RIVER OTTER POPULATION MONITORING IN NORTHEASTERN PENNSYLVANIA USING NON-INVASIVE GENETIC SAMPLING AND SPATIAL CAPTURE-RECAPTURE MODELS

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by
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ABSTRACT

River otter (*Lontra canadensis*) populations in Pennsylvania experienced a range reduction and subsequent expansion of the remnant population, as well as re-colonization of parts of the state through reintroduction efforts and expansion of neighboring populations. There are currently no estimates of population size or densities for river otter populations in Pennsylvania, and large-scale monitoring efforts are hampered by the elusive behavior of river otter. Non-invasive genetic sampling has been used to survey river otter populations, but given the river otter’s unique distribution across the landscape, estimation of population size and densities has been limited to linear habitats in river systems or along coastlines. Spatial capture-recapture models incorporate spatial information from captures into the estimation process, and estimates are more explicitly linked to the area in which observations occur. I analyzed the efficacy of non-invasive genetic sampling to identify individual river otter and I used spatial capture-recapture models to estimate river otter population size and density, and in northeastern Pennsylvania.

I surveyed nine counties in northeastern Pennsylvania, opportunistically collecting samples from latrine sites on public and private land. Latrines were visited on three to four occasions at 6–14 day intervals, clearing latrines after each visit, in a capture-recapture framework. I amplified DNA extracted from the samples at ten microsatellite markers, to generate a genotype for each sample. I matched genotypes using program CERVUS to identify individuals.

My first analysis compared amplification success rates and error rates for samples of different type and time of environmental exposure or freshness, and compared my
amplification success rates to other studies. Previous studies on river otter had lower genotyping success rates than those for other otter species, and did not follow a common sampling protocol despite laboratory studies for the river otter and recommendations from field studies on other otter species. My amplification success rates were most comparable to those from studies on otter species conducted in the winter with samples collected in a storage buffer. I observed similar patterns of success rates as other studies for different sample types and samples classified for different categories related to lengths of environmental exposure, but had higher success rates for every category. Amplification error rates for the different sample types and environmental exposure categories were not reported in the literature, but I included them in the study as another measure of sample quality and to better inform future studies. The importance of comparing success rates and error rates is to better inform future studies on the preferred sampling protocol, and give measures for the amount of effort necessary for studies looking to use non-invasive genetic sampling to identify individual river otter for population analyses.

To estimate population size and density in spatial capture-recapture models, I compiled spatial encounter histories given the location and occasion of collection of each sample assigned to an individual. I also used full likelihood models in program MARK to test for differences in capture and recapture probabilities. I reported the first density estimates for a river otter population in northeastern Pennsylvania (2.1 otter/100 km², 1.4–5.0 otter/100 km² 95% Asymptotic Wald-type CI). The estimates of capture and recapture probabilities in the MARK model with those parameters estimated separately indicated that capture and recapture probabilities were not different, but that the
probability of capturing an individual did vary by occasion. I observed a difference in density estimates for my SCR and MARK models. I would recommend using SCR models because of the spatial justification for density estimates, and the ability to include landscape covariates to build more informed models, which may prove to be useful for river otter given their unique space use.

Future studies conducting non-invasive genetic sampling for river otter should conduct their studies in winter and use a storage buffer for samples. Sample type and length of environmental exposure should be considered when considering the amount of sampling effort to derive a genotype for identification of individual otter. NGS and SCR can be used to generate reliable population or density estimates, but as I documented from my MARK estimates of capture probability, numerous sampling occasions are desirable because of the variation in capture probability between occasions. Spatial capture-recapture models are preferable for river otter in Pennsylvania because the area for which density is being estimated is directly tied into the model, which is ideal given the diversity of linear and non-linear habitat types in northeastern Pennsylvania.
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Chapter 1

An introduction to monitoring river otter in Pennsylvania
Introduction
River otter biology and monitoring

The North American river otter (*Lontra canadensis*) is described as an elusive species due to its cryptic behavior (Melquist and Hornocker 1983, Kruuk 2006). Direct observation of the species is made difficult by its aquatic lifestyle and nocturnal habits (Melquist and Hornocker 1983). The relatively large size of home range, low densities, and difficulty to trap limit the use of live trapping and radio-telemetry in monitoring efforts (Melquist and Hornocker 1983, Kruuk 2006). The species is dependent on and closely aligned with aquatic habitat, but frequently, especially in winter, will travel across terrestrial landscapes as well (Melquist and Hornocker 1983). The non-uniform distribution of river otter across the landscape confounds assumptions necessary for estimating otter densities over large areas with a mosaic of aquatic and terrestrial habitats. Density is not likely uniform across the entire landscape but cannot be solely estimated for one body of water or river system because of the potential to cross over-land to other water bodies or watersheds.

The river otter is a species of interest to management and conservation because of its ecological role as a top-level predator, its value as a furbearer, and the reintroduction efforts that went into reestablishing the species in 21 states (Raesly 2001). In Pennsylvania, river otter became rare or extirpated across much of their range by the early 20th century as a result of unsustainable harvest, pollution, and habitat loss, with a remnant population restricted to the northeast part of the state (Serfass et al. 1993). Concomitant with reintroduction efforts that began in the 1980’s with river otter sourced from other states (e.g. Louisiana and New York) as well as the remnant Pennsylvania population (Serfass et al. 1998), natural expansion of the remnant population and
neighboring populations in adjacent states enabled river otter populations to expand across Pennsylvania (Lovallo and Hardisky 2011).

Populations of river otter in the northeast have not been extensively sampled for population-level analyses (Swimley et al. 1998, Gallant et al. 2009, Stevens et al. 2011, Just et al. 2012). Current monitoring for otter is often conducted using presence-absence of otter sign at bridge sites (Crimmins et al. 2009), radio-telemetry (Blundell et al. 2002), scat and latrine counts (Swimley et al. 1998, Gallant et al. 2009), and track surveys in snow (Kruuk 2006, Arrendal et al. 2007). These techniques are not ideal for the monitoring of river otter because of their invasive nature (i.e. radio-telemetry), cost and limited scope (live-trapping mark-recapture) (Johnson et al. 2013), or inability to produce consistent and robust results (i.e. track surveys and scat counts) (Arrendal et al. 2007). States with a regulated trapping season for river otter have the option to utilize harvest data as a means of monitoring populations (Chilelli et al. 1996). However, for those states without a harvest, an effective and comprehensive monitoring technique is needed to assess the status of populations and establish management needs of the species.

Non-invasive genetic sampling

An increasingly used method for the detection and analysis of rare and elusive populations is noninvasive genetic sampling (NGS), which minimizes disturbance of the animal and does not require direct detection of individuals (Waits 2004). Noninvasive genetic sampling is the collection of hair, feces, skin, or urine, which contain sloughed epithelial cells and thus deoxyribonucleic acid (DNA) of the target species (Waits 2004). This method has been used for species identification, examination of evolutionary relationships or hybridization, and population-level analyses (Allendorf et al. 2006).
Thorough reviews of NGS and descriptions of its use have been conducted (Kohn and Wayne 1997, Allendorf et al. 2006), and the technique has been used on multiple species such as bear (Ursus arctos; Taberlet and Bouvet 1992), wombats (Lasiorhinus kreftii; Banks et al. 2003), elephants (Eggert et al. 2003), badgers (Wilson et al. 2003), coyotes (Canis latrans; Prugh et al. 2005), and giant otter (Pteronura brasiliensis; Pickles et al. 2012).

The DNA obtained from NGS is extracted from the sample, and the polymerase chain reaction (PCR) is used to amplify a genetic fragment specific to the species. The sources of the fragment will either be mitochondrial DNA or nuclear DNA, and the type of fragment used can vary from a large fragment of genetic material (i.e. restriction fragment length polymorphism) to smaller fragments of genetic material (microsatellites and single nucleotide polymorphisms) (Waits and Paetkau 2005).

The basis for using these fragments as a means of identification is that species-specific fragments can be amplified and thus the resulting DNA that is visualized for analysis is known to be that species and not the DNA from any of the prey or bacterial species that could also be found on the feces. Natural variation and mutations in genetic material create unique combinations of fragments of different sizes (alleles) in individuals, and for diploid organisms there are two alleles, one for each of the two homologous chromosomes on which the microsatellite locus is found.

When the goal is individual identification for population analysis, it is common to use multiple microsatellite loci found on the nuclear DNA (Waits 2004, Allendorf et al. 2006). These microsatellite loci are fragments of non-conserved (i.e. non-coding and thus not detrimental to health if they do have mutations), repetitive base-pair compositions at
specific locations in the genome (Allendorf et al. 2006). These paired alleles, one allele inherited from each parent, may both be the same size allele (homozygous, the same number of base pairs), or two alleles of different size (heterozygous, different number of base pairs) (Allendorf et al. 2006). The number of repetitions of base pairs varies between individuals because of natural variation and mutations, and inheritance of one allele from each parent, thus resulting in polymorphic alleles in a large enough, free-mixing population (Allendorf et al. 2006). Individuals can be identified by their unique composition of these polymorphic alleles across a number of microsatellite loci (Allendorf et al. 2006). However, a sufficient number of microsatellite loci needs to be used in combination to reduce the probability of two individuals sharing the same genotype, with the recommended number being at least six to eight (Waits 2004). The more genetically similar a population is, for instance due to inbreeding, more microsatellite loci are needed for differentiation, because closely related individuals are likely to share genetic material. The unique microsatellite composition of an individual then serves as a reliable mark or tag that is never lost and can be sampled over its lifetime (Lucaks and Burnham 2005).

