrine sites along the sections of coastline we surveyed in 2004. Of these, 153 were active (i.e., contained new scats or at least 10 scats; Figure 1). This number of active latrines represents an average density of 0.43 latrines per km of shoreline (Table 1). Latrine densities appeared higher at the mouths of bays away from the glaciated heads of the bays (Figure 1). Among the active sites we counted 5,046 old and 297 new (24 hrs old) scats. The maximum number of old scats found on a site was 235 and the maximum number of new scats on a site was 20. Based on these counts, we estimated that fecal deposition rate in KEFJ in 2004 was 32.98 feces per site for old scats and 1.94 fresh scats per site. We collected 267 fresh scats for DNA analysis.

Of the 153 latrine sites sampled in KEFJ in 2004, 94 were active in 2005. A total of 4,912 old feces were counted and marked, and 416 new feces were collected (total of 260 in first and 156 on second visit for a deposition rate of 2.8 and 1.8 scats per site per day, respectively; Table 1).

In 2006, we were able to complete our sampling of the habitat characteristics for all pre-selected 403 random sites in 5 days (Figure 2).

During the survey of the KATM coastline, 58 river otter latrines were identified (latrine density of 0.35 sites per km) and a total of 63 fresh fecal samples were collected (deposition rate of 1.1 scats per site per day; Table 1).

Our inventory of the shoreline of LACL in 2006 resulted in the location of only 4 latrine sites that collectively contained 11 old scats. These scats were unsuitable for DNA analysis. Much of the coastline consisted of muddy tidal flats that were not compatible with habitat characteristics known to be favored by river otters.

In PWS, we sampled 286 latrine sites in 2004 (Figure 5). Of those sites, 109 were new sites (i.e., unknown from previous surveys). On the recapture occasion we re-sampled 254 of these sites that we considered active. This number of active latrines represents an average density of 0.27 latrines per km of shoreline. The lowest density of latrines was observed in Valdez Arm and Culross Pass (Figure 5). Among all latrine sites we counted 1,048 new scats and saved 302 of those for DNA analyses. For the recaptures, we counted 458 new scats and saved 263. Based on these counts, fecal deposition rate of new scats in PWS is 4.13 feces per site for the capture session and 1.80 fresh feces per site for the re-capture session (Table 1). A total of 565 fresh scats were collected for DNA analysis.

In 2006 we identified 320 river otter latrines along the shoreline of Herring Bay (HB), Lower Passage (LP), and Eleanor Island (EI). We used stratified random sampling with bay (HB, LP and EI) and number of feces (<100 or ≥ 100) as the strata, to select 100 active latrine sites. We monitored these 100 sites for fecal deposition (number of feces per day) in 9 occasions. In all, we counted a total of 17,585 fecal deposits and collected 964 fresh feces yielding a rate of 2.3 scats per site per day (Table 1).

In the Kodiak Island Archipelago in 2007 we identified 183 latrines along 376 km of shoreline for an overall latrine site density of 0.5 sites per km, ranging from 0.2 sites per km in Chiniak Bay/Ouzinkie Narrows to 1.0 site per km in Big Bay (Figure 7; Table 1). At each site, we counted the number of scats. We also collected all fresh scats (characterized by their distinct glossy appearance and strong smell) and preserved them in 100% ethanol. Each sample was collected in a separate a 50-ml sterile tube. All samples were labeled and stored in coolers packed with ice. Among all sites, we counted 9,428 scat deposits for an average deposition rate of 51.5 (SD = 49.1) scats/site, ranging from 19.6 (13.5) scats/site in Chiniak Bay/Ouzinkie Narrows to 79.3 (61.8) scats/site in Blue Fox Bay (Table 1). We collected 261 fresh feces.

Genotyping success and reliability of genotyping results

Between 22 – 65% of the samples we collected yielded DNA. Higher yield (i.e., higher proportion of samples) occurred in PWS (2006) and KOD (2007) after we concentrated on collecting samples that contained anal sac secretions (i.e., anal-jellies, Rostain et al. 2004). Of 565 fecal samples collected in PWS in 2004 we were able to establish full

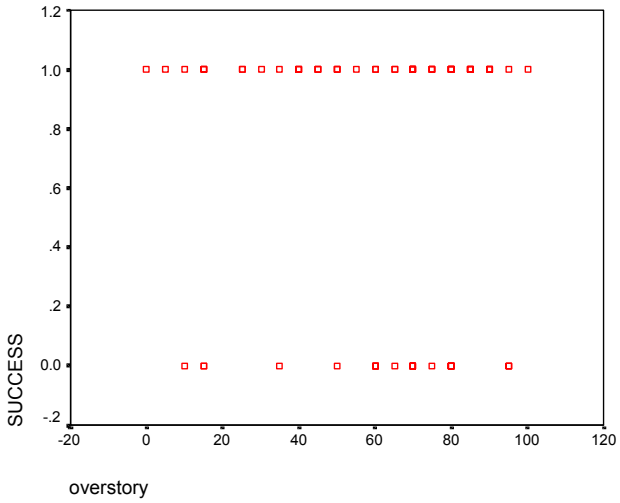
genetic profiles at seven or more loci for 113 samples, representing 106 individuals, which translates to a success rate of 20.0%. From 695 total fecal samples collected in KEFJ in 2004 and 2005 we obtained consensus genotypes at seven or more loci for 120 samples representing 103 individuals and yielding a 17.3% success rate. For PWS 2006 we generated 261 genetic fingerprints of 131 individuals from an original sample set of 963 for a success rate of 27.1%. In KATM, 15 of 63 samples were successfully genotyped (or 23.8%) representing 12 unique individuals, and in KOD, 82 unique individuals were represented in the sample of 94 samples that yielded consensus genotypes. In KOD success rate was the highest at 36% (94 of 261 samples).

The probability of obtaining an incorrect multilocus genotype after replication at all eight microsatellite loci ranged from 0.0004 to 0.009 across all populations. Allelic richness and heterozygosity were high in all areas except KOD (Table 2). The probability that two individuals drawn at random from a given population share identical genotypes at all loci was low (KEFJ – 1 in 15,948,963; PWS – 1 in 7,575,758; KATM – 1 in 333,333; KOD – 1 in 38,760).

Effects of habitat, environmental conditions, diet, and parasite load on genotyping success

Despite large variation in site characteristics, no habitat feature could explain the differences we observed in genotyping success of river otter fecal samples (Figures 8 – 11; Logistic regression, $P > 0.316$). We found no relation between overstory or understory cover and genotyping success despite the potential of such cover to reduce exposure of samples to direct sunlight. Similarly, we found no relation with slope, occurrence of bedrock, or aspect.

Figure 8 – Distribution of percent overstory in relation to successful genotyping (coded 1) and unsuccessful genotyping (coded 0) for river otter fecal samples collected in KEFJ in July 2004.



In contrast, genotyping success declined with an increase in temperature (Figure 12; $R^2 = 0.22$, $P = 0.05$). Genotyping success was higher on days with temperatures bellow 16˚C (44.2% ± 2.5), compared with days when the temperature exceeded 16˚C (30.2% ± 3.7; ANOVA, $P = 0.005$). This suggests that samples collected on hot days should be discarded to reduce efforts and costs of DNA analyses.

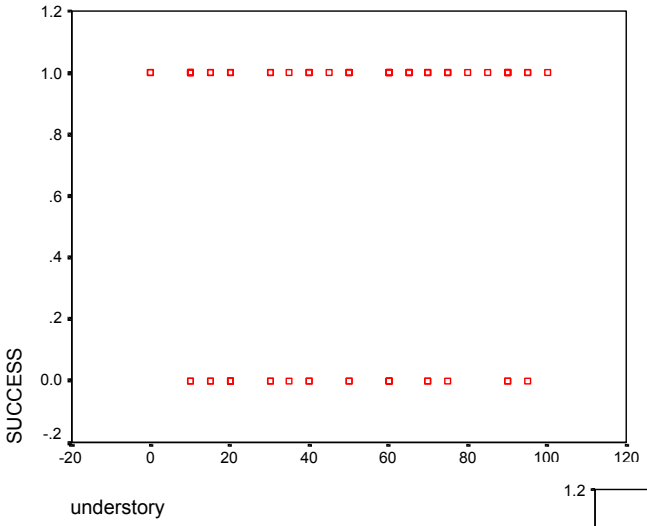
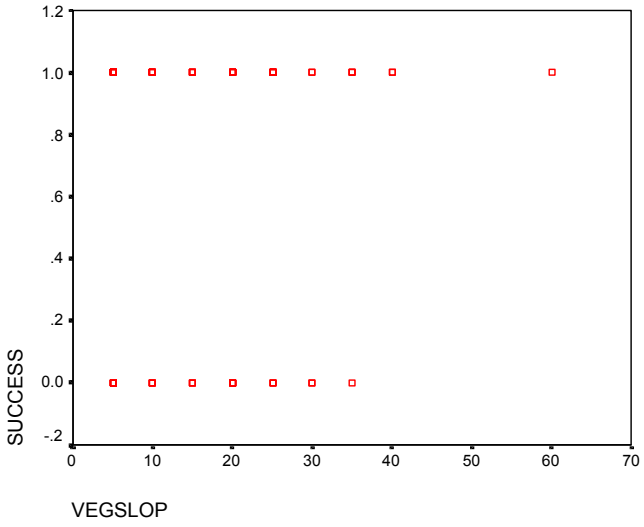


Figure 9 – Distribution of percent understory cover in relation to successful genotyping (coded 1) and unsuccessful genotyping (coded 0) for river otter fecal samples collected in KEFJ in July 2004.

