Original investigation

Use of non-invasive techniques to determine population size of the marine otter in two regions of Peru

Daniella Biffi *, Dean A. Williams

Department of Biology, Texas Christian University, 2955 S University Dr., Fort Worth, TX 76133, USA

ARTICLE INFO

Article history:
Received 5 March 2016
Accepted 16 December 2016
Handled by Frank L. Zchos
Available online 23 December 2016

Keywords:
Fecal DNA
Lontra felina
Microsatellites
Peru
Noninvasive genetic sampling

ABSTRACT

The marine otter (Lontra felina) can be found on rocky shores from the northern coast of Peru (9°S) to the extreme south of Argentina (56°S). This species is currently classified as endangered but there is little information on population size because marine otters are very difficult to observe and count. Between June and August 2012 we collected 240 samples of marine otter feces from seven localities in Peru. All locations were visited four times. One-hundred and thirty-three samples (55%) were successfully amplified at five to seven microsatellite loci and a sex-linked marker. We identified a minimum of 80 individuals (41 males and 39 females) across all locations for a density estimate of 4.4 otters per km, a value about 2X higher than estimates based on previous visual counts. Estimates using the program CAPWIRE averaged 12.6 otters/km, a value six times higher than estimates based on previous visual counts, although confidence limits were large due to the low number of recaptures. There was a strong positive relationship between the number of fresh scats and the number of unique genotypes, suggesting scat counts might be used to estimate the minimum number of otters at a site. Non-invasive genotyping of marine otter feces and scat counts will be valuable tools for estimating population sizes and monitoring movements of this secretive species.

© 2016 Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. All rights reserved.

Introduction

The marine otter (Lontra felina) can be found on rocky shores from the northern coast of Peru (9°S) to the extreme south of Argentina (56°S). Along the Peruvian coast, suitable habitat like rocky shores, alternate with patches of non-suitable habitats like sandy beaches. Marine otters utilize rocky patches for shelter in caves and crevices, and for their proximity to food sources like rocky shore fishes and invertebrates (Medina-Vogel et al., 2006, 2008). Marine otters are mostly solitary but sometimes occur as pairs or trios and very rarely in larger groups (Lariviere, 1998; Medina-Vogel et al., 2007).

In Peru, marine otter populations are at risk due to habitat loss (Sielfeld and Castilla, 1999) and artisanal fisheries (Pizarro, 2008). Marine otters are considered Endangered by the Red List of the International Union for Conservation of Nature (IUCN) because of population declines due to habitat loss and exploitation (Valqui et al., 2010). They are currently listed in Appendix A of the International Trade in Endangered Species of Wild Fauna and Flora (CITES), and are considered Endangered by Peruvian law.

There is little information for this species in Peru although recently, studies of diet (Biffi and Iannaccone, 2010), behavior (Ruiz, 2009; Valqui, 2004) and genetics (Valqui et al., 2010; Vianna et al., 2010) have been conducted. Nevertheless, due to the difficulties in observing this species there is limited information about population sizes and sex-ratio.

In 2005, Apaza surveyed 78 localities along 510 km of potential suitable habitat and a total of 756 ± 86 individuals were estimated with a mean density of 1.48 ind/km (Apaza and Romero, 2012). Valqui (2012) using research conducted between 2008 and 2011, estimated there were 789–2,131 individuals in Peru along 789 km of suitable habitat with a density of 1–2.7 ind/km (Valqui, 2012). Densities between 1 and 2.7 ind/km are the more frequently reported densities in previous studies in Peru and Chile (Valqui, 2012).

Counting elusive animals like otters is challenging. Behavioral studies of marine otters, based on direct observation during eight hours of daylight, conclude that they spend 80% of their time out of view (Medina-Vogel et al., 2006). Some authors have suggested that marine otter abundance may be much higher than currently estimated by visual methods (Sielfeld and Castilla, 1999). In some locations, the only evidence of their presence is the spraints (otter feces) found in latrines in rock caves and cracks (Biffi, personal observation). Genetic analyses of non-invasive samples like feces...
have been used successfully to study population abundance in
mustelids like the Eurasian otter Lutra lutra (Hung et al., 2004;
Park et al., 2011) and river otter Lontra canadensis (Mowry et al.,
2011). Mowry et al. (2011) first identified individuals by geneti-
cally typing feces, and then asked if measures of fecal abundance
could predict the number of individuals detected through genetic
typing. They found that both the number of feces at a latrine and
the density of latrines were accurate predictors of otter abundance.
Using the estimators developed by Mowry et al. (2011) researchers
can now simply monitor feces abundance to determine the abun-
dance of river otters without the need for the more expensive and
labor intensive genetic analyses.