There are a number of considerations that have to be made when considering NGS for study design. The benefits of NGS in terms of animal welfare and detection of rare and elusive species is conceptually straightforward, and studies have shown that there are comparable results for population analyses between data collected by NGS and data collected using more traditional methods (Banks et al. 2003, Waits 2004, Ruibal et al. 2009). The ability to identify individuals without direct contact or invasive procedures is one of the benefits of NGS. There is also less of a chance of ‘trap shyness’ or bias that
is often seen in invasive trapping regimes, as animals avoid a trap after their first exposure or are attracted to traps as a result of baiting (Marucco et al. 2011). In a comparison between traditional trapping methods and population estimation from fecal DNA sampling, Ruibal et al. (2009) found NGS of latrines for a spotted-tailed quoll (*Dasyurus maculatus*) yielded 81% of individuals from the mark-recapture trapping regime, and 3 additional individuals not trapped. Radio-telemetry did not have any added value when used in addition to a NGS regime for coyote (*Canis latrans*) in Alaska (Prugh et al. 2005). Additional studies with a variety of species (Northern hairy-nosed wombats, *Lasiorhinus krefftii*, Banks et al. 2003; tigers, *Panthera tigris*, Mondol et al. 2009) have found similar results indicating that NGS is comparable to both traditional methods and other non-invasive methods of wildlife monitoring.

**Mitigating issues with non-invasive genetic sampling**

Considerations must be made for the potential biases that may come from NGS, and many studies have investigated methods and study designs that can mitigate or eliminate these biases. Many of the issues associated with NGS stem from the genetic material being low quality and low-quantity, which can lead to misidentification or increase the amount of effort needed to obtain results. The quantity of genetic material is low because of the low numbers of epithelial cells typically coating the sample, although this depends on the sample type (i.e. feces, hair, urine; Kohn and Wayne 1997) and species (Frantzen et al. 1998). The amount of material also varies based on the type of DNA with which the fragment is associated, with mitochondrial DNA being in higher quantities in a cell than nuclear DNA (Waits and Paetkau 2005).
The quality of the DNA contained in these samples is typically degraded because of exposure to environmental conditions such as heat, humidity, and ultra-violet light. Temperature and humidity can result in increased bacterial or enzymatic DNA degradation (Fike et al. 2004), while exposure to ultraviolet light is a common decontamination method in genetics laboratories because of its ability to destroy genetic material (Champlot et al. 2010).

Starting with low-quality and low quantity source DNA leads to PCR amplification errors that cause missing alleles (allelic dropout), or false alleles due to stutter bands or PCR misprinting (Creel et al. 2003). Allelic dropout is the misidentification of a heterozygous genotype at a locus as homozygous, because of errors, typically in the PCR amplification, which causes the loss of one of the unique sized alleles (Broquet and Petit 2004). The missing allele may not amplify at all, or may amplify at a much lower magnitude such that it isn’t recognized as occurring (Broquet and Petit 2004). False alleles or null alleles are PCR artifacts, the fabrication of a unique-sized allele by misprinting of a true allele (Broquet and Petit 2004). Typically null alleles are described as the occurrence of an additional allele for a homozygous genotype at a locus that causes it to appear heterozygous. These errors in amplification can then cause misidentification of the genotype of a sample (Creel et al. 2003, Piggot et al. 2003, Broquet and Piquet 2004). Advancements in technology and protocol, or incorporation of these errors into modeling, have mitigated the effects of these errors to some extent (Piggot et al. 2003, Waits 2004, Lucaks and Burnham 2005, Allendorf et al. 2006).

Another way to mitigate the errors associated with NGS is to minimize exposure of samples to unfavorable environmental conditions. Temporal considerations such as
collecting samples within hours of deposition or collecting feces in the morning for nocturnal species reduces the amount of time the samples are exposed. Avoidance of sampling during the summer, when UV light and heat are typically elevated compared to autumn\winter, will also reduce exposure to harmful environmental exposure (Prigioni et al. 2006). Adjusting the sampling season to minimize exposure to UV and high temperatures is a simple step to improving sample quality, if sampling during non-summer months is feasible.

Studies focusing on feces need to make considerations for the behavior of the species in relation to feces deposition because the amount and location of samples can vary seasonally due to behavioral differences (Ruibal et al. 2009, Just et al. 2012). Behavioral differences may exist between the sexes when feces deposition is linked to sexual or territorial behavior. Seasonal shifts in detection of different sex and age classes from fecal samples were a result of absence of adult females during synchronous birthing seasons, increased presence of sub-adults during dispersal, and skewed presence of sexually mature individuals during breeding season in spotted-tailed quoll (Ruibal et al. 2009). Therefore studies should be conducted when fecal deposition is at its peak and there is limited seasonal bias amongst sex and age groups, and the duration of the study must also be adequate to allow for a large proportion of individuals in the population to be available for sampling (Ruibal et al. 2009).

**Spatial Capture-recapture models**

Non-invasive genetic sampling is unique in respect to its ability to capture an individual at multiple times within a sampling occasion, but traditional capture-recapture models cannot incorporate multiple encounters in the same sampling occasion (Miller et
Spatial capture-recapture (SCR) models make use of multiple encounters within an occasion by incorporating temporal and spatial information from the capture of an individual. A comprehensive explanation of the background, development, and variations of SCR models can be found in Royle et al. (2014). In essence, SCR models are hierarchical models in which the observation process is conditionally related to the ecological process (i.e. population size and density) and both these processes are inherently spatial (Royle et al. 2014).

Spatial capture-recapture models use a spatial encounter history that includes both the occasion an individual was captured and the location it was captured (Royle et al. 2014). The spatial encounter history can thus handle multiple captures of an individual within an occasion, if these observations are at different locations, which is typical for non-invasively collected samples.

The spatial encounter history for each individual is used in the observation model that conditions all observations of an individual on the estimated ‘activity center’ of that individual, which is assumed to be the average location of an individual throughout the sampling duration (Royle et al. 2014). Encounter probability, for individual $i$ at trap $j$, is modeled as a function of the probability of detection if the activity center occurred at the trap location, $\alpha_0$, and the movement coefficient, $\alpha_1$:

$$p_{ij} = \text{logit}^{-1}(\alpha_0) \exp(-\alpha_1 \cdot D^2)$$

where $D$ is $|x_j - s_i|$, the distance between the trap, $x_j$, and the individual’s activity center, $s_i$. The movement coefficient, $\alpha_1$, is defined as:

$$\alpha_1 = \frac{1}{2\sigma^2}$$
where $\sigma$ is the spatial scale parameter that describes the decay of probability of detection with distance (Borchers and Efford 2008, Royle et al. 2014).

The points at which activity centers can occur are pre-defined as a set of points known as the state space, which is the ‘area’ in which estimation is occurring and in the model represents the area sampled. The ecological process defines the distribution of these activity centers, modeled by a point process (Illian et al. 2008, Royle et al. 2014). The point process estimates the distribution of activity centers and an estimated number of augmented activity centers, $N_a$, representative of individuals not observed through sampling but likely to occur across the state space. The sum of the observed individuals and unobserved individuals is the population estimate for the area, $\hat{N}$. The density of activity centers, $\hat{D}$, across the state space is then the product of the population estimate over the size of the state space.

The estimation of activity centers at points within an area pre-defined by the ‘trap’ locations intricately links the ecological process and space where observations occurred by a formal model. For traditional capture-recapture models, estimation and definition of the study area for which density will be estimated are two separate analytical steps (Royle et al. 2014). This link is important when considering that there are landscape level effects on spatial variations in estimates of a population. These models are able to incorporate landscape-level covariates to investigate spatial variations in abundance caused by phenomena such as landscape connectivity and resource selection (Royle et al. 2014). Spatial capture-recapture models are well suited for population analyses of rare or elusive species surveyed using NGS given that these models incorporate multiple captures within an occasion, the intricate link between estimation and the area of
observation, and the ability to then incorporate landscape covariates to more realistically model density given a species’ interactions with surrounding habitat. The combination of NGS and spatial capture-recapture modeling has been used in previous studies to estimate density for an elusive species (Kery et al. 2010), and investigate the difference in density estimates when estimation is made using SCR models as compared to traditional capture-recapture models (Gardner et al. 2009).

**River otter in Pennsylvania**

In Pennsylvania, current monitoring of river otter only assesses trends in distribution through Wildlife Conservation Officer Furbearer Questionnaires, which reports river otter complaints, incidental trappings, and road kills (Lovallo and Hardisky 2011). Given the history of extirpation and recovery in the state, an assessment of population dynamics and baseline population estimates is needed. The sampling method for such an assessment would need to be practical for coverage of a large area. A geographically broad survey in Pennsylvania would be ideal considering movement patterns of river otter (Melquist and Hornocker 1983), and given that management decisions are made over large areas.