Figure 10 – Distribution of vegetative slope in relation to successful genotyping (coded 1) and unsuccessful genotyping (coded 0) for river otter fecal samples collected in KEFJ in July 2004.



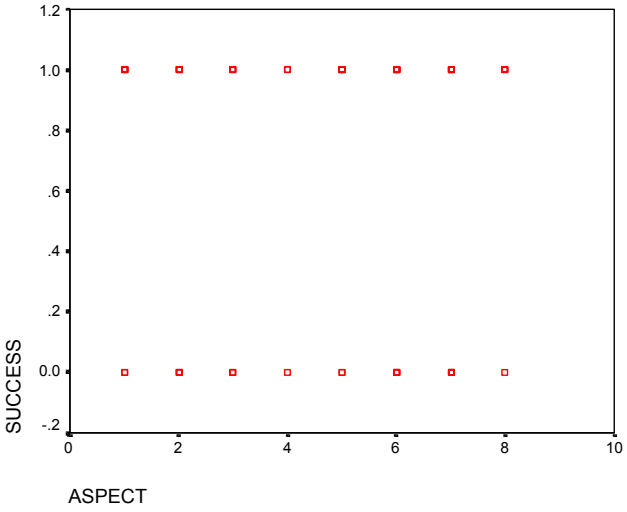


Figure 11 – Distribution of aspect as measured in 8 compass directions in relation to successful genotyping (coded 1) and unsuccessful genotyping (coded 0) for river otter fecal samples collected in KEFJ in July 2004.

Table 1. Shoreline lengths and number of river otter latrine sites surveyed among different bays in southwestern and southcentral Alaska between 2004 - 2007. Latrine densities were calculated based on the number of sites located along the coast. Fecal deposition was estimated based on the number of scats counted per site in each bay.

Area	Bay	Year	Shoreline length	Number of latrines	Latrine density	Scats/site/day	Minimum number known alive
Katmai		2005	168	58	0.35	1.10	12
Kenai Fjords	Aialik Cape	2004	213	106	0.50	2.17	39
	Nuka Bay*	2004	129	56	0.43	1.31	4
	Aialik Cape	2005	213	106	0.50	2.76	39
	Nuka Bay	2005	129	56	0.43	3.72	21
Kodiak	Big	2007	22	23	1.05	3.17	15
	Blue Fox	2007	27	24	0.89	1.21	15
	Paramanof/Foul/ Malina	2007	158	71	0.45	1.00	29
	Uganik	2007	92	49	0.53	1.35	21
Prince William Sound	Jackpot	2004	154	159	1.03	1.88	15
	Olson	2004	184	93	0.51	0.83	13
	Valdez Entrance	2004	154	43	0.28	3.37	17
	Unakwik Inlet	2004	207	146	0.71	4.77	28
	Herring[@]	2006	76	138	1.81	2.69	20
	Lower Passage[@]	2006	46	88	1.90	0.97	7
	Eleanor Island[@]	2006	21	36	1.72	0.82	9

*Data excluded from further analyses because of low genotyping success

[@]Only data from the first occasion were included to ensure compatibility with the other datasets

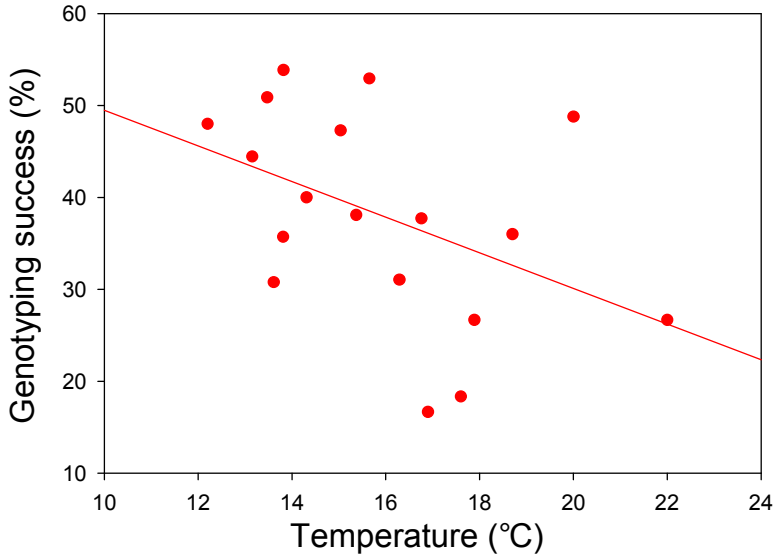
Diet analyses revealed that genotyping success was lower for samples containing the remains of Cottidae and Stichaeidae (Binomial test, $P < 0.05$; Table 2). Samples containing the remains of Syngnathidae yielded no consensus genotypes. No other fish remains appeared to significantly affect genotyping success although samples containing Pleuronectiformes, Salmonidae and Scorpaenidae exhibited consistently higher genotyping success. Thus, evaluation of diet composition may assist in pre-screening of fecal samples for DNA analyses.

Through sieving we found a difference in the incidence of intestinal parasites in river otter feces from KEFJ (parasites occurred in 36% of samples) and PWS (parasites occurred in 10% of samples). The occurrence of parasites negatively affected genotyping success. Whereas 49.1% of samples that were free of parasites yielded usable DNA, only 19.8% of infected ones were successful (Z-test of 2 proportions, $P < 0.01$).

Observer bias

We detected no observer bias (Z-test of multiple proportions, $P = 0.38$). On average 38% (± 8%) of samples collected by HH yielded consensus genotypes. Similarly, 42% (± 9%) collected by MBD, and 49% (± 9%) collected by KEO yielded consensus genotypes. Thus, it will be possible to assign the task of sample collection to multiple observers in future monitoring efforts.

Figure 12 – Genotyping success (percent of samples that yielded DNA from the total collected on each day) declined with increasing temperatures. Data was collected in KEFJ and PWS 2004.



Population genetics

In the following analyses we included data obtained by Guertin et al. (*In review*) for river otters inhabiting the southern part of Vancouver Island, British Columbia (BC), Canada (Table 3). Pairwise F_{ST} values suggested significant differentiation among all sampled populations (Table 4). Cavalli-Sforza chord distance with BC rooted as an out-group indicated strong divergence of KEFJ and PWS from KOD and KATM, as well as well-supported divergence between KEFJ and PWS. In contrast, divergence of KOD

from KATM was weak, with less than half of the projected trees supporting separation of the 2 populations (Figure 13). These results suggest that KATM and KOD otters likely differentiated from one ancestral stock that inhabited the Pleistocene southwestern shores of Alaska, and was isolated from other more easterly populations by distance.

Table 2. Percent occurrence of prey items in scats that yielded consensus genotypes (Successful) and in scats that failed to produce consensus genotypes (Unsuccessful). Statistical comparisons were conducted only for families that occurred in at least 10 of the 104 analyzed samples (*). Bolded numbers represent overall values for families with multiple species.