Determining the distribution and population trends of marine
otters is critical information for the formulation of conservation
strategies in Peru and elsewhere in South America (Valqui, 2012).
The goals of this project were to 1) estimate the number of marine
otters utilizing seven sites in central and southern Peru using non-
invasive genetic sampling of their feces and 2) determine if it is
possible to use the number of feces as an indirect estimator of
marine otter abundance.

Material and methods

Study sites

We searched for marine otter feces in two regions along the
coast of Peru separated by ~730 km. The central region was
located in the Department of Lima and had two sampling localities and the
southern region included the Departments of Moquegua and Tacna
and encompassed five localities (Fig. 1, Table 1). A total of 18.14 km
of coastline was searched regularly for spraints (Table 1).

In the central region, Pucusana Bay (PU) is an artisanal fishing
port and yacht club. There is constant traffic in the bay by fish-
ing and recreational boats. The bay is composed of rocky shores
alternating with small patches of sandy beaches. We collected feces
inside the recreational boats anchored in front of the club. The sec-
ond site in the central region is Punta Corrientes (PC) located 60 km
south of PU and is a summer residential site. It has a 450 m sandy
beach surrounded by rocky shores. There is a low cave with easy
access where feces were collected.

The Southern Ilo site (SI) was made up of three localities, the
pier of the thermal power plant of Enersur, and the rocky shores
of La Higuera and Chorrillos. The first section is a 100 m stretch of
tetrapods (concrete structures used in breakwaters) located at the
beginning of the pier, a 1.5 km extension of sandy beach with rocky
patches, and an area of shallow rocky shores. Feces were collected
in all three sites but due to their proximity (less than 5 km apart) we
merged the sites in this area since Medina-Vogel et al. (2007) deter-
mined the home ranges of marine otters were from 1.4 to 4.1 km of
seashore. Punta Picata (PP) is 5 km south of SI and is a 4 km rocky
shore where feces were found and is located between 5 km of sandy
beach on its north end and shallow rocky shores to the south. Puerto
Grau (PG) is 28 km south of PP. PG is an artisanal fishing port
and feces were located mainly along a man-made breakwater of large
rocks. To the north of this area were rocky shores and to the south
were low rocky shores where no otters or feces were seen. Que-
brada Burros (QB) is 7 km south of PG and is composed of nearly
3 km where feces were found. Vila Vila (VV), the southernmost site,
is a fishing village located 11 km south of QB. This site was com-
posed of two man-made water breaks composed of large rocks and
the rocky shore of Canepa, a beach located north of the village. Feces
were found in both water breaks and along the rocky shore. To the
south of this site we ~ 10 km of low rocky shores and then a 40 km
sandy beach extending to Chile.

Marine otters are patchily distributed along the coast of Peru and
Chile depending on the presence of suitable rocky shores (Medina-
Vogel et al., 2008; Valqui, 2012). Long stretches of sandy beach
appear to limit otter movement. The two sites in the north are sepa-
rated by six sandy beach areas that range from 1.2 to 7.8 km in
length (Fig. 1). In the south there are fewer long stretches of sandy
beach between the sites. The longest stretch (14 km) is located
between PP and PG which is the zone of the artificial lte wetlands
(Fig. 1). Between PG and QB there are no extensive stretches of
beach.