With a feasible sampling method of river otter in Pennsylvania and proper sampling coverage, a comprehensive statistical model would be needed for estimation of population size and density. The model would need to be able to incorporate the complex mosaic of habitat and non-habitat for river otter in Pennsylvania, and have the capability to accommodate non-uniform distribution of individuals across the landscape. Spatial capture-recapture models are adaptable in that sense. The capacity for these models to include landscape data and account for spatial variation in density because of a species’
habitat requirements or movement is ideal for river otter. The diversity of aquatic habitat in Pennsylvania, which includes lakes, rivers, streams, and wetlands, make estimation over linear distance of watercourse unreasonable. The river otter’s tendencies to move over land and through non-aquatic habitat compound this issue of accurately depicting river otter distribution across the landscape (Melquist and Hornocker 1983). Given that the data from NGS has been successfully used to generate robust estimates in SCR models, use of these methods for monitoring river otter in Pennsylvania would be ideal for estimating density in the northeastern part of the state.
LITERATURE CITED


Chapter 2

Non-invasive genetic sampling of the North American river otter: review and implementation of recommended practices.
ABSTRACT River otter (*Lontra canadensis*) have been studied in North America with non-invasive genetic sampling (NGS) using fecal samples, but these studies often have low success rates for amplification and genotyping of recovered DNA. My objective was to assess practices from the literature for NGS on a variety of otter species, and provide recommendations for field and laboratory methods based on data collected in a field study. I collected fecal samples and stored them in 99% ethanol during the winters of 2013 and 2014 in northeastern Pennsylvania. I extracted DNA and amplified 10 microsatellite markers to genotype individual otter from samples. My genotyping success rate was higher than previous NGS studies on river otter (53.5%), and was comparable to studies on other otter species that sampled in the winter and used a storage buffer for preservation. I observed similar patterns to those in the literature for the effects of sample type and time since deposition of samples or freshness on amplification of DNA, which would strengthen recommendations for collecting samples containing anal jellies and samples deposited within 24 hours. Overall, I demonstrated that relatively high genotyping success rates for otter fecal samples could be achieved, but that success is contingent on sampling in the correct season and using the correct preservation method.

KEYWORDS non-invasive sampling, fecal genotyping, *Lontra canadensis*, winter, buffer

Introduction

The use of molecular techniques in wildlife management and conservation has enabled study of rare and elusive species. The collection of deoxyribonucleic acid (DNA) from
sources such as hair and feces, known as non-invasive genetic sampling (NGS), allows for a range of analyses (Kohn and Wayne 1997, Waits 2004). Assigning a genotype to a non-invasively collected sample is hindered by the low quantities of DNA present (Waits and Paetkau 2005), however, and the exposure of samples to environmental and ecological conditions that degrade DNA (Frantzen et al. 1998, Broquet and Petit 2004). As a result, there may be fewer samples to work with or misidentification of alleles and thus misidentification of individuals or biased allele frequencies (Kohn and Wayne 1997, Waits et al. 2001). The errors associated with low-quality DNA can lead to biases and errors when analyzing microsatellite markers for population estimation (Creel et al. 2003, Marucco et al. 2011).

Numerous studies have given general suggestions for mitigating the effects of low-quality and low-quantity DNA from NGS (Waits and Paetkau 2005). Most common are recommendations for storage and preservation of samples (Frantzen et al. 1998) or protocols for polymerase chain reaction (PCR) amplification (Taberlet et al. 1996). The inherent errors of amplifying low-quality and low-quantity DNA have also led to development of methods for quality control and error checking (Marucco et al. 2011). The changes in protocol necessary to mitigate these effects can increase the amount of effort needed or the costs associated with sampling and laboratory work. Therefore, it is important to invest careful consideration into each step of the NGS process, from sampling design and laboratory procedures to analysis methods and models, resulting in clear recommendations to inform future research. The best practices vary by species (Frantzen et al. 1998, Waits and Paetkau 2005), however, and for a given species there
are typically sparse or conflicting results, and unclear suggestions (Fike et al. 2004, Mowry et al. 2011, Brzewski et al. 2013).

The North American river otter (*Lontra canadensis*) is described as an elusive species (Kruuk 2006), and it was rare or extirpated across much of its range in the continental United States by the early 20th century. River otter have been studied in North America with NGS using fecal samples (Mowry et al. 2011, Brzewski et al. 2013), but success rates for amplifying and genotyping otter DNA from feces have been lower than for other mammalian species (Dallas et al. 2003, Hansen et al. 2008). Studies frequently describe genetic material found on otter feces as low quantity and low quality without direct explanation of the cause of this low recovery of DNA (e.g., PCR inhibitors present in prey or low number of intestinal epithelial cells shed compared to other mammal species; Dallas et al. 2003, Hansen et al. 2008). Studies have focused on the effects of a variety of factors on genotyping success rates and genotyping errors, including: time since deposition of samples or the freshness (Lampa et al. 2008, Mowry et al. 2011), type (i.e., scat or anal jelly), and section (i.e., ends or center of the scat) of the sample taken (Fike et al. 2004, Lampa et al. 2008, Mowry et al. 2011), length and method of storage (Fike et al. 2004, Hájková et al. 2006, Lampa et al. 2008), extraction method (Hájková et al. 2006, Lampa et al. 2008), and amplification method (Lampa et al. 2008). While there have been studies looking into the best practices for NGS of river otter in North America (Fike et al. 2004, Hansen et al. 2008), in general the sample success rates for field studies have been low when compared to otter species in other countries (Mowry et al. 2011, Brzewski et al. 2013). Despite recommendations from studies with animals in captivity and studies of otter species in other countries (Fike et al. 2004, Hájková et al. 2006,
Lampa et al. 2008), the recommended practices have not been thoroughly followed in NGS field studies for river otter in North America.

Marucco et al. (2011) suggested that to increase precision, decrease biases, and increase efficiency using NGS for population estimation, three steps require careful consideration: (1) designing the appropriate sampling scheme; (2) conducting the genetic lab analysis; (3) and applying the capture-recapture (CR) analysis to the molecular dataset. The sampling design should focus on collecting samples in an efficient way that minimizes contamination and collection of samples with expected errors. The genetic lab analysis should minimize genotyping errors through multiple approaches, and reduce the number of samples that need to be eliminated from analysis (Marucco et al. 2011). Based on these recommendations, my objectives were to: (1) compare success rates for DNA detection, amplification, and error rates to previous NGS studies on river otter, (2) further develop guidelines for sampling in the field, specifically which samples to take based on freshness and type, and (3) determine whether additional extractions and additional amplifications for samples in which no results or poor results were observed generated reliable data and information that may have otherwise been lost. By comparing my results from a field study to other studies in the literature, I hope to give strict guidelines to the protocols that are essential to performing a NGS study for North American river otter.

METHODS

Field Sampling

I collected samples January–March 2013 and 2014 in northeastern Pennsylvania (Fig. 2-1). I surveyed all accessible public and private land to collect samples on lakes,
impoundments, rivers, streams and wetlands. From hereon I refer to all otter excrement as feces, while distinguishing scat samples as excrement and anal jellies as secretions from the otters’ anal glands. Freshness of samples was estimated when possible based on position within or on top of snowfall. I developed a general impression of freshness by leaving samples estimated as deposited within 24 hours because of their position on top of fresh snowfall, at latrines I revisited throughout the season. My criteria for assessing freshness aside from position in snowfall, were that scat retained organic digested material other than bones, scales and carapaces of prey, and retained a dark color. For revisits to latrines, the time since deposition was known to be at maximum the total amount of days since the previous visit. I then estimated the time since deposition based on these criteria as the number of days from deposition, and I fully acknowledge the possibility of overlap or inaccuracies with my estimations. I then assigned samples to a freshness category (freshness category 1 - less than one day since deposition, freshness category 2 - between one and six days since deposition, and freshness category 3 - greater than six days since deposition).

I collected the ends of feces, about 2 cm in length, from between one and four individual feces within a distinct pile at latrines using tweezers flamed for sterilization prior to and after collections. Samples were immediately stored in 99% ethanol in 15 mL screw-top vials. Samples remained in the collection vials at room temperature for a maximum two weeks while in the field, and were stored in the collection vials in the lab at 4°C for a maximum of four months.

Laboratory Protocol
Prior to the extraction process I removed about 2 cm of the sample and placed it on filter paper to absorb excess ethanol from the sample (Koelewijn et al. 2010). Extractions followed the protocol described in the QIAamp Stool Kit (QIAGEN), modified only by increasing the amount of the time spun in the centrifuge to six minutes after the addition of the Inhibitex©. I performed a negative extraction control with every extraction run. The final extraction product was stored in a 2 mL screw top tube at −40°C.

I used the ten primers used by Mowry et al. (2011) for two multiplex PCR’s (Table 2-1), but modified their reaction as follows: 5.6 uL of Multiplex PCR Master Mix (QIAGEN) was used instead of 5.0 uL, and I did not use bovine serum albumin and instead added deionized water to bring the reaction volume to 10 uL. I used a modified thermocycler profile from Mowry et al. (2011), changing the number of cycles for denaturation to forty-two. Reactions were run in a 96-well plate. Three replicates for each sample were run per plate, following the multiple-tubes approach (Taberlet et al. 1996). A positive tissue control and negative extraction control were run with each 96-well plate to confirm amplification and detect contamination. Fragment analyses were performed at Pennsylvania State University’s Huck Institute Genomics Core Facility on an ABI3730XL DNA sequencer (Applied Biosystems), using the LIZ500 allelic size ladder. Results from the fragment analysis were analyzed in GeneMapper (Applied Biosystems) to score alleles.

I optimized the multiplex conditions and determined which multiplex would be used to screen for usable DNA by running the most recent 29 samples through both multiplexes. I determined that Multiplex 2 was the best to screen samples for DNA and eliminate those without adequate DNA from further analysis. Samples were chosen for
further analyses if: (1) amplification occurred at least twice at marker RIO-08R and two times at any combination of other loci, or (2) amplification occurred at least twice for at least two other loci (occurring two times at both loci). Samples that passed the screening multiplex were then run in Multiplex 1. Samples that needed further amplifications to provide clarity for un-amplified or under-amplified loci were always run in multiplex PCR’s for the multiplex that contained the needed loci. Samples were re-run for at most 12 iterations.