Family	Common name	Scientific name	Percent occurrence	
			Successful	Unsuccessful
Agonidae	Poacher	Agonidae	0.00	1.18
Ammodytidae*	Sand lance	*Ammodytes hexapterus*	10.53	9.41
Anarhichadidae	Wolf eel	*Anarrichthys ocellatus*	0.00	1.18
Bathymasteridae	Searcher	*Bathymaster signatus*	5.26	0.00
	Searcher	*Bathymaster* sp.	0.00	1.18
Clupeidae	Pacific herring	*Clupea pallasi*	0.00	1.18
Cottidae*	Padded sculpin	*Artedius fenestralis*	5.26	9.41
	Scalyhead sculpin	*Artedius harringtoni*	5.26	5.88
	Smoothhd sculpin	*Artedius lateralis*	10.53	3.53
	Buffalo sculpin	*Enophrys bison*	0.00	2.35
	Leister sculpin	*Enophrys lucasi*	0.00	1.18
	Gymnocanthus	*Gymnocanthus* sp.	5.26	0.00
	Red irish lord	*Hemilepidotus hemilepidotus*	0.00	10.59
	Irish lord sp	*Hemilepidotus* sp.	15.79	15.29
	Shorthorn sculpin	*Myoxocephalus scorpius*	0.00	1.18
	Great-type sculpin	*Myoxocephalus* sp.	0.00	4.71
	Tidepool sculpin	*Oligocottus maculosus*	0.00	2.35
	Sculpin	*Oligocottus* sp.	0.00	9.41
	Sculpin	Cottidae	0.00	5.88
			42.11[a]	**71.76**
Gadidae*	Saffron cod	*Eleginus gracilis*	0.00	1.18
	Pacific cod	*Gadus macrocephalus*	0.00	2.35
	Tomcod	*Microgadus proximus*	5.26	1.18
	Pollock	*Theragra chalcogramma*	0.00	1.18
	Gadid (not hake)	Gadidae	0.00	3.53
			5.26	**9.41**
Gasterosteidae	Three-spined stickleback	*Gasterosteus aculaeatus*	0.00	1.18
Hemitripteridae	Crested sculpin	*Blepsias bilobus*	0.00	1.18
	Silverspot sculpin	*Blepsias cirrhosus*	0.00	1.18
	Sailfin sculpin	*Nautichthys* sp.	0.00	1.18
Hexagrammidae*	Kelp greenling	*Hexagrammos decagrammus*	10.53	11.76
	Rock greenling	*Hexagrammos lagocephalus*	0.00	3.53
	W-s greenling	*Hexagrammos stelleri*	0.00	1.18
	Greenling	*Hexagrammmos* sp.	0.00	3.53
			10.53	**20.00**
Liparididae	Snailfish	Liparidinae	0.00	3.53
Pholidae*	Crescent gunnel	*Pholis laeta*	5.26	30.59

	Red gunnel	*Pholis schultzi*	0.00	1.18
	Gunnel	Pholididae	15.79	7.06
			21.05	**38.82**
Pleuronectiformes*	Arrowtooth flounder	*Atheresthes stomias*	5.26	1.18
	Pacific sanddab	*Citharichthys sordidus*	0.00	1.18
	Sanddab	*Citharichthys* sp.	0.00	1.18
	Halibut	*Hippoglossus stenolepis*	5.26	0.00
	Rock sole	Lepidopsetta sp.	21.05	20.00
	Dover sole	*Microstomus pacificus*	0.00	2.35
	English sole	*Parophrys vetulus*	5.26	0.00
	Starry flounder	*Platichthys stellatus*	0.00	1.18
	C-o turbot	*Pleuronichthys coenosus*	0.00	1.18
	Sand sole	*Psettichthys melanostictus*	5.26	1.18
	Flatfish	Pleuronectiformes	5.26	3.53
			47.37	**32.94**
Salmonidae*	Salmon	*Oncorhynchus* sp.	47.37	32.94
Scorpaenidae*	Rockfish	*Sebastes* sp.	26.32	17.65
Stichaeidae*	Slender cockscomb	*Anoplarchus insignis*	0.00	2.35
	Cockscomb	*Anoplarchus sp.*	0.00	11.76
	Daubed shanny	*Lumpenus maculatus*	0.00	1.18
	Snake prickleback	*Lumpenus sagitta*	0.00	4.71
	Arctic shanny	*Stichaeus punctatus*	0.00	5.88
	Black prickleback	*Xiphister atropurpures*	0.00	7.06
	Rock prickleback	*Xiphister mucosus*	5.26	0.00
	Prickleback sp.	Stichaeidae	0.00	3.53
			5.26[a]	**36.47**
Syngnathidae*	Bay pipefish	*Syngnathus leptorhynchus*	0.00[a]	11.76
Zaproridae	Prowfish	*Zaprora silensus*	0.00	1.18
Cephalopoda	Squid unidentified		0.00	1.18
Polycaeta	Polycaete unidentified		5.26	0.00

[a] significantly different at $p = 0.05$

These results seemingly contradict those provided by the STRUCTURE analysis. Log-likelihood estimates suggested the existence of 4 distinct populations: KOD, KEFJ, PWS (with KATM individuals included in that cluster), and BC. Values of ΔK, however, implied the existence of only 3 populations, combining KEFJ, KATM and PWS into a single cluster (Figure 14). Nonetheless, none of the KEFJ individuals was mis-assigned to the PWS-KATM cluster with probability greater than 0.5 and only 16 individuals from PWS (or 6.7%) were mis-assigned to the KEFJ cluster with probability greater than 0.7. The conclusion that river otters from KEFJ are genetically distinct from those inhabiting PWS is further supported by results from MIGRATE (Table 5). Migration rates across all 5 populations were low and similar, suggesting little genetic exchange among the study populations.

Similarly, F_{ST} values indicated that population sub-structuring occurred within all sampled areas (Tables 6 and 7), although STRUCTURE analysis detected sub-structuring only in KEFJ (Figure 15) with isolation between Resurrection Bay (in the Northeast) to

Nuka Bay (in the Southwest; Figure 15). This was supported by an F_{ST} value of 0.091 between Aialik Cape (AC) and Nuka Bay (NB; 95% Confidence interval 0.036 - 0.159). Based on these observations, we calculated all population parameters separately for each of the sub-populations (Table 1).

Table 3. Sample size (*n*) mean number of alleles frequency (A ± SD), expected heterozygosity (He), observed heterozygosity (Ho), and inbreeding coefficient (F_{IS}) for five river otter populations along the Pacific coast. Genotypes were obtained from 471 fecal samples representing unique individuals. Samples were collected between 2004 and 2007.

Location	*n*	A (SD)	H_e	H_o	F_{IS}
Kodiak	82	4.00 (1.86)	0.420	0.433	0.098[b]
Katmai	12	4.50 (1.69)	0.622	0.718[a]	-0.168[b]
Kenai	103	6.13 (2.23)	0.683	0.674	0.013
Prince William Sound	237	6.63 (2.36)	0.659	0.695	-0.054[b]
British Columbia*	49	4.38 (2.00)	0.570	0.520[a]	0.106[b]

*Adopted from Guertin et al. (*In review*)
[a] significantly different than expected
[b] significantly different than zero

Table 4. Pairwise F_{ST} values (upper diagonal) and 95% confidence intervals (lower diagonal) for 5 river otter populations along the Pacific coast (Figure1). Genotypes were obtained from 471 fecal samples representing unique individuals. Samples were collected between 2004 and 2007. Adopted from Seymour et al. (*In prep*).

	KOD	KATM	KEFJ	PWS	BC
KOD	-	0.254	0.248	0.190	0.394
KATM	0.088-0.449	-	0.070	0.059	0.150
KEFJ	0.126-0.371	0.011-0.130	-	0.076	0.197
PWS	0.081-0.287	0.020-0.110	0.035-0.126	-	0.159
BC	0.160-0.472	0.083-0.153	0.100-0.314	0.047-0.279	-

Table 5. Migration rates (as calculated by program MIGRATE) among 5 river otter populations along the Pacific coast (Figure1). Genotypes were obtained from 471 fecal samples representing unique individuals. Samples were collected between 2004 and 2007. Adopted from Seymour et al. (*In prep*).