Sample collection

We collected fresh marine otter spraints (~1 day old) and dis-
carded older ones for every visit. GPS coordinates were taken for
all feces collected. Two sampling periods were conducted in each
region between June — August 2012 separated by ~10 days. Dur-
ing each sample period, each location was visited a first time and
then a second time three days later. Using surgical gloves, a small
part from the surface of the feces was extracted with a straw and
immediately placed in a vial with 1 ml 8 M urea preservative buffer
(10 mM Tris pH 7.5. 125 mM of NaCl, 10 mM EDTA pH 8.0, 1% SDS
and 8 M urea) (Asahida et al., 1996).

Genetic analysis

DNA extraction

DNA was extracted using the QiAamp DNA Stool Mini-kit (Qiag-
gen Genomics, Valencia, CA). We followed the manufacturer’s
protocols with the following modifications: at the beginning of the
extraction we spun the via with the sample, removed the super-
natant and added 1.6 ml of Buffer ASL. Negative DNA extraction
controls were included in each extraction batch to test for contam-
ination of reagents. Extractions were conducted in an extraction
dedicated AirClean® 600 PCR workstation.

Microsatellite marker development

We selected 16 microsatellite loci developed for L. longicaudis
(Beheler et al., 2005), Pteronura brasiliensis (Ribas et al., 2011),
and L. lutra, (Dallas and Pietrney, 1998; Hung et al., 2005) to be
tested on fecal samples of marine otters. Nine markers either did
not amplify or were monomorphic: Lut435, Lut453, Lut701, Lut782,
Phra21, Rio06, Rio12, Rio16 and 040702. Seven microsatellite loci
amplified and were polymorphic in marine otters: 040717, Phra01,
Phra02, Phra24, Rio11, Rio13, and Rio18 (Table 2). PCR products for
all seven loci were gel purified using the Wizard® SV Gel and PCR
Clean-Up System (Promega, USA). PCR products were cloned with
the pGEM-T Easy Vector System (Promega, USA). We sequenced clones using ABI Big Dye Terminator Cycle Sequencing v 3.1
chemistry (Applied Biosystems, USA) using pGEM vector primers.
Sequences were electrophoresed on an ABI 3130XL Genetic Ana-
lizer (Applied Biosystems, USA). Sequences were trimmed and
edited using Sequencher v. 5.0. We verified the sequences con-
tained the expected microsatellite region and then used Primer3
(Rozen and Skaletsky, 2000), for five of the seven loci to design
primers flanking the microsatellite region in order to amplify frag-
ments less than 200 bp. We did not design primers for Rio11 and
Rio18 because these were in the appropriate size range and there
was not sufficient sequence flanking the microsatellite repeat to
design high quality primers. Each locus was fluorescently labeled
with one of four dyes (Table 2).

Genotyping

Sets of 16 samples were amplified at each single locus with three
to nine PCR replicates each (1178 total PCR reactions). PCR reactions
were conducted in a separate room from DNA extractions in a PCR
Fig. 1. Study sites sampled for marine otter scats in Peru, (a) sites in the central region, (b) sites in the southern region. Hatched lines along the coast indicate the presence of beach areas 1 km or greater in length.

Table 1
Localities where marine otter spraints were collected.

<table>
<thead>
<tr>
<th>Region</th>
<th>Department</th>
<th>Site abb.</th>
<th>Site</th>
<th>Km searched</th>
<th>Type</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>Lima</td>
<td>PU</td>
<td>Pucusana</td>
<td>0.14</td>
<td>Fishing Port</td>
<td>12°28’42''</td>
<td>76°47’45''</td>
</tr>
<tr>
<td>Central</td>
<td>Lima</td>
<td>PC</td>
<td>Pta. Corrientes</td>
<td>0.10</td>
<td>Cave, Rocky Shore</td>
<td>12°57’17''</td>
<td>76°30’53''</td>
</tr>
<tr>
<td>South</td>
<td>Moquegua</td>
<td>SI</td>
<td>Ilo</td>
<td>7.90</td>
<td>Tetrapods, Rocky Shore</td>
<td>17°46’38''</td>
<td>71°11’32''</td>
</tr>
<tr>
<td>South</td>
<td>Tacna</td>
<td>PP</td>
<td>Pta. Picata</td>
<td>4.04</td>
<td>Rocky Shore</td>
<td>17°51’59''</td>
<td>71°05’49''</td>
</tr>
<tr>
<td>South</td>
<td>Tacna</td>
<td>PG</td>
<td>Pta. Grau</td>
<td>0.82</td>
<td>Fishing Port</td>
<td>18°59’36''</td>
<td>70°53’40''</td>
</tr>
<tr>
<td>South</td>
<td>Tacna</td>
<td>QB</td>
<td>Qda. Burros</td>
<td>2.94</td>
<td>Rocky Shore</td>
<td>18°03’54''</td>
<td>70°50’26''</td>
</tr>
<tr>
<td>South</td>
<td>Tacna</td>
<td>VV</td>
<td>Vila Vila</td>
<td>2.20</td>
<td>Fishing Port, Rocky Shore</td>
<td>18°05’31''</td>
<td>70°45’10''</td>
</tr>
</tbody>
</table>