**Fragment Analysis**

After amplification in each multiplex, results were compared amongst replicates to reach a consensus genotype (Frantz et al. 2003, Hansen et al. 2008). A consensus genotype at a locus was conditional upon an identical allele pair observed at least twice to confirm a heterozygote genotype, or the same allele observed three times to confirm a homozygote genotype. Samples were re-run in a second or third PCR when a consensus genotype was not amplified across all loci in the first PCR. I followed the standards of Koelewijn et al. (2010) for genotype calling across more than three replicates.

I used program CERVUS (Kalinowski et al. 2007) to calculate allelic diversity, null allele rates by locus, observed and expected heterozygosities for the entire population, and deviations from Hardy-Weinberg Equilibrium I calculated allelic dropout (ADO) for each microsatellite locus using the method described in Broquet and Petit (2004), which uses only individuals that are heterozygous at a locus for allelic dropout calculation. Allelic dropout was determined by comparing scored allele calls for each successful amplification at each locus to the consensus genotype for that locus for the individual to which the sample was assigned (Frantz et al. 2003, Lampa et al. 2008).
Allelic dropout rate was then the proportion of amplifications in which an allele was missing for a heterozygous locus to the total number of amplifications with observable product.

To investigate differences in sample quality based on sample type and freshness, I used sample type (scat, anal jellies, and scat containing jellies classified as mixed) and freshness category to make comparisons using analysis of variance (ANOVA) and Tukey’s Honest Significant Difference test to distinguish between categories. I used amplification rate, the proportion of microsatellite loci able to be genotyped per sample, to make the statistical comparisons between exposure and sample type categories, as well as allelic dropout rate. I analyzed the effects of sample exposure and sample type by combining data from both years with a nested ANOVA to account for the difference in amplification rate and error rate between years.

RESULTS

In 2013, I collected 627 samples from 182 latrines. The mean temperature was $-2.2^\circ\text{C}$ (mean min.: $-6.3^\circ\text{C}$; mean max.: $1.6^\circ\text{C}$). Over that time period the temperature dropped below freezing each day, the number of days with a maximum temperature lower than $0^\circ\text{C}$ was 35 days and there were 83 days with a minimum temperature less than $0^\circ\text{C}$. I collected 156, 182, and 252 samples in January, February and March 2013, respectively.

In 2014, I collected 334 samples from 64 latrines. The mean temperature was $-5.1^\circ\text{C}$ (mean minimum $-10.7^\circ\text{C}$; mean maximum $0.5^\circ\text{C}$). Over that period the temperature dropped below freezing each day, with 46 days with a maximum temperature lower than $0^\circ\text{C}$, and 83 days with a minimum temperature less than $0^\circ\text{C}$. Monthly totals of samples
collected were 42, 42, and 238 samples collected in January, February, and March 2014, respectively.

Overall, 69.8% of the samples were found to have adequate DNA after amplification in the screening multiplex (71.7% in 2013, 69.8% in 2014). All of the microsatellite loci used were polymorphic in this population (Table 2-2), and only one (RIO-11) was found to deviate from Hardy-Weinberg Equilibrium and was excluded from analysis. The genotyping success rate at seven or greater loci over both years was 53.5% (56.3% in 2013, 47.3% in 2014), with an ADO rate averaged across all loci of 0.414 (0.329 in 2013, 0.499 in 2014) (Table 2-2).

Of those samples that passed the screening multiplex, the average amplification rate, calculated as the proportion of loci genotyped out of all loci amplified, was 0.804 (SD=0.269). Between years, the amplification rates for 2013 (0.842) and 2014 (0.730) were different (ANOVA, F=25.75, P<0.001), and the ADO rates for 2013 (0.329) and 2014 (0.472) were different (ANOVA, F=30.35, P<0.001). There was a difference in amplification rate between anal jellies (0.925) and scat (0.773) (ANOVA, F=25.67, P<0.001). The ADO rate was lower for anal jelly (0.103) than mixed (0.293) and scat samples (0.379; ANOVA, F=50.472, P<0.001; Fig. 2-2).

Amplification rates for samples in freshness category 1 (0.899) were higher than in freshness category 2 (0.79) and freshness category 3 (0.787; ANOVA, F=7.811, P=0.005, Fig. 2-3). Allelic dropout rates were higher for freshness category 3 samples (0.426) than freshness category 2 (0.349), but not different from freshness category 1 (0.357) (ANOVA, F=7.136, P=0.007; Fig. 2-4).
Of the 90 samples from 2014 that were re-extracted after failing to pass the screening multiplex using the DNA from their first extraction, 73% were confirmed to have DNA after amplification in the screening multiplex, and 60% were genotyped at seven or more loci. The average amplification rate was 0.783 (SD=0.295), and the average allelic dropout rate was 0.598 (SD=0.244).

The average number of PCR runs needed to genotype samples at seven or more loci was six for all samples across both years. Of the 2014 samples that were genotyped at seven or more loci, 36% of those samples only had to be run in multiplex 1 for three runs, whereas 46% of those samples had to be run nine times in multiplex 2.

**DISCUSSION**

My genotyping success rate of 53.5% at seven or more loci was comparable to studies done on Eurasian otter in the winter using a storage buffer for sample preservation (*Lutra lutra*, Koelewijn et al. 2010, Prigioni et al. 2006, Hájková et al. 2011 ) (Table 2-3). My genotyping success rate was slightly lower than seen for some of those comparable studies (65% Hung et al. 2004, 60% Hájková et al. 2011), but this could be because I included the first failed extraction of subsequently successful re-extracted samples in the calculation. This could also be a result of less lenient sample-selection in which samples deposited for longer times than allowed in other studies were collected. Sampling season and preservation method are the most apparent differences between my study and others conducted on North American river otter. Future NGS studies of river otter should focus on collection of fecal samples during the winter or during cooler months, and use a storage buffer, either ethanol or DET buffer (Fike et al. 2004, Hájková et al. 2006). To
account for decreased genotyping success for fresh samples, Mowry et al. (2011) proposed that collection in plastic bags may have facilitated activity of mold and bacteria on the samples, which may hasten the rate of DNA degradation, inhibit PCR amplification, or drown out target DNA. Prigioni et al. (2006) attributed low genotyping success rates to sampling during warm summer months. Increased defecation at latrines in winter (Kruuk 2006, Stevens and Serfass 2008, Just et al. 2012) and potentially improved detection of feces on top of snow (Gallant et al. 2009) would further support improved sampling efficiency during winter months. I collected the most samples in February and March both years, and would suggest concentrating sampling efforts to those months.

Other studies used genotyping success rate, the percentage of samples that were able to be genotyped at or above the threshold level of loci for individual comparisons based on PID calculations (Table 2-3). I used amplification rate to make the comparisons because it allowed for more samples to be included in the analysis, any that passed the screening multiplex (Frantz et al. 2003, Lampa et al. 2008). This appeared to be a good indicator of amplification success because analysis could be done on samples of all qualities, not just those that were able to be genotyped at seven or more loci. Amplification rate gives an indication of how many loci are able to be genotyped, not just whether enough loci were genotyped using a threshold number unique to each study. I still calculated genotyping success rates by sample type and freshness category, however, to make comparisons to other studies.

When I calculated genotyping success rates for each sample type and freshness category I found similar patterns to those I observed with amplification rates (Fig. 2-4).
Genotyping success rate was highest with anal jellies, as documented in previous research (Mowry et al., 2011 and Brzewski et al. 2013), and genotyping success decreased as estimated time since deposition increased, consistent with Brzewski et al. (2013; Fig. 2-4). Of note is that for any given category, or sample type, the genotyping success rate was higher than that seen in the other studies on North American river otter studies, which further supports the suggestion of sampling during months with lower temperatures and using a storage buffer for preservation.

The pattern of higher amplification for anal jellies and lower rates for scat was seen in all other fecal DNA studies of river otter (Fike et al. 2004, Hansen et al. 2008, Mowry et al. 2011, Brzewski et al. 2013). Not reported in other field studies were the differences in allelic dropout between sample types. I observed less allelic dropout for anal jellies than for scat. Fike et al. (2004) reported similar overall error rates (0.40) in a study with captive otters, but also reported that anal jelly samples had higher ADO rates, opposite to the study. The mechanism behind the differences in amplification rate and error rates between scat and anal jellies has not been investigated, but could be a result of the contents of the anal secretions reducing exposure of DNA to environmental degradation, or some compound in the secretions preserving and stabilizing the DNA or those target epithelial cells.

When comparing preservation methods, samples stored in ethanol at room temperature had higher error rates than those stored in ethanol and frozen, which had the lowest error rates (Fike et al 2004). The initial storage in the field, at room temperature in ethanol, may have contributed to the high ADO rates. The amplification rate and allelic dropout rate for mixed scat-anal jelly samples was indistinguishable from the other two
categories, however, the amplification rate was closer to that of anal jellies, while the allelic dropout rate was closer to that of scat. This could indicate these samples could be higher priority for collection, but they should be analyzed using multiple error checking methods to ensure accurate genotyping. Of interest would be whether these mixed samples lead to identification of more individuals than anal jellies, as found for scat samples (Mowry et al. 2011). I found an effect of freshness on amplification rate and error rate, with a higher amplification rate for samples in freshness category 1 and 2 (Figure 2-3). The pattern I observed for the effect of freshness on amplification was similar to Brzewski et al. (2013), but opposite to that observed in Mowry et al. (2011). The allelic dropout rate was higher in 2014, and by combining data from both years a difference in allelic dropout rate between freshness categories that was observed for the data from 2013 was lost.