Receiving population	Source Population				
	KOD	KATM	KEFJ	PWS	BC
KOD		1.155	0.957	1.572	0.725
KATM	1.209		0.761	1.814	0.986
KEFJ	1.306	1.260		1.900	1.378
PWS	1.878	1.656	1.852		1.138
BC	1.201	1.184	0.986	1.726	

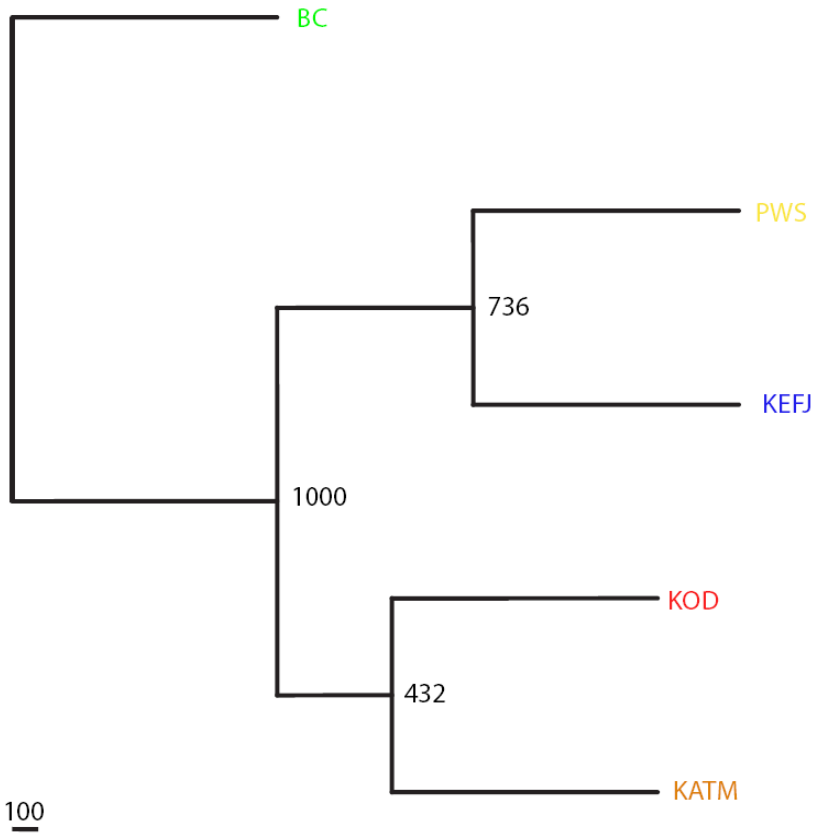


Figure 13 – Cavalli-Sforza chord distance tree (as calculated by program PHILYP) with southern Vancouver Island, British Columbia (BC) rooted as an out-group, indicated strong divergence of Kenai Fjords National Park (KEFJ) and Prince William Sound (PWS) from the Kodiak Island Archipelago (KOD) and Katmai National Park and Preserve (KATM), high support for divergence between KEFJ and PWS, and weak support for divergence of KOD from KATM. Genotypes were obtained from 471 fecal samples representing unique individuals. Samples were collected between 2004 and 2007. Adopted from Seymour et al. (*In prep*).

Figure 14. Posterior probability assignments of river otters to two genetic clusters inferred by STRUCTURE for 5 river otter populations sampled along the Pacific coast between 2004 and 2007. No individuals from the Kodiak Island Archipelago (KOD) or British Columbia (BC) were mis-assigned to any other population. Animals from Katmai National Park and Preserve (KATM) largely clustered with those from Prince William Sound (PWS). Adopted from Seymour et al. (*in prep*).

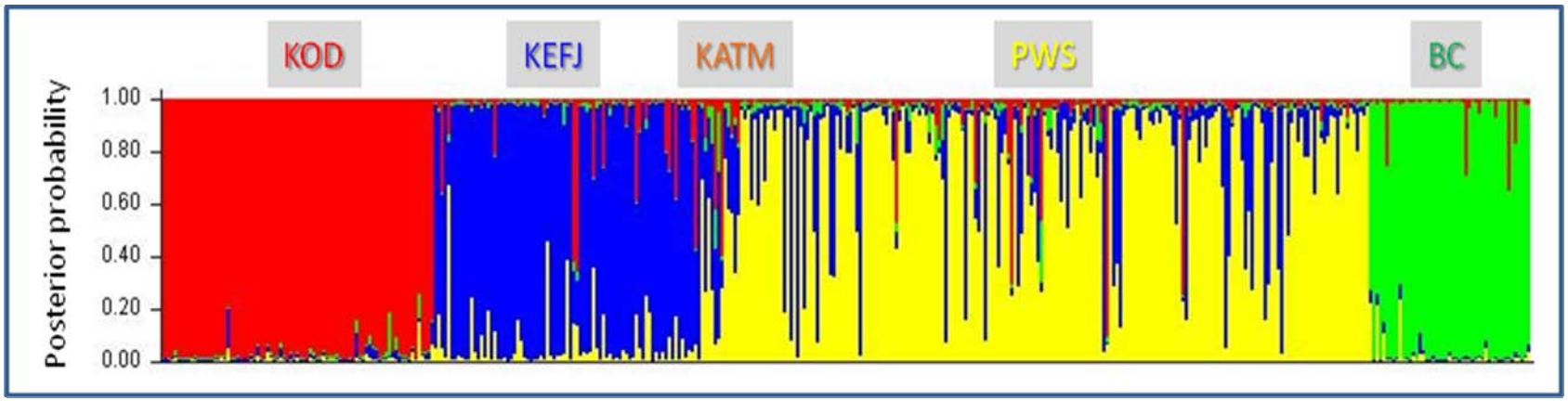


Table 6. Pair-wise genetic distances (F_{ST} upper diagonal, 95% confidence interval lower diagonal) between subpopulations of river otters in Prince William Sound (sound-wide survey in 2004 [a] and Northern Knight and Eleanor islands in 2006 [b]). We designated these areas based on distance and previous demarcation of populations by Blundell et al. (2002). Northern Knight Island complex (NKIC) encompassed Drier Bay, Herring Bay, Lower Passage, Eleanor Island and Naked Island; Jackpot Bay (JB) included samples collected in Lower Chenega Island, Icy bay, Jackpot Bay and Culross Passage; Olson Bay (OB) included the area from Port Gravina to Cordova; Valdez Entrance (VE) the area from Sawmill Bay east to Port Fidalgo; and Unakwik Inlet (UI) from Ester Passage East to the Columbia Glacier. Adopted from Ott et al. (*In prep* a).

(a)

Study area	NKIC[a]	Jackpot Bay	Olson Bay	Valdez Entrance	Unakwik Inlet
NKIC[a]		0.062	0.086	0.046	0.035
Jackpot Bay[a]	0.018-0.112		0.125	0.074	0.051
Olson Bay	0.039-0.134	0.066-0.181		0.024	0.026
Valdez Entrance	0.007-0.091	0.019-0.123	0.002-0.049		0.017
Unakwik Inlet	0.006-0.064	0.019-0.092	0.004-0.045	0.011-0.021	

(b)

Study area	Herring Bay	Lower Passage	Eleanor Island
Herring Bay[a]		0.012	0.021
Lower Passage[a]	0.002-0.023		0.025
Eleanor Island[a]	0.007-0.037	0.007-0.046	

[a] Includes Herring Bay, Lower Passage, Eleanor and Naked Islands

Table 7. Pairwise F_{ST} values (upper diagonal) and 95% confidence intervals (lower diagonal) for river otter sub-populations along the coast of the Kodiak Island Archipelago (Figure 7). Genotypes were obtained from 80 fecal samples representing unique individuals. Samples were collected in 2007. Adopted from Seymour et al. (*In prep*).

	Big Bay	Blue Fox Bay	Paramanof Bay	Uganik Bay
Big Bay		0.085	0.133	0.026
Blue Fox Bay	0.003-0.159		0.068	0.052
Paramanof Bay	0.053-0.226	0.008-0.132		0.054
Uganik Bay	0.002-0.126	0.023-0.094	0.027-0.091	

Estimation of abundance

These analyses were performed only on the PWS 2006 dataset because there was an insufficient number of true recaptures in the other datasets. In KEFJ and PWS 2004 and 2005, 17 individuals were observed twice (seven in PWS and ten in KEFJ). Two individuals in KEFJ were observed three times and one was observed four times. However, these recaptures usually occurred on the same collection dates and in most cases on the same latrine site (only six individuals were recaptured on the same day at different latrine sites, and only two were observed on different latrines on separate days). The 261 samples collected in PWS 2006 and for which we had genetic profiles at six or more loci represented 131 individuals (minimum number known alive). Of these, 58 individuals were recaptured between two and eight times.

Models with constant apparent survival and recapture probability that varied with time ([φ (.), p(t)]) had the best fit to our data in both datasets (131 and 58) based on AIC_c and AIC weights, regardless of whether we used the default or other link functions (Table 8). The estimate of apparent survival for the full dataset was 0.90, and 1.00 for recaptures only dataset (Table 9). Ben-David et al. (2002) estimated survival rate of 1.0 for river otters in PWS during a similar time period over the summer. Thus, we believe we identified the resident population with our "residents only" capture-recapture dataset.