Table 2
Characterization of the microsatellite loci used for marine otters.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer Sequence (5’–3’)</th>
<th>Cloned PCR product (bp)</th>
<th>Size range in L. felina (bp)</th>
<th>Annealing temperature C</th>
<th>Dye label</th>
<th>Coload</th>
</tr>
</thead>
<tbody>
<tr>
<td>04OT17Lf</td>
<td>GCCGCACATAGTGAATTACAGG CCGGATTTGAACCCTACCTTC</td>
<td>190</td>
<td>163–221</td>
<td>55</td>
<td>6-FAM</td>
<td>–</td>
</tr>
<tr>
<td>Pbra01Lf</td>
<td>ACAGTCTCCATTGCCTTGTCG CCAACAGGGGTCTACCTAAA</td>
<td>181</td>
<td>161–185</td>
<td>55</td>
<td>NED</td>
<td>B</td>
</tr>
<tr>
<td>Pbra02Lf</td>
<td>GTCTGAGCGAGATGTTGTCG AGGGGCAACATGACATCC</td>
<td>114</td>
<td>88–127</td>
<td>55</td>
<td>6-FAM</td>
<td>A</td>
</tr>
<tr>
<td>Pbra24Lf</td>
<td>AAGATTATTCCCTGCCTCTCTCTTT TGGTAACCTGAATGTTACTGAT TCTCCACTTTCATTTAGGTA</td>
<td>120</td>
<td>75–148</td>
<td>60</td>
<td>6-FAM</td>
<td>B</td>
</tr>
<tr>
<td>Rio11</td>
<td>GCCGAAGTCTTTGCTATTGAGA</td>
<td>NA</td>
<td>115–159</td>
<td>55</td>
<td>VIC</td>
<td>B</td>
</tr>
<tr>
<td>Rio13Lf</td>
<td>GCACATGGCTTTATGAGA</td>
<td>139</td>
<td>103–139</td>
<td>60</td>
<td>NED</td>
<td>A</td>
</tr>
<tr>
<td>Rio18</td>
<td>TTCCATGCTCTCGCGCTGTC</td>
<td>NA</td>
<td>123–157</td>
<td>60</td>
<td>VIC</td>
<td>A</td>
</tr>
</tbody>
</table>

NA – primers were not redesigned for these loci. Loci with the same letter in “Coload” were run together during capillary electrophoresis.
Table 3  
Genetic diversity estimates at microsatellite loci for marine otters across all sites.

<table>
<thead>
<tr>
<th>Locus</th>
<th>N</th>
<th>Na</th>
<th>H0</th>
<th>H1</th>
<th>FIS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>O04071Lf</td>
<td>70</td>
<td>11</td>
<td>0.529</td>
<td>0.529</td>
<td>-0.006</td>
<td>0.100</td>
</tr>
<tr>
<td>Pbra01Lf</td>
<td>52</td>
<td>7</td>
<td>0.577</td>
<td>0.594</td>
<td>0.020</td>
<td>0.004</td>
</tr>
<tr>
<td>Pbra02Lf</td>
<td>79</td>
<td>10</td>
<td>0.392</td>
<td>0.387</td>
<td>-0.020</td>
<td>0.100</td>
</tr>
<tr>
<td>Pbra24Lf</td>
<td>78</td>
<td>9</td>
<td>0.449</td>
<td>0.454</td>
<td>0.006</td>
<td>0.670</td>
</tr>
<tr>
<td>Rio11</td>
<td>78</td>
<td>9</td>
<td>0.513</td>
<td>0.460</td>
<td>-0.121</td>
<td>0.160</td>
</tr>
<tr>
<td>Rio13Lf</td>
<td>80</td>
<td>10</td>
<td>0.788</td>
<td>0.651</td>
<td>-0.218</td>
<td>1.000</td>
</tr>
<tr>
<td>Rio18</td>
<td>70</td>
<td>10</td>
<td>0.586</td>
<td>0.742</td>
<td>0.205</td>
<td>0.001</td>
</tr>
</tbody>
</table>