I reported higher error rates than other studies on North American river otter, but had better success genotyping samples. The high error rates could be a result of increased effort for problematic samples and running samples through more PCR amplifications until a consensus genotype could be determined. The mean number of PCR amplifications for samples that were genotyped at seven or more loci in 2014 was seven runs (21 reactions), while Mowry et al. (2011) only ran samples for up to five runs. The maximum number of PCR amplifications to genotype samples in other studies was typically 15 (Hájková et al. 2009, Mowry et al. 2011). The number of reactions necessary to genotype enough loci could have been related to conditions of the multiplex PCR, and not the quality of the samples. For multiplex 1 in 2014, the number of samples genotyped at seven or more loci that only needed to be run for three PCR runs was 56 out of 156.
While for multiplex 2 there were only 27 out of 156 samples that only needed to be run for three PCR runs. Splitting multiplex 2 into two separate reactions would not have necessarily decreased the amount of effort, because there would be additional PCRs to run for the new multiplex, however, further investigation into optimizing the PCR conditions may have resulted in better performance in the multiplex reaction.

Of those samples I re-extracted (for which the first and second extractions were included in the counts for overall calculations of amplification, error, and genotyping success rates), I observed similar results for genotyping success rate, and the number of amplifications to reach a consensus genotype at seven or more loci was also similar. This would indicate that samples that may be discarded after one extraction because they don’t appear to have adequate DNA could in fact be usable. Re-extractions could be worthwhile if there is uncertainty about the first round of extractions. For the initial extractions, I kept the sample in the tube with the ethanol and selected only what could be removed without spilling ethanol or sample, often times limiting us to selection of only bones, carapaces, and fish scales. For the re-extractions, I emptied the entire scat sample and selected the very tips or most unrecognizable digested material, and looked for any anal jelly that might be in the sample. Both anal jellies and the tips of feces have been found to more consistently yield DNA (Fike et al. 2004, Mowry et al. 2011), and thus emptying the sample onto filter paper allowed us to select for parts more likely to yield DNA. This method does not need to be used for samples that are entirely, or in large part, anal jelly because of the ease of selecting just that substance for extraction. The drawbacks to this method are the increased amount of time to process samples, and an increased risk for contamination as samples are completely exposed on the laboratory
bench. However, this method could help increase the amount of samples to be used in analysis.

**MANAGEMENT IMPLICATIONS**

Management of wildlife populations requires monitoring of both the population dynamics and the genetic structure and trends for the population, and NGS enables these components while also increasing the detection of rare and elusive species. The use of NGS was estimated to cost more than hair sampling and radio-telemetry (Johnson et al. 2013), however, this comparison was from previous research that had low overall success rates and no quantification on effort (Mowry et al. 2011). Increased laboratory effort may be necessary to maximize success rates, but this alternative is preferable to invasive sampling techniques such as radio telemetry that are limited in the spatial range of their application and the number of individuals captured. A sampling design that incorporates amplification success and error rates, collection of feces during months of low temperatures and humidity, and a storage buffer for preservation, along with a budget that factors in the amount of effort needed per sample are integral components to the successful application of NGS to population assessment of river otter.

**ACKNOWLEDGMENTS**

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trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.
LITERATURE CITED


Waits LP (2004). “Using noninvasive genetic sampling to detect and estimate abundance of rare wildlife species” pp 211–228. *Sampling Rare or Elusive Species:*


Figure Legends

Figure 2-1. Study area and survey sites for non-invasive genetic sampling for river otter in northeastern Pennsylvania in 2013 and 2014.

Figure 2-2. Comparisons of amplification rate and allelic dropout (ADO) rate for different types of otter scat.

Figure 2-3. Comparisons of amplification rate and allelic dropout rate (ADO) for different freshness categories of otter scat.

Figure 2-4. Comparison of genotyping success rate between different types and freshness categories for river otter scat, as reported in the literature.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Fluorescent Tag</th>
<th>Multiplex</th>
</tr>
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<tbody>
<tr>
<td>RIO-01R2</td>
<td>6-FAM</td>
<td>1</td>
</tr>
<tr>
<td>RIO-04R</td>
<td>PET</td>
<td>1</td>
</tr>
<tr>
<td>RIO-13R</td>
<td>6-FAM</td>
<td>1</td>
</tr>
<tr>
<td>RIO-15R</td>
<td>NED</td>
<td>1</td>
</tr>
<tr>
<td>RIO-18</td>
<td>PET</td>
<td>1</td>
</tr>
<tr>
<td>RIO-02R</td>
<td>VIC</td>
<td>2</td>
</tr>
<tr>
<td>RIO-06R</td>
<td>NED</td>
<td>2</td>
</tr>
<tr>
<td>RIO-07R</td>
<td>NED</td>
<td>2</td>
</tr>
<tr>
<td>RIO-08R</td>
<td>VIC</td>
<td>2</td>
</tr>
<tr>
<td>RIO-11</td>
<td>PET</td>
<td>2</td>
</tr>
<tr>
<td>RIO-16R</td>
<td>VIC</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 2-2. Number of unique alleles (AO), observed (H₀) and expected (Hₑ) heterozygosity, null allele calculation (NA), and allelic dropout rate (ADO) for all microsatellites used for identification of individual river otter in northeastern Pennsylvania, 2013–2014.

<table>
<thead>
<tr>
<th>Marker</th>
<th>AO</th>
<th>HO</th>
<th>HE</th>
<th>NA</th>
<th>ADO</th>
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<tr>
<td>RIO-01R2</td>
<td>9</td>
<td>0.708</td>
<td>0.785</td>
<td>0.055</td>
<td>0.41</td>
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<td>RIO-02R</td>
<td>6</td>
<td>0.703</td>
<td>0.725</td>
<td>0.011</td>
<td>0.19</td>
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<tr>
<td>RIO-04R</td>
<td>8</td>
<td>0.588</td>
<td>0.623</td>
<td>0.023</td>
<td>0.34</td>
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<tr>
<td>RIO-06R</td>
<td>4</td>
<td>0.594</td>
<td>0.607</td>
<td>0.005</td>
<td>0.41</td>
</tr>
<tr>
<td>RIO-07R</td>
<td>7</td>
<td>0.589</td>
<td>0.599</td>
<td>0.008</td>
<td>0.45</td>
</tr>
<tr>
<td>RIO-08R</td>
<td>6</td>
<td>0.692</td>
<td>0.756</td>
<td>0.048</td>
<td>0.25</td>
</tr>
<tr>
<td>RIO-13R</td>
<td>10</td>
<td>0.811</td>
<td>0.832</td>
<td>0.011</td>
<td>0.31</td>
</tr>
<tr>
<td>RIO-15R</td>
<td>3</td>
<td>0.673</td>
<td>0.856</td>
<td>0.023</td>
<td>0.36</td>
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<tr>
<td>RIO-16R</td>
<td>6</td>
<td>0.681</td>
<td>0.743</td>
<td>0.043</td>
<td>0.51</td>
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<tr>
<td>RIO-18</td>
<td>8</td>
<td>0.673</td>
<td>0.856</td>
<td>0.109</td>
<td>0.43</td>
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<td>Author/Year</td>
<td>Species</td>
<td>Duration of Study</td>
<td>Sampling Season</td>
<td>Storage Method</td>
<td>Genotyping Success Rate</td>
</tr>
<tr>
<td>-------------</td>
<td>---------</td>
<td>-------------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Hung et al. 2004</td>
<td>Lutra lutra</td>
<td>16/30</td>
<td>Winter</td>
<td>100% Ethanol</td>
<td>24% (6/30)</td>
</tr>
<tr>
<td>Kalz et al. 2006</td>
<td>Lutra lutra</td>
<td>60/365</td>
<td>Year-round</td>
<td>Q-tip swabs at room temp; full scat frozen</td>
<td>65% (222/343)</td>
</tr>
<tr>
<td>Prigioni et al. 2006</td>
<td>Lutra lutra</td>
<td>135</td>
<td>Summer</td>
<td>95% Ethanol</td>
<td>41.2% (77/187)</td>
</tr>
<tr>
<td>Arrendal et al. 2007</td>
<td>Lutra lutra</td>
<td>17</td>
<td>Winter</td>
<td>Freezing at −22°C</td>
<td>30% (46/150)</td>
</tr>
<tr>
<td>Hansen et al. 2008</td>
<td>Lontra canadensis</td>
<td>N/A</td>
<td>N/A</td>
<td>100% Ethanol</td>
<td>68%</td>
</tr>
<tr>
<td>McElwee 2008</td>
<td>Lontra canadensis</td>
<td>52/365</td>
<td>Year-round</td>
<td>Freezing at −25°C</td>
<td>18% *</td>
</tr>
<tr>
<td>Koelewijn et al. 2010</td>
<td>Lutra lutra</td>
<td>48/152</td>
<td>Winter</td>
<td>99% Ethanol</td>
<td>46% (582/1,265)</td>
</tr>
<tr>
<td>Valqui et al. 2010</td>
<td>Lontra felina</td>
<td>61</td>
<td>Winter</td>
<td>96% Ethanol</td>
<td>47% (41/87)</td>
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<td>Hájková et al. 2011</td>
<td>Lutra lutra</td>
<td>210</td>
<td>Winter</td>
<td>90% Ethanol</td>
<td>60%</td>
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<tr>
<td>Mowry et al. 2011</td>
<td>Lontra canadensis</td>
<td>20/97</td>
<td>Winter</td>
<td>Freezing at −20°C</td>
<td>24%</td>
</tr>
<tr>
<td>Williams, 2011</td>
<td>Lontra canadensis</td>
<td>2/14</td>
<td>Fall</td>
<td>Dried under fume hood, paper bag at room temp.</td>
<td>18.2% (53/292)</td>
</tr>
<tr>
<td>Ortega et al. 2012</td>
<td>Lontra longicaudis</td>
<td>&lt; 45</td>
<td>Winter</td>
<td>Silica dessicant</td>
<td>24.40%</td>
</tr>
<tr>
<td>Brzewski et al. 2013</td>
<td>Lontra canadensis</td>
<td>46/166</td>
<td>Summer</td>
<td>Freezing at −20°C</td>
<td>25% (124/490)</td>
</tr>
<tr>
<td>Forman and Walter, unpublished</td>
<td>Lontra canadensis</td>
<td>84</td>
<td>Winter</td>
<td>99% Ethanol</td>
<td>56.3% (353/627)</td>
</tr>
</tbody>
</table>

*Signifies a success rate only for positive detection of DNA from extractions, not amplifications or genotyping.
Figure 2 - 3.