Recapture probabilities were generally higher and similar in the dataset containing only animals that were observed at least twice, although the temporal pattern of change in recapture probabilities was similar in both datasets. In contrast, the temporal pattern of the post hoc estimates of abundance differed between the full dataset and the two datasets excluding transients and edge-otters. Abundance estimates for the full dataset ranged from 85 in occasion 6 to 182 in occasion 9 (a 114.1% difference), while estimates from the recaptures only dataset for the same occasions varied between 57 to 67 individuals (or a 17.5% difference). Average density of otters in the areas is estimated at 1 otter per 1.18 km of shoreline for the full dataset and 1 resident otter per 2.33 km.

The model with the best fit to the data using closed-population models was one estimating one uniform capture probability but two different recapture probabilities. This was true when we estimated these parameters for the full dataset (131 individuals; AIC weight 1.00) as well as the resident only dataset (58 individuals; AIC weight 0.99; Table 10). As with open population models, capture and recapture probabilities were

higher for the resident only dataset although the patterns were similar for both. With these models abundance of river otters in Herring Bay, Lower Passage, and Eleanor Island was estimated at 163 (144 – 207) individuals for the full dataset, and 58 (58 – 65) for residents only. This translates to a density of 1 otter per 0.89 km of shoreline. Excluding transients the density of otters in our study area was 1 resident per 2.5 km of shoreline.

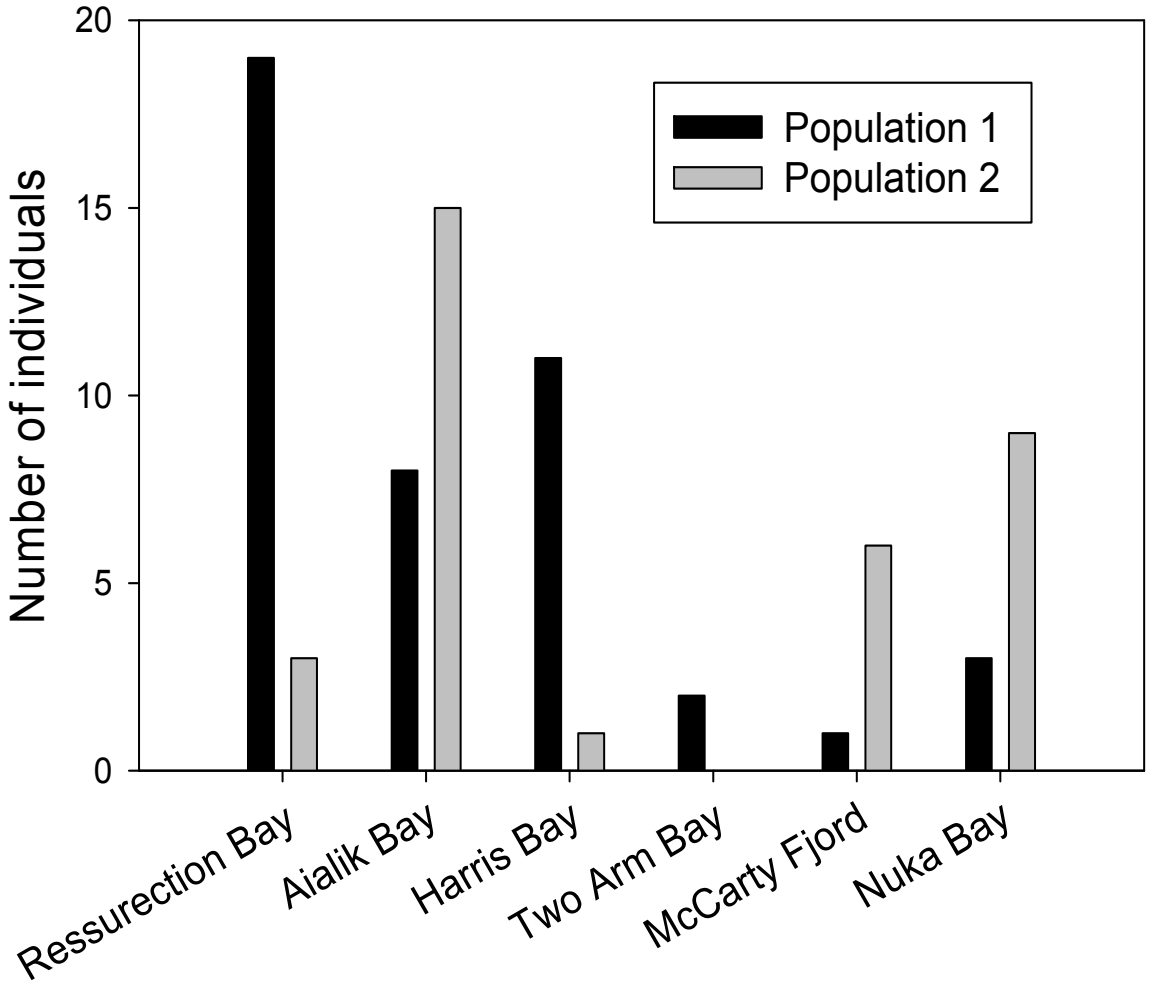


Figure 15. Number of individuals assigned to each sub-population in KEFJ at a threshold of 0.7. Survey locations are arranged from east to west with Resurrection Bay representing the Northeastern most extent of our KEFJ study area and Nuka Bay in the Southwest. Adopted from Ott et al. (*In prep* a).

Table 8. Open-population CJS models used to estimate river otter abundance from capture-recapture data derived from fecal genotyping of river otters in Herring Bay, Lower Passage, and Eleanor Island in Prince William Sound, Alaska, USA. Samples were collected in nine occasions between May 25 and August 15, 2006. Adopted from Ott et al. (*In prep* b).

.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
[φ (.) p (t)]†	5322.5	0.0	1.000	1.000	9	1589.1
[φ (t) p (.)]†	5451.1	129.0	0.000	0.000	9	1718.1
[φ (.) p (.)]†	5547.0	224.5	0.000	0.000	2	1827.7
[φ (.) p (t)] ‡	4174.1	0.0	0.975	1.000	9	1672.1
[φ (t) p (t)] ‡	4181.5	7.3	0.025	0.025	15	1667.2
[φ (t) p (.)]‡	4353.1	179.0	0.000	0.000	9	1851.1
[φ (.) p (.)]‡	4462.6	288.5	0.000	0.000	2	1974.7

† = Full dataset (131 individuals)

‡ = Recaptured otters only (58 individuals)

Table 9. Estimates of apparent survival (φ ± SE), recapture probability (*p* ± SE) and mean population size (N ± 95% confidence interval) produced by the best fit model ([φ (.), p(t)]), from capture-recapture data derived from fecal genotyping of river otters in Herring Bay, Lower Passage, and Eleanor Island in Prince William Sound, Alaska, USA. Samples were collected in nine occasions between May 25 and August 15, 2006. Adopted from Ott et al. (*In prep* b).

	Full dataset (131 individuals)		Recaptures only (58 individuals)	
	estimate	SE	estimate	SE
φ	0.904	0.012	1.000	1.39×10^{-6}
p_{1-2}	0.273	0.024	0.360	0.029
p_{2-3}	0.199	0.018	0.270	0.022
p_{3-4}	0.167	0.015	0.217	0.018
p_{4-5}	0.376	0.023	0.490	0.022
p_{5-6}	0.077	0.011	0.094	0.012
p_{6-7}	0.173	0.017	0.203	0.017
p_{7-8}	0.122	0.014	0.145	0.014
p_{8-9}	0.159	0.018	0.179	0.015
N	123	99 – 147*	62	58 – 66*

*95% confidence intervals

Table 10. Estimates of capture (*p* ± SE), recapture probability (*c* ± SE) and population size (N ± 95% confidence interval) produced by the best fit model from capture-recapture data derived from fecal genotyping of river otters in Herring Bay, Lower Passage, and Eleanor Island in Prince William Sound, Alaska, USA. Samples were collected in nine occasions between May 25 and August 15, 2006. Adopted from Ott et al. (*In prep* b).

	Full dataset (131 individuals)		Recaptures only (58 individuals)	
	estimate	SE	estimate	SE
p	0.163	0.030	0.392	0.046
C_1	0.207	0.025	0.338	0.038
C_2	0.082	0.013	0.155	0.025
N	163	144 – 207*	58	58 – 65*

*95% confidence intervals

Assessing the effect of sampling occasions on abundance estimates

For all datasets with truncated number of occasions the best fit in open-population models was for constant apparent survival and recapture probability that varied with time ([φ (.), *p*(t)]). For closed population models the best fit was for one uniform capture probability but two different recapture probabilities, except for the 4 occasion dataset where the best fit was a model with one capture and one recapture probabilities.