N = number of individuals typed, Na = number of alleles, H0 = observed heterozygosity, H1 = expected heterozygosity, FIS = inbreeding coefficient, P = probability of departure from Hardy-Weinberg expectations.

Table 4  
Success rate, proportion of allelic dropout (ADO) and false alleles (FA) by locus.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Success rate</th>
<th>ADO</th>
<th>FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>O04071Lf</td>
<td>0.340</td>
<td>0.481</td>
<td>0.017</td>
</tr>
<tr>
<td>Pbra01Lf</td>
<td>0.195</td>
<td>0.228</td>
<td>0.002</td>
</tr>
<tr>
<td>Pbra02Lf</td>
<td>0.550</td>
<td>0.348</td>
<td>0.013</td>
</tr>
<tr>
<td>Pbra24Lf</td>
<td>0.495</td>
<td>0.400</td>
<td>0.015</td>
</tr>
<tr>
<td>Rio11</td>
<td>0.555</td>
<td>0.423</td>
<td>0.025</td>
</tr>
<tr>
<td>Rio13Lf</td>
<td>0.600</td>
<td>0.221</td>
<td>0.013</td>
</tr>
<tr>
<td>Rio18</td>
<td>0.410</td>
<td>0.272</td>
<td>0.022</td>
</tr>
<tr>
<td>Mean</td>
<td>0.450</td>
<td>0.339</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Results

Genotyping

We collected 240 samples (68% fresh feces, 18% anal jelly [anal sac secretions], and 14% feces with anal jelly) which represented 45% of all feces present. A total of 133 samples (55%) were successfully amplified at five to seven loci (Online Appendix A). The remaining 107 samples were discarded, since they amplified at fewer than five loci. The genotyping success rate of anal jelly was higher (74% of 43) than that of feces with jelly (61% of 36) and feces (50% of 161) (χ² = 7.98, df = 2, P = 0.02).

The seven loci were moderately polymorphic with an average of nine alleles (range 7–11) and an average observed heterozygosity of 0.55 (range 0.392–0.788) (Table 3). When all sites were analyzed as a single population, Rio18 and Pbra01Lf exhibited significant heterozygote deficits (P < 0.001) and Rio13Lf had a significant heterozygote excess (P = 0.003). None of the pairs of loci exhibited genotypic linkage disequilibrium. When analyzing each sampling site separately, Rio18 exhibited a significant heterozygote deficit only in S1 (P = 0.001) and Pbra01Lf and Rio13Lf no longer exhibited deficits or excesses so we kept these loci in the consensus genotypes. The presence of a possible null allele in Rio18 may have reduced our ability to differentiate some individuals if they had the null allele present.

PCR success rate at a locus averaged 45% and ranged from a minimum of 19.5% for Pbra01 to a maximum of 60% for Rio13 (Table 4). Allelic dropout was high, averaging 34% and ranging from 22 to 48% while the incidence of false alleles was only 1.5% (Table 4). The probability of identity (PI) was 0.000048 for all sites combined and ranged from 0.00004–0.00160 across sites for genotypes with all seven loci and ranged from 0.021–0.002 for genotypes with only five loci (Table 5). The probability of discerning full siblings from each other (P(sibs)) was not very high, averaging 0.024 (range 0.01–0.05) across the seven sites for genotypes with seven loci. For
genotypes with only five loci there was no ability to differentiate siblings at a site with an average $P_{\text{hbs}}$ of 0.10 (Table 5).