Age Category

Rate

Rate Type

ADO Amplification Rate

6 > Days

1-6 Days

>1 Day

Rate
Figure 2.4

<table>
<thead>
<tr>
<th>Type</th>
<th>&lt;1 Day</th>
<th>1-6 Days</th>
<th>&gt;6 Days</th>
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<td></td>
<td></td>
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<tr>
<td>Mixed</td>
<td></td>
<td></td>
<td></td>
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<td>Genotyping Success Rate</td>
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</tbody>
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Studies:
- Our Study
- Mowry et al. 2011
- Brzewski et al. 2013
Chapter 3

A framework for spatial capture-recapture models for estimation of river otter population size and density
ABSTRACT Management and conservation for a species that is rare from a sampling standpoint, or has a unique distribution across the landscape, requires a sampling design and method of estimation that can account for scarcity and spatial variation in abundance. The North American river otter (*Lontra canadensis*) is difficult to monitor, and density estimation in areas containing a variety of aquatic habitat types is not straightforward, but non-invasive genetic sampling and spatial capture-recapture methods can account for both of these issues. My objective was to use non-invasive genetic sampling as a means of monitoring, and spatial capture-recapture models to estimate river otter densities in northeastern Pennsylvania. I collected river otter feces during winter months across six counties, sampling locations multiple times in a mark-recapture framework. I extracted DNA from samples, amplified 10 microsatellite markers using the polymerase chain reaction, and identified individuals by matching samples. I then used those captures in a spatial capture-recapture model to estimate population size and density, and in the program MARK to investigate patterns in capture probability. The density estimates (2.01 otter/100 km$^2$, 95% Asymptotic Wald-Type CI 1.49–4.94 otter/100 km$^2$), are the first reported for Pennsylvania. Non-invasive genetic sampling was successful in capturing a high number of individuals (103), and recapturing individuals (60 individuals recaptured, 182 recapture events).

KEYWORDS noninvasive genetics, river otter, *Lontra canadensis*, spatial capture-recapture
INTRODUCTION

Management and conservation depends on a comprehensive understanding of demographic parameters of a population. Species with low densities, cryptic behavior, or that occupy inaccessible habitat may be inaccurately surveyed, and biases due to sample design or estimation may add considerable error to population estimates. The North American river otter (*Lontra canadensis*) is typically described as an elusive species due to its cryptic behavior, but monitoring river otter can be facilitated by non-invasive methods (Kruuk 2006, Gallant et al. 2009). Previous studies have demonstrated that non-invasive genetic sampling (NGS) using deoxyribonucleic acid (DNA) has been used for the Eurasian otter (*Lutra lutra*) (Hájková et al. 2009), the Neotropical otter (Ortega et al. 2012), the Giant Otter (*Pteronura brasiliensis*) (Pickles et al. 2012), the marine otter (*Lontra felina*) (Valqui et al. 2010), and the North American river otter (Mowry et al. 2011, Brzewski et al. 2013).

Molecular techniques have made it easier to monitor river otter, but traditional capture-recapture models for population estimation are not ideal because of the non-uniform distribution of otter across the landscape. Population estimation is straightforward for linear water systems, where density can be measured as individuals per kilometer of water system (Mowry et al. 2011), or wetland habitats or areas with a high density of aquatic habitat where space use can occur across the landscape uniformly and density is estimated as individuals per square-unit of area (Hájková et al. 2006). For a species that is distributed across the landscape in various types of aquatic habitats (e.g., lakes, rivers), traditional capture-recapture models assume an even distribution of
individuals over the landscape, and this does not account for the influence of spatial variation on abundance (Royle et al. 2014).

Banks et al. (2003) suggested that models that include information about multiple recaptures of an individual during a sampling session would more accurately estimate population abundances for a study involving NGS. Spatial capture-recapture models are able to incorporate spatial information in both the observation process and the estimation of unobserved individuals, and thus multiple captures within an occasion can be used in the model if they are made at different locations or trap sites (Royle et al. 2014). Spatial capture-recapture models are hierarchical models with an observation model informed by the temporal and spatial components of observations of individuals. The models relate observations to the detection process and an activity center, which indicates the average location of an individual throughout the sampling duration (Borchers and Efford 2008, Royle et al. 2014). The observation model is a function of probability of detection and a movement coefficient, which describes the decay in detection as detector devices are further away from the activity center (Borchers and Efford 2008, Royle et al. 2014). The activity centers for individuals are estimated over the state space, a pre-defined set of values that is representative of the area sampled, and the distribution of estimated activity centers for observed individuals is augmented with an estimated number of unobserved individuals to derive a population estimate (Royle et al. 2014). These models are more ecologically intuitive in the sense that estimation is occurring over a pre-defined space that is defined by the trap array or sampled area. These models can be used to test ecological assumptions about variations in spatial distribution due to habitat covariates and individual covariates (Royle et al. 2014).
River otter populations in Pennsylvania underwent a considerable range reduction, with a remnant population restricted to the northeast corner of the state by the mid-20th century (Serfass et al. 1993). River otter have now re-colonized most of the state through natural range expansion and reintroduction efforts (Serfass et al. 1993, Raesly 2001). Little is known about the current river otter population in Pennsylvania except their general distribution across the majority of the state. In northeast Pennsylvania, there is a mosaic of major rivers, tributaries, lakes and wetlands where river otter have been observed. With such a diversity of habitat occupied by river otter, estimation of densities should not assume uniformity across the landscape and thus spatial capture-recapture models may be the best option for population estimation.

My objectives were to (1) use NGS to identify individual otter using microsatellite genotypes from fecal samples and (2) to estimate population size and density for river otter in northeastern Pennsylvania with spatial capture-recapture modeling. Additionally, I wanted to compare NGS using traditional mark-recapture models to spatial capture-recapture models for estimating river otter population size over a large, spatially diverse area.

METHODS

Study Area

I sampled seven counties in northeast Pennsylvania (Figure 3-1). The area is a mosaic of lentic and lotic habitat, including major rivers such as the Delaware and Susquehanna Rivers and their major and minor tributaries. There are also glacial lakes and wetlands and impounded lakes and ponds that are all separated by large expanses of
non-habitat, including mountains, farmland and urban-suburban areas. I sampled both public and private lands opportunistically, surveying all accessible water bodies for river otter feces, with the exception of large rivers and lakes.

The study was conducted from 6 January to 31 March, 2013 where mean temperature was $-2.2^\circ C$ (mean min.: $-6.3^\circ C$; mean max.: $1.6^\circ C$) and dropped below freezing each day. The number of days with a maximum temperature lower than $0^\circ C$ was 35 days and there were 83 days with a minimum temperature less than $0^\circ C$. I did not use samples collected during a 2014 sampling season to the west of the area covered in 2013 because of limited and irregular coverage of the area.

**Sample Collection**

I collected samples daily, whether at latrines, dens, or in random locations and the location was treated as a ‘trap’ in the capture-recapture framework. A point was also assigned to water bodies that were searched but no samples were found, and were treated as ‘trap’ locations as well. A point was assigned at roughly 250-meter increments along the distance of the shoreline that was surveyed. A sampling occasion included the first visit to a site with feces, and any subsequent revisits to that site. Upon the initial discovery of the latrine, I judged the freshness of the sample (i.e. stage of decomposition, position on snowfall, moisture content, sheen or glossiness, and gelidity) to assess whether it was deposited within an acceptable time period to be included in the study. At the end of collection for each latrine visit, I cleared any remaining fecal material. Latrines were then resampled approximately six to nine days after initial discovery for the first recapture occasion, and resampled up to two more additional times between 12–28 days later for the third and fourth occasions.
I collected the proximal and distal ends of scat, about 2 cm sections in length (1–4 sections), from individual piles at latrines using tweezers flamed for sterilization prior to and after collections. Samples were immediately stored in the field in 15 mL screw-top vials with 7 mL of 99% ethanol at room temperature.

**Laboratory Methods**

The laboratory methods for extraction, PCR multiplex amplification, and sample screening and additional amplification followed those described in the previous chapter (Chapter 2 – Laboratory Methods), with the exception that the microsatellites used were nine of the ten used by Mowry et al. (2011)(Table 3-1)

To determine the sex of individuals, after samples were genotyped and individuals were identified (see below subsection), I used the primers and laboratory protocol for SRY and ZFXY sex-linked microsatellite amplification (Mowry et al 2011). I added a restriction digest for the ZFXY fragment because of poor amplification of the SRY fragment (Mucci and Randi 2007). I visualized all sexing PCR products using gel electrophoresis on a 2% agarose gel at 115 V for 75 minutes. I chose the sample that amplified at the most loci and in the least amount of runs as the representative sample for each individual to be used in the sexing PCR. I also sexed problematic samples or samples that needed additional confirmation for assignment to an individual as an additional check (Marucco et al. 2011).