Population estimates for the full datasets with closed-population models were higher than for the same dataset with open population models only when the 9 occasions were included, suggesting that data obtained in that occasion introduced bias into closed-population estimates. This is likely a result of a recruitment pulse that occurs at the end of summer, when young otters join their dames foraging and scent marking at latrines. There was little change in point estimates when the dataset was truncated between 8 and 5 occasions using open-population models, although confidence intervals were broader when only 5 occasions were used to produce the estimates. The residents only dataset exhibited similar patterns and here too precision was lower with only 4 and 5 occasions. These analyses suggest that unbiased and precise population estimates for coastal river otters can be achieved with a minimum of 6 occasions (Figure 16).

Relating latrine density and fecal deposition rate to minimum number known alive

We found no relation between latrine density and fecal deposition rate and MNKA (Multiple regression, $R^2 = 0.207$, $p = 0.156$; p latrine density = 0.101, p fecal deposition = 0.523). Similarly, we found no relation between fresh fecal deposition at each occasion and the estimated number of otters for the PWS 2006 dataset ($R^2 = 0.001$, $p = 0.935$). There was a marginally significant relation between recapture probability and fecal deposition rate in that dataset ($R^2 = 0.459$, $p = 0.065$), likely reflecting the fact that genotyping success is a function of the number of samples collected.

Discussion

Our results suggest that latrine density and fecal deposition rates relative to minimum number known alive may be poor predictors of the status of populations of coastal river otters. Furthermore, because sampling of entire sections of coastline is impractical, the best approach for monitoring river otters should adhere to the requirements of open-population models. Such models depend on a minimum of 3 sampling occasions. Our data, however, indicate that 6 or more sampling occasions will be required to obtain unbiased and precise estimates of river otter abundance.

Although the costs of monitoring otter abundance and population status through time with fecal DNA analyses may be more costly than relative abundance indices and may seem prohibitive, this technique offers important benefits:

1. With fecal DNA analyses it is possible to delineate meaningful study populations, because sub-populations and geneflow between them can be identified. For example, using this extensive dataset we were able to identify not only population level differentiation between KOD, KATM, KEFJ and PWS but also identify sub-

population structuring within KOD, KEFJ, and PWS. From our analyses it appears that ocean currents may be responsible for much of the isolation of river otter populations along the Alaska coast. Strong ocean currents and tidal fluxes associated with the Alaska Coastal Current (ACC) and Shelikof Straights likely limit dispersal of otters between KOD and KATM (Seymour et al. *in review*). Strong currents of the ACC in combination with a high frequency of storms along the Kenai Peninsula potentially contributed to the patterns we observed, on a smaller scale, within KEFJ. For example, Aialik Cape may represent a geographic barrier to otter movement because it is precipitously rocky and exposed to storms and swells from the Pacific Ocean. In contrast, PWS is more sheltered from the ACC by Hinchinbrook, Latouche, and Montague Islands (Bang and Moores 2003). Therefore, river otter populations within PWS may not be subject to the extreme effects of the ACC and may experience enhanced geneflow as suggested by the lower values of F_{ST} among sub-populations (Ott et al. *In prep* a). It is not too surprising that geneflow in semi-aquatic mammals such as river otters could be influenced by ocean currents given that ocean currents can dictate geneflow even in marine fishes (such as the rosethorn rock fish, *Sebastes helvomaculatus*; Rocha-Olivares and Vetter 1999). Indeed, our results parallel those reported by Almeida et al. (2005) for the semi-aquatic Neotropical water rat inhabiting off-shore islands in Brazil.

2. With fecal DNA analyses it is possible to identify important source populations worthy of conservation. For example, our data suggest that river otters produced in Paramanof, Foul, and Malina Bay on Afognak Island may immigrate to Blue Fox Bay and replenish that population, which is exposed to relatively high levels of trapping (Golden et al. 2009).
3. By assessing geneflow between source populations it is possible to identify geographical locations that serve as corridors and thus merit special protection. For example, much of the coastline in LACL consists of muddy tidal flats that are selected against by river otters. In our survey of the shoreline we found few latrines and none of the samples we collected yielded viable DNA. Nonetheless, our results suggest that this area may be an important corridor for geneflow among otter populations in KATM and PWS. Thus, this area merits enhanced status of protection.
4. While monitoring river otter populations with fecal DNA analyses it is possible to evaluate their effects on the carbon sequestration capacity of Alaskan coastal forests. Recently we were able to demonstrate that conifers growing on river otter latrines have 2.45 times higher photosynthetic capacity than their conspecifics growing on adjacent non-fertilized sites (Roe et al. *In review*). By estimating fecal deposition rate at latrines we were able to unequivocally link this higher photosynthetic capacity to marine-derived nutrients brought to latrines by otters (Roe et al. *In review*).

To reduce time, effort, and cost associated with monitoring river otters with fecal DNA analyses, we recommend observers preferentially collect samples that contain anal gland secretions. Also, any sample should be discarded if it does not amplify after three PCRs with the most reliable primers (e.g., RIO-19, LUT-733 and LUT829), and one or more of the following conditions apply: 1) it contains parasites, 2) it contains remains of

Cottidae and Stichaeidae, or 3) it was collected when ambient temperatures exceeded 16˚C. Our analyses demonstrated that there was no observer bias in the identification of samples that would yield DNA. Also, none of the habitat features that we measured could explain genotyping success or failure. Thus, it will be possible to train novice observers to identify otter latrines based on habitat features as well as to collect high quality samples.

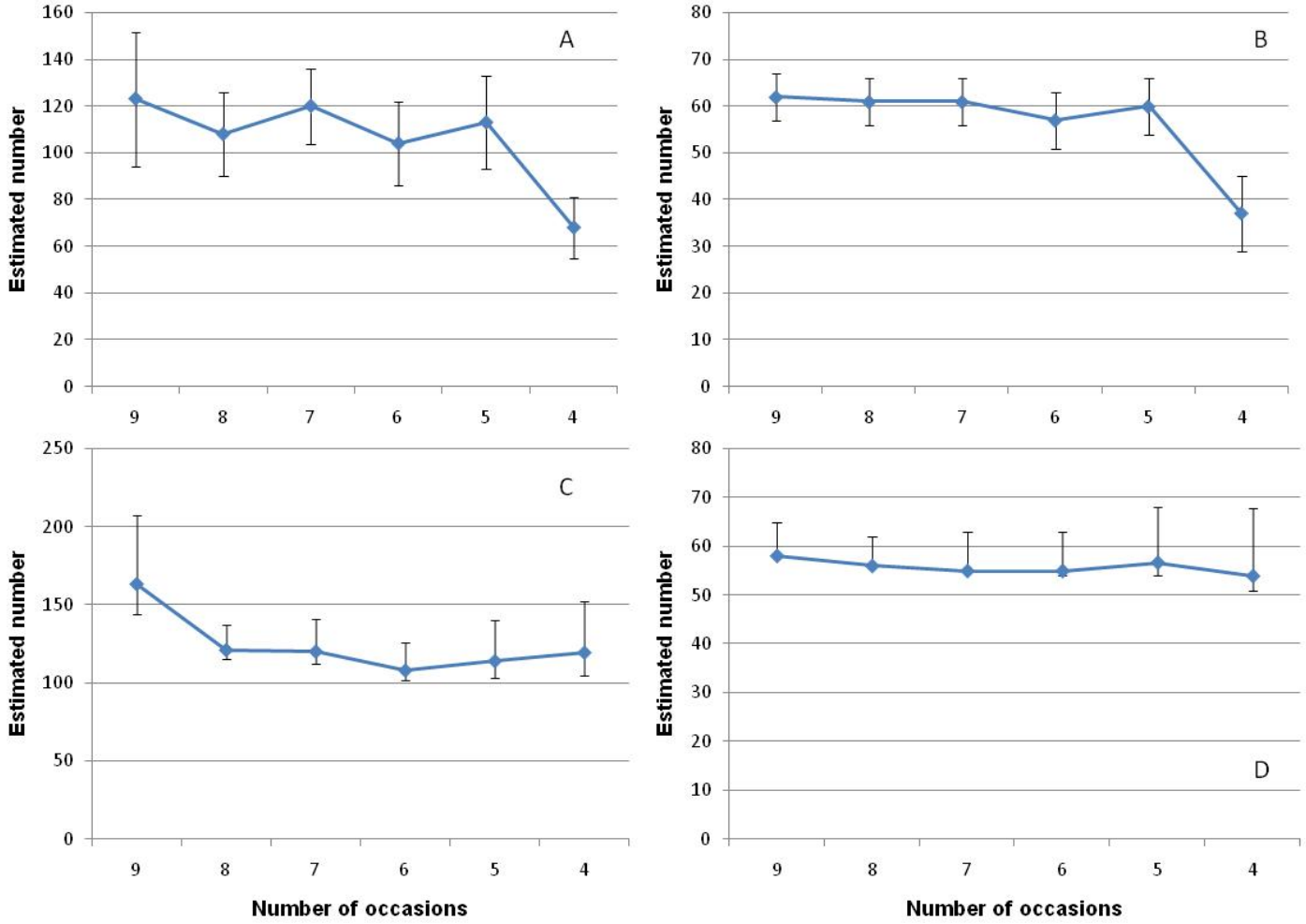
It is unfortunate that we were unable to formally estimate abundance of river otters in all sampled populations. Our conclusion that latrine densities and fecal deposition rate were poor predictors of otter abundance relied only on values of MNKA, which likely do not represent abundance (Amstrup et al. 2005). Unfortunately, to obtain formal estimates, it is likely that we would have needed to conduct at least 6 sampling occasions in each area at each survey. In PWS 2006, the first 6 occasions were conducted between May 25 and July 25, a period too long for most monitoring studies. Recently (June 2009), we sampled 60 river otter latrines in Herring Bay, Lower Passage, and Eleanor Island over 11 consecutive days to explore whether formal estimates can be obtained over a shorter time period. Results from the 787 samples we collected in that effort are pending.