Population estimation

A total of 80 individuals were identified across all sites and visits, ranging from four otters in PU to 16 otters in PC (Table 6). Of the 80 individuals identified, 41 were typed at all seven loci, 25 were typed at six loci, and 14 were typed at five loci. Overall, the number of unique individuals at a site does not stabilize by four visits although the sampling curve suggests the addition of new individuals is reduced by the fourth visit and may stabilize with several more visits to each site (Fig. 2). New individuals were identified during every visit, with the second visit having the highest number of individuals being detected for the first time. Most individuals ($N = 63$) were sampled only a single time whereas 17 individuals were sampled multiple times either during the same visit or across multiple visits. The average number of samples found for these individuals was $4.1 \pm 0.70$ SE (range 2–14). For these 17 individuals, distances between samples of the same individual ranged from less than a meter up to 6.4 km apart. Most (90.9%) of these samples were within 150 m of each other (159 of 175 pairwise distances within sites).

The sex ratio across sites was close to 1:1 with 39 females and 41 males. Within sites, the numbers of males and females were also similar (Table 6). Most of the individuals that were sampled multiple times were male (13 males, four females), although the individual with the greatest number of samples ($N = 14$) was a female. Overall, there was no difference in the number of samples collected from males and from females (58 female samples and 75 male samples, $X^2 = 1.19$, df = 1, $P = 0.28$). All of the individuals that were detected at distances greater than 200 m apart were male ($N = 4$ individuals). One male was at PP (520 m), one at QB (240 m) and two were from SI and ranged from 1.64–6.4 km apart.

We were able to estimate population size using CAPWIRE for 14 site visits that had recaptures during the same visit (Online Appendix B). For the remaining 14 site visits, all individuals were sampled only a single time during a visit or no samples were collected (Online Resources 2). We also estimated the total population size at a site by combining all data for the four visits. For most sites, the CAPWIRE point estimate of $N$ was greater than the minimum estimated from the genetic data and was considerably higher than previous estimates based on sightings (Apaza and Romero, 2012; Valqui, 2012) (Table 6). The confidence intervals (95%) often included the minimum estimate from the genetic data but were fairly wide for most estimates, reflecting the high incidence of single captures in the data as evidenced by an average recapture of individuals of less than two during most visits with recaptures (Online Appendix B, Table 6). Our minimum estimate from the genetic data (80 individuals) was similar to visual estimates, about 1.3 fold greater than Apaza and Romero’s (2012) visual estimate (31 individuals) and 0.95 fold greater than Valqui’s (2012) visual estimate (35 individuals, excluding SI). Estimates of density ranged from 4.4 otters per km using only the minimum genetic estimate ($N = 80$ otters) to 12.6 otters per km when using all CAPWIRE estimates for each site across all four visits ($N = 231$ otters).

Both PU and especially PC had very high numbers of otters detected in a small area (Table 6). We removed these data points from most of the following regression analyses because they strongly influenced the overall results. There was a strong positive relationship between the minimum number of otters estimated from the genetic data across all four visits, even after both PC and PU were removed from the analysis ($n = 5$, $y = 0.231x + 0$, $R^2 = 0.93$, $F_{1,4} = 52.77$, $P = 0.005$). Adding PU into the analysis gives a similar relationship ($n = 6$, $y = 0.186x + 0$, $R^2 = 0.98$, $F_{1,5} = 300.05$, $P = 0.00006$) (Fig. 3a). The relationship based on estimates from CAPWIRE across all four visits was non-linear when both PC and PU were removed ($n = 5$, $y = 0.024x^2 + 1.261x + 2.92$, $R^2 = 0.36$) (Fig. 3b) and strongly linear ($y = 0.376x + 0.75$) when PU was included although this data point has high leverage in the regression model.