**Genotyping and Individual Analysis**

Genotyping was performed as described in the previous chapter (Chapter 2 – Genotyping and Individual Analysis) to reach a consensus genotype for each sample (Frantz et al. 2003, Hansen et al. 2008, Koelewijn et al. 2010). I used the program
CERVUS (Kalinowski et al., 2007) to calculate allelic diversity, null allele rate for each locus, observed and expected heterozygosities for the entire population, and deviations from Hardy-Weinberg Equilibrium to test for any homozygote or heterozygote excess suggestive of loci under selection, non-random mating, or population substructure. I calculated allelic dropout (ADO) for each microsatellite locus using the method described in Broquet and Petit (2004), which uses only individuals that are heterozygous at a locus for allelic dropout calculation. Allelic dropout was determined by comparing scored allele calls for a sample to the consensus genotype of the individual to which it was assigned (Frantz et al. 2003, Lampa et al. 2008). I used the PID and PID_{sib} calculations from program CERVUS (Kalinowski et al., 2007) and GenAlEx (Peakall and Smouse 2012), which calculate the power for increasing number of locus combinations to differentiate individuals (PID) and individuals that are siblings (PID_{sib})(Waits et al. 2001). I discarded samples that amplified at less than seven loci based on PID_{sib} calculations, the more stringent calculation (see Results section). Samples then had to match at seven or more loci to be considered the same individual. I used multiple checks in addition to genotype to confirm assignment of samples to an individual, including sex and geographic location (Marucco et al. 2011).

**Population Estimation**

I used likelihood analysis spatial capture-recapture (SCR) models to estimate population size over the entire study area (Borchers and Efford 2008, Royle et al. 2014). The models were run using the ‘intlik3’ models from the *scrbook* package (Royle et al. 2014) in the program R (R Core Team, Vienna, Austria). These models estimate as a parameter the number of unobserved individuals (N_0) occurring within the state space,
and allow for user definition of the state space shape and resolution of the integration grid. The standard error for these models was calculated from the Hessian Matrix of the likelihood function, and from this I calculated an asymptotic Wald-type confidence interval. I calculated density as the model population estimate over the area of the state space.

I fit a buffered (20 km) polygon around a local convex hull (Getz and Wilmers 2007) of the latrine and survey sites as the area for the state space. In this model, all sites sampled were treated as trap locations, and I included the occasions for which sites were surveyed. I set the integration grid resolution, the grid defining the set of points for the state space, at 0.2 km.

I also ran closed population models in the program MARK using the ‘Full Likelihood p and c’ class models (White and Burnham 1999). I ran one model with capture and recapture probabilities set as equal, and constant over time, as a comparison for the density estimates derived from the spatial capture-recapture model. I estimated density for this MARK model by calculating the area in two ways: (1) as the area defined by the trap array fitted with a buffer equal to the maximum observed distance moved by individual, and (2) as the area defined by the trap array fitted with a buffer equal to half the maximum observed distance moved by individual. I ran two models to test for differences in capture and recapture probabilities, one with capture \(p\) and recapture \(c\) probabilities set equal and varying over time \(p=c\) model) and one with \(p\) and \(c\) estimated separately and varying over time \(p(t)c(t)\) model). For the model \(p(t)c(t)\), the \(p\) and \(c\) for the last occasion were set equal because they are not separately estimable.
To assess the efficiency of NGS for capturing river otter individuals, I calculated a redundancy rate that was a proportion of the number of capture occasions for an individual over the total number of samples genotyped for an individual, averaged across all individuals. I considered the redundancy rate an indication of excess effort that went into genotyping samples that duplicated capture occasions already described by another sample.

**RESULTS**

I collected 625 samples across nine counties, with a genotyping success rate, measured as amplification at seven or more loci, of 58.7% (354/625). All loci were polymorphic with an overall observed heterozygosity of 0.63 across all loci, an overall expected heterozygosity of 0.68, and only one of the loci (RIO-11) was found to be out of Hardy-Weinberg Equilibrium and was excluded from analysis (Table 3-1). Estimates of null alleles for each locus were low (0.01–0.16), and allelic dropout rates were relatively high compared to the literature (0.05–0.50). Across the nine microsatellites used, PID was $3.25 \times 10^{-9}$ and $\text{PID}_{\text{sibs}}$ was $3.06 \times 10^{-4}$, and for a combination of seven loci, my threshold number for accepting a genotype, PID was $1.7 \times 10^{-6}$ and $\text{PID}_{\text{sib}}$ was $3.7 \times 10^{-3}$.

I identified 103 individuals (23F:59M:21 unidentified), of which 60 individuals were recaptured. The mean number of recaptures for recaptured individuals was $3.34 \pm 1.71$, with a maximum of eight recapture occasions for a single individual. The average number of individuals captured per latrine was 1.9, and the average number of latrines used by sex were 1.5 for female and 3.5 for male. The number of individuals captured at multiple latrines was 46. The mean number of samples assigned to an individual was 3.37
± 3.34, and the redundancy rate was 0.65 (2.19 capture occasions: 3.37 samples genotyped) across all individuals. By feces type, 44 individuals were identified solely from feces, 55 individuals were identified from anal jellies, and 22 individuals were identified using both types of samples.

The spatial capture-recapture model estimate of population size was $\hat{N} = 259$ individuals (175–603, 95% asymptotic Wald-type CI) (Table 3-2). The estimate for density given the size of the state space (15,120 km$^2$), was $\hat{D} = 1.7$ otter per 100 km$^2$ (1.1–3.9, 95% asymptotic Wald-type CI). The most comparable traditional model from the program MARK (p=c(.) model) estimated population size $\hat{N} = 155$ (134–191), with a density estimate using the maximum distance moved buffer of 1.4 otter per 100 km$^2$ (1.2–1.7 otter per 100 km$^2$), and a density estimate using the half-maximum distance moved buffer of 1.7 otter per 100 km$^2$ (1.4–2.1 otter per 100 km$^2$).

The best-supported model in program MARK was model $p = c(t)$ (Table 3-3), with capture and recapture probability equal, but varying by occasion. Estimates of capture probabilities were different between the first occasion and the second and third ($p_1 = 0.57$, $p_2 = 0.22$ and $p_3 = 0.28$, $p_4 = 0.06$).

**DISCUSSION**

To my knowledge, this is the first estimation of population size and density for an otter species using the combination of NGS and spatial capture-recapture modeling. The density estimate from the spatial capture-recapture model was close to the density estimate from the most similar MARK model. Given that the estimates were close, I feel this validates the use of spatial capture-recapture models for estimating river otter
densities, especially given the ability to incorporate landscape level covariates to test for spatial variation in density, which is expected for otter populations.

This study is also unique in its estimation of otter density over a large area with a mosaic of non-uniformly distributed habitats types, both aquatic and terrestrial. Most studies on otter species estimate population size along linear habitats, as a number of individuals per distance of river or stream (Mowry et al. 2011). Previous research that estimated otter densities as individuals per square area were conducted in small study areas with productive habitat (≤100 km²), on populations suspected to have high densities, and on populations that had been recently reintroduced (Hájková et al. 2006, Koelewijn et al. 2010). The study area contained large swaths of non-habitat including forested mountains, urban-suburban development, and agricultural fields. This could be the reason my estimates were low (2.6 otter/100 km², 95% CI 1.6–5.9 otter/100 km²) compared to other studies (13–81 otter/100 km²; Hajkova et al. 2006; 28 otter/100 km²; Koelewijn et al. 2010). Preliminary analysis of suitable otter habitat using a data layer created for Pennsylvania (Myers et al. 2000) indicated an otter density of 12 otter per 100 km² of suitable habitat. A more detailed assessment of suitable habitat would be needed for comparison to previous research on population density of otter in suitable habitat.

There were a few caveats in my sampling design for monitoring river otter in northeastern Pennsylvania. First, I was unable to sample the large rivers in the area (Delaware River, Susquehanna River, and lower Lehigh River) as well as the largest lake, Lake Wallenpaupak, because of difficulties in access. These habitats represent a large, presumably productive, habitat that remains partially or completely ice free during the winter. A study looking into the use of these water bodies during the winter in
Pennsylvania and the northeast would indicate whether they act as a winter refuge for otter, or whether densities are higher on these water bodies compared to surrounding smaller water bodies. Otters from the surrounding landscape, which I sampled, could be using these water bodies in addition to otters that maintain home ranges solely on these water bodies. Conversely, it would be interesting to investigate whether individuals that center their activity on these large water bodies travel up tributaries and into smaller neighboring water bodies, and thus my sampling may have captured them. Any temporal variation in otter movement between the largest water bodies and surrounding mid to small sized water bodies could introduce bias if sampling regimes omit certain habitat. I observed no deviation from Hardy-Weinberg Equilibrium, which would suggest that this was a representative sampling of the population, however the overall observed heterozygosity (0.63) was slightly lower than the calculated expected heterozygosity (0.68). Low heterozygosity levels could be a result of a bottleneck effect, a result of the historic range reduction for the river otter in Pennsylvania, or could be tied to the sampling effort and the loss of heterozygosity from individuals that were unable to be sampled, i.e. from larger water bodies.

Second, only marking individuals that used latrines were recorded. The literature is unclear whether juvenile otter defecate and mark with anal jellies at latrines. Defecation and marking at latrines increases during the breeding seasons (February–April) (Crimmins et al. 2009, Stevens and Serfass 2008, Just et al. 2012) suggesting that adults in breeding condition are more likely to be observed at latrines in the breeding season, but not individuals out of breeding condition. Yearling river otter have smaller home ranges than adults (Foy 1984), which could impact availability to sampling. I did
observe a sex ratio similar to that from previous research (Mowry et al. 2011, Brzewski et al. 2013), which would suggest a non-biased sampling procedure in terms of sexual differences in sample availability. In winter, sex ratios for harvest were male biased in multiple states across the northeast (Chilelli et al. 1996), which was attributed to increased vulnerability during the breeding season and larger home ranges. Studies on the Eurasian otter found that there were no sex or age class biases in fecal deposition year round (Kruuk 1992, Dallas et al. 2003). With sampling limited to mid-size and small water bodies, and the potential omission of certain age classes because of fecal sampling, the population and density estimates produced in this study were likely a conservative estimate for the area.