Despite our failure to obtain formal population estimates for KEFJ, KATM, KOD, and most of PWS, our results provide the first large-scale description of the distribution of river otters in southcentral and southwestern Alaska. It is a first glimpse at the range of naïve densities and the differences in such measures between several geographic areas. It is also the first rigorous attempt to assess the validity of indices of relative abundance. Future efforts should be dedicated to refining of monitoring protocols and their implementation in advance of environmental change.

Other accomplishments

In 2005, we tested the efficacy of deploying non-invasive hair snares in KEFJ. Between 1-3 snares were set on 48 latrines on the mark occasion and collected 24 hours later during the recapture occasion. Hair snaring success was 1 capture per 3.6 trap-nights and yielded a total of 20 samples for DNA analyses (DePue and Ben-David 2007).

Figure 16. Estimated number (± 95% confidence interval) from capture-recapture data derived from fecal genotyping of river otters in Herring Bay, Lower Passage, and Eleanor Island in Prince William Sound, Alaska, USA. Samples were collected in nine occasions between May 25 and August 15, 2006. (A) Estimates derived from open-population models with the full dataset (131 individuals); (B) estimates derived from open-population models with the residents only dataset (58 individuals); (C) estimates derived from closed-population models with the full dataset (131 individuals); (D) estimates derived from closed-population models with the residents only dataset (58 individuals).



Acknowledgements

The following people were instrumental in data collection and analyses: Shannon Albeke (UGA), Alan Bennett (NPS-SWAN), Jessica Boyd (UW), Aaron Christ (ADFG), David Crowley (ADFG), John DePue (UW), Greg Fahl (KATM), Matt Gray (KEFJ), Dan Guertin (SFU), Heidi Hansen (UW), Michael Harrington (ADFG), Matthew Hayes (UW), Jason Herreman (UW), Tahzay Jones (KATM), Mandy Kauffman (UW), Jennie Lewis (UW), Dan Logan (USFS), Ian Martin (KEFJ), Wayne Melquist (Idaho), Nathan Nibbelink (UGA), Kaithryn Ott (UW), Aaron Poe (USFS), Todd Rinaldi (ADFG), Mathew Seymour (UW), Carol Scott (Northstar Borough School District), Elizabeth Solomon (ADFG), Michael Tetreau (KEFJ), Marci Trana (UW), and James Wendland (ADFG). We thank the crew of the M/V Serac for providing logistical support for our work in KEFJ and KATM. Similarly we thank the crew of the M/V Babkin for their help in the PWS and KOD surveys. Without the enthusiasm, willingness to help and accommodate, and the wonderful food they cooked, this project could not have been done. We very much appreciated the efficient logistical support provided by the staff of KEFJ, KATM and LACL. We also thank the crew of the M/V Chigmit for providing logistical support, safe passage, and expert guide service along the LACL coast and crossing Cook Inlet. Field quarters in LACL were provided by Silver Salmon Creek Lodge. Funding for the project was provided by the National Park Service, Southwest Alaska Network Inventory & Monitoring Program, the US Forest Service – Chugach National Forest, and the Oil Spill Recovery Institute. Funding for the work in Prince William Sound in 2006 was provided by the National Science Foundation. Additional support was provided by the University of Wyoming NSF-EPSCoR program, The College of Arts & Sciences, and the Department of Zoology and Physiology. The Alaska Department of Fish and Game provided logistical support and personnel.

Literature Cited:

Almeida, F. C., L. S. Maroja, M. A. M. Moreira, H. N. Seuánez and R. Cerqueira. 2005. Population structure and genetic variability of mainland and insular populations of the Neotropical water rat, *Nectomys squamipes* (Rodentia, Sigmodontinae). Genetics and Molecular Biology 28: 693-699.

Amstrup, S. C., T. L. McDonald, and B. F. J. Manly, Eds. 2005. *Handbook of Capture-Recapture Analysis*. Princeton University Press. Princeton, New Jersey.

Bang, I. and C. N. K. Moores. 2003. The influence of several factors controlling the interactions between Prince William Sound, Alaska and the northern Gulf of Alaska. American Meteorological Society 33: 19-36.

Beerli, P. and Felenstein, J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences of The United States of America 98: 4563-4568.

Beheler, A. S., J. A. Fike, L. M. Murfitt, O. E. Rhodes Jr., T. S. Serfass. 2004. Development of polymorphic microsatellite loci for North American river otters (*Lontra canadensis*) and amplification in related Mustelids. Molecular Ecology Notes 4: 56-58.

Beheler, A. S., J. A. Fike, G. Dharmarajan, O. E. Jr. Rhodes, and T. S. Serfass. 2005. Ten new polymorphic microsatellite loci for North American river otters (*Lontra canadensis*) and their utility in related mustelids. Molecular Ecology Notes 5**:** 602-604.

Ben-David, M., R. T. Bowyer, and J. B. Faro. 1996. Niche separation by mink and river otters: coexistence in a marine environment. Oikos 75: 41–48.

Ben-David M., G. M. Blundell, and J. E. Blake 2002. Post-release survival of river otters: effects of exposure to crude oil and captivity. Journal of Wildlife Management 66: 1208-1223.

Ben-David, M., G. M. Blundell, J. W. Kern, J. A. K. Maier, E. D. Brown, and S. C. Jewett. 2005 Communication in coastal river otters: creation of a variable resource shed for terrestrial communities. Ecology 86: 1331-1345.

Blundell, G. M., M. Ben-David, P. Groves, R. T. Bowyer, and E. Geffen. 2002. Sex-biased dispersal and gene flow in coastal river otters: implications for natural recolonization of extirpated populations. Molecular Ecology 11: 289–303.

Bowyer, R. T., J. W. Testa, J. B. Faro, C. C. Schwartz, and J. B. Browning. 1994. Changes in diets of river otters in Prince William Sound, Alaska: effects of the *Exxon Valdez* oil spill. Canadian Journal of Zoology 72: 970–976.

Bowyer, R. T., J. W. Testa, and J. B. Faro. 1995. Habitat selection and home ranges of river otters in a marine environment: effects of the *Exxon Valdez* oil spill. Journal of Mammalogy 76: 1-11.

Bowyer, R. T., G. M. Blundell, M. Ben-David, S. C. Jewett, T. A. Dean, and L. K. Duffy. 2003. Effects of the *Exxon Valdez* oil spill on river otters: injury and recovery of a sentinel species. Wildlife Monographs 153: 1–53.

Broquet, T. and E. Petit. 2004. Quantifying genotyping error in noninvasive population genetics. Molecular Ecology 13: 3601-3608.

Burnham, K. P., and D. R. Anderson. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer, New York, New York, USA.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21: 550–570.

Crait, J. R., G. M. Blundell, K. E. Ott, J. K. Herreman, and M. Ben-David. 2006. Late seasonal breeding of river otters in Yellowstone National Park. American Midland Naturalist 156:189-192.

Dallas, J. F., and S. B. Piertney. 1998. Microsatellite primers for the Eurasian otter. Molecular Ecology 7: 1247-1251.