Discussion

Our data suggests that otters are more abundant in the areas we sampled than previously estimated by visual counts. Estimates of otter density using all combined visits and either the minimum genetic estimate or CAPWIRE estimate (4.4 and 12.6 otters/km, respectively) are two to six times higher than estimates based on visual sightings in this region of Peru and elsewhere in Chile. The CAPWIRE estimates were often not very precise since the confidence intervals were wide, however the lower 95% CI often included or was close to the minimum estimate from the genetic data. The lack of precision for these estimates is probably due to the low number of recaptures of individuals within and between visits. The assumption of population closure for CAPWIRE may also not be met.
in this situation because we detected new individuals in most visits to a site.

When genotyping errors are high and recaptures are low, misidentification of individuals can lead to an overestimation of population size (Hansen et al., 2008). In extreme cases, genotyping error can overestimate population size by as much as 300% which is why the multiple tube approach and negative controls are important to implement in non-invasive genetic studies (Creel et al., 2003; Broquet and Petit, 2004). Our overall genotyping success (45%) falls within the range of other studies of mustelids. Prigioni et al. (2006) obtained a 41.2% genotyping success rate from L. lutra when using 10 microsatellites, while Mowry et al. (2011) reported a genotype success of 24% for samples that amplified at seven to 10 loci. Hansen et al. (2008), achieved a 56% success rate, however only one locus was used in the analysis, and it drops to 8% when four loci were considered in the analysis. More than half of the samples we collected (55%) amplified from five to seven loci. A similar value was found by Hung et al. (2004) in their study of L. lutra, where 65% of the samples collected worked (222 out of 343 samples). Allele dropout rates (34%) in this study were higher than those reported in other studies. For L. lutra, Hájková et al. (2009) reported 18% allelic dropout for all samples and Arrendal et al. (2007) obtained an allelic dropout of 7.7% for samples that yielded complete genotypes and 13.6% for samples with incomplete genotypes. Hung et al. (2004) obtained an allele dropout rate of 30.7%. In contrast, our false allele error rate (1.5%) was comparable to other studies. Hájková et al. (2009) obtained a 2.9% false allele rate and Arrendal et al. (2007) reported a 9% false allele rate.

We therefore believe that genotyping error is not responsible for our high estimates of otters at these sites. Our ability to distinguish siblings was low and we conservatively assumed that genotypes differing at only one locus were the same and so we may have underestimated the number of otters at some sites. Developing more marine-otter-specific loci will be useful to increase the power to differentiate individuals, especially parent-offspring and siblings. The use of next generation sequencing (NGS) technologies has increasingly been utilized to cost-effectively develop 100 s of microsatellite marker loci in non-model organisms and could prove useful for marine otters (e.g. Jennings et al., 2011; Yu et al., 2011). Recently, Vianna et al. (2010) found a strong genetic break in marine otters between Peru and Chile consistent with earlier morphological studies (Housse, 1953; Sielfeld, 1983) suggesting there may be two separate subspecies or species and so marker development should be conducted using samples collected from both regions. Mitochondrial markers are also diverse in marine otters and could potentially help distinguish individuals in conjunction with microsatellite markers (Valqui et al., 2010; Vianna et al., 2010).

The presence and relative abundance of fresh water otters is often determined by the presence of scats and dens (e.g. Conroy and French, 1987; Kruuk et al., 1989) and recent studies of fresh water otters utilizing genetic profiling of scats have revealed that the number of scats can be correlated with the number of otters in an area (e.g. Mowry et al., 2011). Some researchers have suggested that scat counts may not be an effective way to estimate marine otter abundance because, 1) some of the habitat is inaccessible to check for scats, 2) high tides periodically remove scat from shorelines, and 3) otter movement patterns may vary with age, sex, and reproductive status, and so may result in variable deposition patterns over time at any one site (Medina-Vogel et al., 2006; Alvarez, 2012). All of these potential problems could lead to underestimates of marine otter population sizes, although visual sightings also underestimate marine otter abundance (Medina-Vogel et al., 2006; Alvarez, 2012).