The use of NGS and SCR enables us to document a high number of individuals (103), and 60 of those individuals were recaptured at different locations or sampling events (average of 3.37 recaptures). I recorded 228 captures over 1039 trap nights (2.5 trap nights per capture), which is high compared to traditional live-capture methods (188.0 trap nights per capture Melquist and Hornocker 1983, 25.0 trap nights per capture Blundell et al. 1999). I captured an average of 1.9 individuals per latrine, and 46 individuals were captured at multiple latrines, which is important to consider given the difficulty in trapping otter and the potential for trap shyness. Additionally, I observed that females visited fewer latrines on average (1.5) than males (3.5), which supports results seen with live-trapping and harvest in winter during which males are more susceptible to capture (Melquist and Hornocker 1983, Chilelli et al 1996). This bias for females to visit fewer latrines would indicate that a sampling design for NGS for otter over a large area should try and avoid geographic gaps in the search effort to avoid missed detection of
females. This would correspond with the male biased sex ratio I observed, and could be tied to the opportunistic sampling effort that did not result in a uniform coverage of all water bodies in the study area. The reduced number of latrines visited could be caused by denning behavior of female with young resulting in less distance travelled, coupled with the fact that females also have smaller home ranges (Kruuk 2006).

The number of individuals detected by the different sample types was similar, with 55 individuals detected by anal jellies, 44 individuals detected by fecal, and 22 with both sample types. This is inconsistent with Mowry et al. (2011), which found that anal jellies only added 2 individuals not accounted for in fecal, but more similar to other studies that found a large proportion of individuals detected can come from these samples (Hájková et al. 2009, Brzewski et al. 2013). Anal jellies also had the highest amplification rate in the study (0.925) as well as in other studies (Fike et al. 2004, Hájková et al. 2009, Mowry et al. 2011). When collecting in the field, and when selecting the portion of fecal samples for extraction in the lab, effort should be put in to selecting portions of fecal samples that have anal jellies to increase genotyping success.

I did not observe a difference between capture and recapture probabilities in the estimates from models in the program MARK that varied capture and recapture probabilities, but did observe a difference in capture probability between each occasion. This would suggest that NGS, specifically clearing away feces after a sampling occasion, does not affect likelihood that an animal will return to that latrine and deposit feces. The difference in capture probability between sampling occasions could be a result of weather or other natural variations, or could indicate a decrease in activity at the latrines as sampling continues. Detection could also be higher for the first visit because I did not
clear latrines before collecting samples, and thus samples that had accumulated but appeared fresh enough to fall within the sampling time frame may have been collected.

To characterize the amount of effort needed to observe individuals, I calculated a redundancy rate (0.65) that described the proportion of genotyped samples that described a capture occasion. The redundancy rate, as well as the number of individuals observed and recapture numbers, are important when planning the logistics and budget of NGS for river otter. A recent study on river otter in British Columbia compared live-capture, telemetry, and non-invasive hair and fecal sampling, and found that NGS for hair was the most cost-effective (Johnson et al. 2013). The study used fecal genotyping success rates from Mowry et al. (2011), however, which were lower than this study and the study was conducted only at 15 previously known latrine sites, which would reduce the amount of effort necessary. Considering the amount of effort to capture the number of individuals I observed, my success rates, and the amount of laboratory effort, NGS for river otter feces can be a feasible method for monitoring.

Management Implications

NGS within spatial capture-recapture models permitted estimation of population size and density for river otter in an area with a high degree of habitat complexity. I was able to generate a high number of captures and recaptures, and estimate population size and density for a large area as opposed to being constrained to estimation along a linear system, both important considerations for future monitoring and management of river otters. Although NGS is easily applied across a large area, the ability for SCR models to accommodate geographic gaps that may occur from the sampling effort may not be beneficial for estimation of river otter because of potential differences in space use
between males and females. One benefit of using the SCR models is the flexibility in incorporating a variety of additional information to better inform the model, including individual covariates such as sex, and landscape characteristics. Future research on the Least Cost Path models described in Royle et al. (2014), could potentially provide more accurate models of the unique space use of river otter and thus better describe their density patterns across the landscape. Additional sampling in 2014 in areas west of this study found individuals in 2014 that were observed in 2013. This would indicate that NGS is suitable for long-term monitoring and the potential for open population models. This has also been documented in previous research for otter using NGS (Koelewijn et al. 2010), but has not yet been used to conduct a study looking at open population models for the species. These open population models would be very informative for a reintroduced population to obtain population demographics for survival, and immigration and emigration from nearby established populations.

ACKNOWLEDGMENTS

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FIGURE LEGEND

Figure 3-1. Locations of latrine sites that were sampled for river otter feces in northeastern Pennsylvania from January to April 2013.
Table 3. Statistics for allele frequencies, Hardy-Weinberg Equilibrium calculations, allelic dropout, and null alleles for each microsatellite locus.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Number of Alleles</th>
<th>Null Alleles</th>
<th>Allelic Dropout</th>
<th>H^0</th>
<th>H^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIO-16R</td>
<td>6</td>
<td>0.72</td>
<td>0.45</td>
<td>0.046</td>
<td>0.66</td>
</tr>
<tr>
<td>RIO-15R</td>
<td>3</td>
<td>0.73</td>
<td>0.36</td>
<td>0.036</td>
<td>0.52</td>
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<tr>
<td>RIO-13R</td>
<td>6</td>
<td>0.82</td>
<td>0.31</td>
<td>0.008</td>
<td>0.13</td>
</tr>
<tr>
<td>RIO-11</td>
<td>9</td>
<td>0.72</td>
<td>0.29</td>
<td>0.006</td>
<td>0.83</td>
</tr>
<tr>
<td>RIO-07R</td>
<td>0.25</td>
<td>0.57</td>
<td>0.45</td>
<td>0.004</td>
<td>0.69</td>
</tr>
<tr>
<td>RIO-06R</td>
<td>0.41</td>
<td>0.57</td>
<td>0.41</td>
<td>0.003</td>
<td>0.56</td>
</tr>
<tr>
<td>RIO-04R</td>
<td>5</td>
<td>0.77</td>
<td>0.36</td>
<td>0.004</td>
<td>0.56</td>
</tr>
<tr>
<td>RIO-03R</td>
<td>4</td>
<td>0.77</td>
<td>0.36</td>
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<td>0.56</td>
</tr>
<tr>
<td>RIO-02R</td>
<td>7</td>
<td>0.86</td>
<td>0.31</td>
<td>0.002</td>
<td>0.64</td>
</tr>
<tr>
<td>RIO-01R</td>
<td>6</td>
<td>0.70</td>
<td>0.31</td>
<td>0.001</td>
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<tr>
<td>RIO-01R</td>
<td>7</td>
<td>1.0</td>
<td>1.0</td>
<td>0.000</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Bonferroni correction.

Because marker was not used in individual identification. *Signifies significant deviation from Hardy-Weinberg Equilibrium with microsatellite locus. Overall observed heterozygosity was 0.63 across all loci. Error rates for marker RIO-11 were not calculated.
<table>
<thead>
<tr>
<th>Model</th>
<th>Population Size (N)</th>
<th>Standard Error</th>
<th>Density (N/km$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCR</td>
<td>131 (118–157)</td>
<td>2.1</td>
<td>48.69</td>
</tr>
<tr>
<td>Traditional Capture-Recapture</td>
<td>148 (129–182)</td>
<td>2.0</td>
<td>31.9 (22.3–75.7)</td>
</tr>
</tbody>
</table>

*Signifies density estimate using maximum distance moved buffer for area calculation. **Signifies density estimate using half maximum distance moved as buffer for area calculation.

Table 3-2. Model estimates of population size, standard error, and density for the spatial capture-recapture and traditional capture models for river otter in northeastern Pennsylvania.
<table>
<thead>
<tr>
<th>V/N</th>
<th>( c=0.25 (0.02-0.92) )</th>
<th>( d=0.25 (0.02-0.92) )</th>
<th>148 (129–182)</th>
<th>4</th>
<th>(-355.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( c=0.26 (0.02-0.92) )</td>
<td>( d=0.26 (0.02-0.92) )</td>
<td>141 (112–212)</td>
<td>3</td>
<td>(-337.8)</td>
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<tr>
<td>( c=0.26 (0.02-0.92) )</td>
<td>( d=0.26 (0.02-0.92) )</td>
<td>131 (118–157)</td>
<td>3</td>
<td>(-337.9)</td>
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</tbody>
</table>

Table 3-3. The model parameterization, second-order Akaike’s Information Criterion Score (AICc), estimation for population size (\( \hat{N} \)), and probability of capture of occurrence in time varying (\( \hat{c}=d \)).

\( c \) When applicable:

- With 95% confidence interval, capture probability (\( c \)) by occasion when applicable.
- With 95% confidence interval, capture probability (\( c \)) by occasion when applicable.
- Time varying, probability of capture and recapture probability (\( c \)) by occasion when applicable.
- Time varying, probability of capture and recapture probability (\( c \)) by occasion when applicable.
- Time varying, probability of capture and recapture probability (\( c \)) by occasion when applicable.
- Time varying, probability of capture and recapture probability (\( c \)) by occasion when applicable.

Model Description