Dallas J. F., K. E. Coxon, T. Sykes, P. R. F. Channin. 2003. Similar estimates of population genetic composition and sex ratio derived from carcasses and faeces of Eurasian otter *Lutra lutra*. Molecular Ecology 12: 275-282.

DePue, J. E. and M. Ben-David. 2007. Hair snaring techniques for river otters: a comparison of three trap designs. Journal of Wildlife Management 71: 671-674.

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

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164: 1567-1587.

Farrell, L. E., J. Roman, and M. E. Sunquist. 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. Molecular Ecology 9: 1583–1590.

Felsenstein, J. 1989. PHYLIP - Phylogeny Inference Package (Version 3.2). Cladistics 5: 164-166.

Foran, D. R., K. R. Crooks, and S. C. Minta. 1997. Species identification from scat: an unambiguous genetic method. Wildlife Society Bulletin 25: 835-839.

Frantz, A. C., L. C. Pope, P. J. Carpenter, T. J. Roper, G. J. Wilson, R. J. Delahay, and T.Burke. 2003 Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Molecular Ecology 12: 1649-1661.

Giblin, A. E., K. J. Nadelhoffer, G. R. Shaver, J. A. Laundre, A. J. McKerrow. 1991 Biogeochemical diversity along a riverside toposequence in Arctic Alaska. Ecological monographs 61: 415-435.

Golden, H. N., M. Ben-David, M. Seymour, and E. Solomon. 2009. Estimating coastal river otter abundance and harvest potential in the Kodiak Island Archipelago. Alaska Department of Fish and Game. Progress Report. 15 Pp.

Goossens, B., L. Chikhi, S. S. Utami, J. de Ruiter, and M. W. Bruford. 2000. A multi-samples, multi-extracts approach for microsatellite analysis of faecal samples in an arboreal ape. Conservation Genetics 1: 157-162.

Goudet, J. 2000. FSTAT. A program to estimate and test gene diversities and fixation indices. Version 2.9.3.2. www.unil.ch/lzea/softwares/fstat.htm.

Guertin et al. *In review*. Faecal genotyping reveals demographic differences between subpopulations of river otters inhabiting a contaminated coastal landscape. Molecular Ecology.

Guo S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics, 48: 361-372.

Hansen H. 2004. The effects of diet, extraction technique and microsatellite primers on genotyping success and error rates of river otter (*Lontra canadensis*) fecal DNA. MSc Thesis. University of Wyoming, Laramie, WY.

Hansen, H., M. Ben-David, and D. B. McDonald. 2008. Effects of genotyping protocols on success and errors in identifying individual river otters (*Lontra canadensis*) from their faeces. Molecular Ecology Resources 8: 282-289

Janssens, X., J. Michaux, M. C. Fontaine, R. Libois, J. de Kermabon, P. Defourny, and P. V. Baret. 2007. Non-invasive genetics at basin scale reveals barriers to the present otter recovery in Southern France. Ecography: .

Kohn, M., F. Knauer, A. Stoffella, W. Schroder, and S. Paabo. 1995. Conservation genetics of the European brown bear – a study using excremental PCR of nuclear and mitochondrial sequences. Molecular Ecology 4: 95-103.

Larsen, D. N. 1983. Habitats, movements, and foods of river otters in coastal southeastern Alaska. Thesis, University of Alaska Fairbanks, Fairbanks, Alaska, USA.

Lebreton, J.-D., K. P. Burnham, J. Clobert, and D. R. Anderson. 1992. Modeling survival and testing biological hypotheses using marked animals: A unified approach with case studies. Ecological Monographs 62: 67-118.

Lilleskov, E. A., T. J. Fahey, T. R. Horton, G. M. Lovett. 2001. Ectomycorrhizal fungal aboveground community change over an atmospheric nitrogen deposition gradient. Ecological Applications 11: 397-410.

Lucchini, V., E. Fabbri, F. Marucco, S. Ricci, L. Boitani, and E . Randi. 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. Molecular Ecology 11: 857 –868.

Melquist, W. E., and M. G. Hornocker. 1983. Ecology of river otters in west central Idaho. Wildlife Monographs 83: 1-60.

Melquist, W. E., and A. E. Dronkert. 1987. River otter. Pages 626-641 *in* M. Novak, J. A. Baker, M. E. Obbard, and B. Malloch, editors. Wild Furbearer Management and Conservation in North America. Ministry of Natural Resources, Toronto.

Mills, L. S., J. J. Citta, K. P. Lair, M. K. Schwartz, and D. A. Tallmon. 2000. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. Ecological Applications 10:283-294.

Morin, P.A., K. E. Chambers, C. Boesch, and L. Vigilant. 2001. Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). Molecular Ecology 10: 1835-1844.

Murphy, M. A, L. P. Waits, K. C. Kendall. 2002. An evaluation of long-term preservation methods for brown bear (*Ursus arctos*) faecal DNA samples. Conservation Genetics 3: 435-440.

Nsubuga, A. M., M. M. Robbins, A. D. Roeder, P. A. Morin, C. Boesch, and L. Vigilant. 2004. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. Molecular Ecology 13: 2089-2094.

Ott, K. E., H. N. Golden, and M. Ben-David. (*In prep* a). Recolonization or local reproduction? An assessment of river otter recovery in previously-oiled areas of coastal Alaska via non-invasive genetic sampling. Journal of mammalogy.

Ott K. E., S. A. Albeke, N. P. Nibbelink, and M. Ben-David. (*In prep* b). Abundance and movements of coastal river otters: implications for nutrient transports from sea to land. Journal of Animal Ecology.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology 12: 1375-1387.

Pledger, S, K. H. Pollock, and J. L. Norris. 2003. Open capture-recapture models with heterogeneity: I. Cormack-Jolly-Seber model. Biometrics 59: 786-794.

Pollock, K. H. 2000. Capture-recapture models. Journal of the American Statistical Association 95: 293-296.

Pradel, R., J. E. Hines, J. D. Lebreton, and J. D. Nichols. 1997. Capture-recapture survival models taking account of transients. Biometrics 53: 60-72.

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

Prigioni, C, L. Remonti, A. Balestrieri, S. Sgrosso, .2006. Estimation of European otter (*Lutra lutra*) population size by fecal DNA typing in southern Italy. Journal of Mammalogy 87: 855–858.

Prugh, L. R., C. E. Ritland, S. M. Arthur, and C. J. Krebs. 2005. Monitoring coyote population dynamics by genotyping faeces. Molecular Ecology 14: 1585-1596.

Raymond, M., and F. Rousset. 1995. Genepop version 1.2: population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.
Rocha-Olivares, A., and R. D. Vetter. 1999. Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). Canadian journal of Fish and Aquatic Science 56: 803-813.
Roe, A. M., C. B. Meyer, N. P. Nibbelink, and M. Ben-David. (*In review*) Differential production of trees and shrubs in response to fertilization and disturbance by coastal river otters in Alaska. Ecology.
Roe, A. M., C. B. Meyer, M. J. Wood, B. J. Meyer, N. P. Nibbelink, and M. Ben-David. (*In prep*) Changes in the diversity and cover of a coastal herbaceous plant community in response to fertilization and disturbance by river otters. PLoS One.
Rostain, R. R., M. Ben-David, P. Groves and J. A. Randall. 2004. Why do river otters scent mark? An experimental test of several hypotheses. Animal Behaviour 68: 703-711.
Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.
Seber, G. A. F. 1982. The Estimation of Animal Abundance, second edition. Macmillan, New York, New York, USA.
Seymour, M. S., K. E. Ott, D. A. Guertin, H. N. Golden, D. B. McDonald, and M. Ben-David. (*In prep*). Early Holocene glacial retreat isolated river otter populations along the Pacific Coast. Molecular Ecology
Swimley, J. T., L. T. Serfass, P. R. Brooks, and M. W. Tzilkowski. 1998. Predicting river otter latrine sites in Pennsylvania. Wildlife Society Bulletin 26: 836-845.
Testa, J. W., D. F. Holleman, R. T. Bowyer, and J. B. Faro. 1994. Estimating populations of marine river otters in Prince William Sound, Alaska, using radiotracer implants. Journal of Mammalogy 75: 1021–1032.
Valière, N. 2002. GIMLET: a computer program for analysing genetic individual identification data. Molecular Ecology Notes 2: 377-379.
Weir, B. S. and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358-1370.
White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46: 120-139.
Williams, B. K., I. D. Nichols, M. J. Conroy. 2002. *Analysis and management of animal populations: modeling, estimation, and decision making*. Academic Press, San Diego.
Zar, J.H. 1999. *Biostatistical Analysis*. Prentice-Hall, Inc, New Jersey, USA.