Our study suggests that fecal counts can provide useful information about marine otter abundance, although this should be tested in other areas of Peru and Chile to determine whether the relationship we found is geographically variable. The relationship we found may not hold in other areas due to differences in coastal architecture or to different behavioral patterns in fecal deposition. There was a strong linear relationship between the density of fresh scats at a site and the density of otters estimated from the number of unique genotypes and so the number of fresh scats would give a minimum estimate of the number of otters utilizing a site. The relationship between the density of fresh scats and the CAPWIRE point estimates was non-linear when not including the two sites with unusually high densities (PC and PU). The non-linear relationship appears to be due mainly to one site (PG) which had a high density of scats but a relatively low density of otters as estimated by CAPWIRE. More intensive sampling may result in more precise point estimates which could reveal a linear relationship with CAPWIRE estimates.

Visit three most closely approximated the time frame used by Mowry et al. (2011) in their study of river otters since this sampling period occurred ~10 days after clearing feces from a site. There was a strong relationship between the density of otters and the density of scats for this sampling period (results not presented). Using this single visit to estimate otter abundance, however, would have underestimated the number of otters utilizing these sites over the course of this study by almost 50%. Our sampling curve indicates that probably more than four visits to a site will be necessary in order to capture all individuals utilizing a site. Increased temporal sampling at a site and nearby areas may reveal the optimal temporal and spatial scale needed to accurately determine the total number of otters utilizing a site by scat counts.
Marine otters are not sexually dimorphic and so the sex ratio of this species has not been previously described. The sex ratio was approximately even across the sites. Most of the individuals for which there were recaptures were male which suggests some individual males may engage in more scent marking behavior than females, however; there was not an overall difference in the number of sprint that belonged to males and females.

The relatively high numbers of individuals utilizing the same site suggests that home ranges may not be very exclusive, similar to the single radio tracking study of six otters by Medina-Vogel et al. (2007) in Chile. There may also be transient individuals or temporary residents as has been described in European otters (Lutra lutra) (Erlinge, 1968; Kalz et al., 2006). The individuals that were recaptured at distances greater than 200 m apart were all male which is consistent with male-biased movement or dispersal as has been described for other otter species (e.g. Blundell et al., 2002; Quaglietta et al., 2013). These long movements also all occurred along continuous rocky seashores consistent with previous observations (Medina-Vogel et al., 2007). One of the highest densities recorded in this study was at site PC which was a small 100 m rocky area with a cave that contained scats from at least 16 otters. It is unknown why this site contained so many different individuals since it was located near a summer residential area and was also frequented by numerous fisherman fishing for near-shore fishes. Sites PG and PU also had relatively high densities of otters. Both of these sites are artisanal fishing ports and so may attract otters because they have a readily exploited resource (fish and fish parts) similar to what has been suggested by Medina-Vogel et al. (2007) for marine otters in Chile. More intensive genetic sampling at a site and immediately surrounding areas could potentially be used to better understand marine otter movements and how they may be related to sex or resource availability.

Our study utilizing non-invasive genotyping of marine otter feces has confirmed earlier suspicions that this species is probably more abundant than visual counts have suggested. Expanding this type of study throughout the marine otter’s range will give more accurate population size estimates which will inform conservation strategies in Peru and Chile. These data could also be used to study individual movements of otters. In the future, researchers may also be able to utilize scat counts to monitor populations of this secretive species.

Acknowledgements

We thank Amanda Hale, John Horner, and Tamie Morgan for their valuable suggestions throughout the study, Juan Valqui, Joanna Alfaro, Manolo Apaza, Thomas Valqui, and Calo Calvo-Mac for their help with the logistics of the fieldwork, and Francisco “Chaval” Bernedo from ProDelphinus, Valeria Verme. Rodrigo Lecca, Patricia Biffi for their assistance with sample collection. This study was funded by The Rufford Foundation, the TCU Adkins Fellowship, the TCU Graduate Student Travel Grant, the TCU Graduate Student Senate, and IdeaWild. This study was conducted under the research authorization No. 357–2012–AG-DGFSS-DGEFFS, the access to genetic resources authorization No. 002-2014-MINAGRI-DGFSS/DGEFFS, and the exporting authorization CITES No. 15-PE-000333/SP, all emitted by the Direcction General Forestal y de Fauna Silvestre de Peru.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mambio.2016.12.006.


Sielfeld, W., 1983. Mamíferos Marinos De Chile. Ediciones de la Universidad de Chile, Santiago (Chile).


