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Original investigation

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

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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.

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 and Rheingantz, 2015). 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 Iannacone, 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

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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 genetically 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 abundance 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 fishing 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 second 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) determined 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. Quebrada 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 composed 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 were ~ 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 separated 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 Ite 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 discarded 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. During 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 (Qiagen Genomics, Valencia, CA). We followed the manufacturer's protocols with the following modifications: at the beginning of the extraction we spun the vial with the sample, removed the supernatant and added 1.6 ml of Buffer ASL. Negative DNA extraction controls were included in each extraction batch to test for contamination 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 Piertney, 1998; Huang 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, Pbra21, Rio06, Rio12, Rio16* and *04OT02*. Seven microsatellite loci amplified and were polymorphic in marine otters: *04OT17, Pbra01, Pbra02, Pbra24, 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 Analyzer (Applied Biosystems, USA). Sequences were trimmed and edited using Sequencher v. 5.0. We verified the sequences contained the expected microsatellite region and then used Primer3 (Rozen and Skaletsky, 2000), for five of the seven loci to redesign primers flanking the microsatellite region in order to amplify fragments less than 200 bp. We did not redesign 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

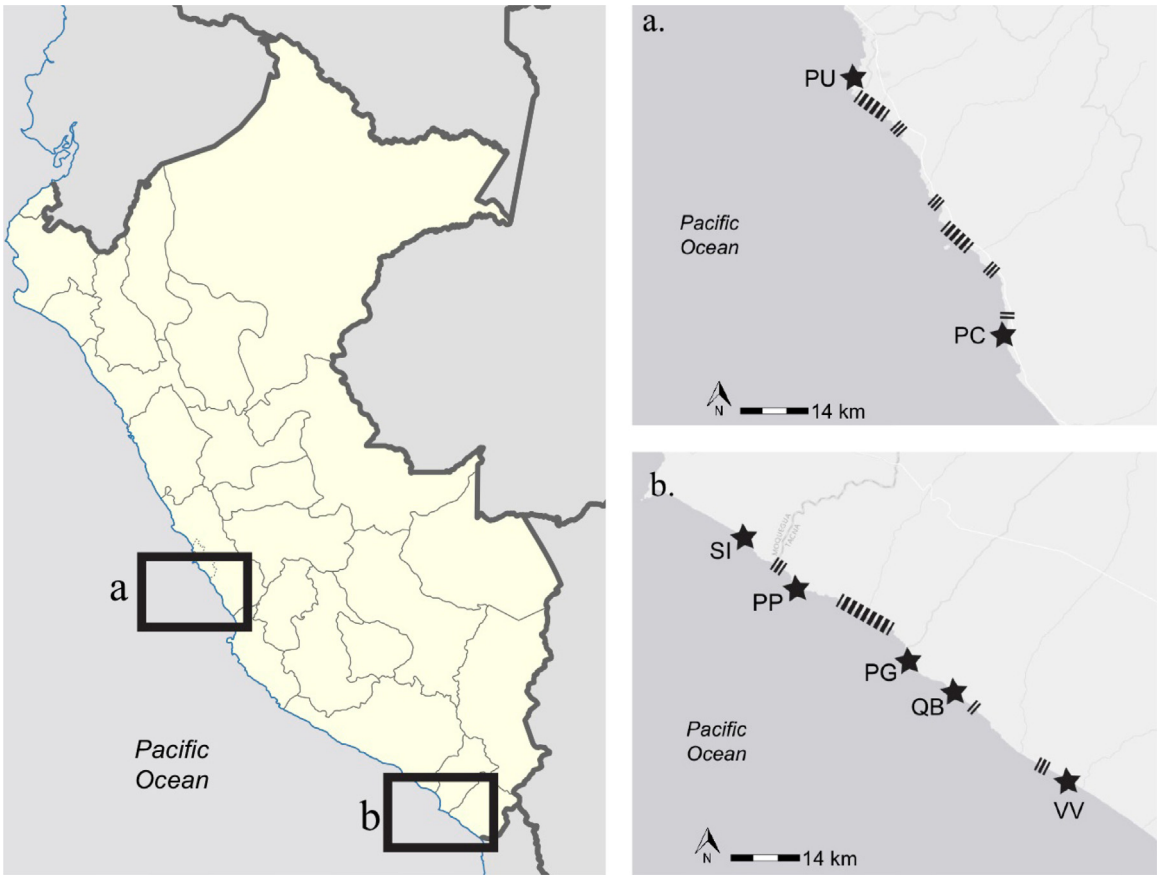

Fig. 1. Study sites sampled for marine otter scats in Peru, (a) sites in the central region, (b) sites in the southern region. Hatched lines along the coast indicate the presence of beach areas 1 km or greater in length.

Table 1
Localities where marine otter spraints were collected.

Region	Department	Site abb.	Site	Km searched	Type	Latitude	Longitude
Central	Lima	PU	Pucusana	0.14	Fishing Port	12°28′42″	76°47′45″
Central	Lima	PC	Pta. Corrientes	0.10	Cave, Rocky Shore	12°57′17″	76°30′53″
South	Moquegua	SI	Ilo	7.90	Tetrapods, Rocky Shore	17°46′38″	71°11′32″
South	Tacna	PP	Pta. Picata	4.04	Rocky Shore	17°51′59″	71°05′49″
South	Tacna	PG	Pto. Grau	0.82	Fishing Port	18°59′36″	70°53′40″
South	Tacna	QB	Qda. Burros	2.94	Rocky Shore	18°01′54″	70°50′26″
South	Tacna	VV	Vila Vila	2.20	Fishing Port, Rocky Shore	18°05′31″	70°45′10″

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

Locus	Primer Sequence (5′–3′)	Cloned PCR product (bp)	Size range in *L. felina* (bp)	Annealing temperature C°	Dye label	Coload
04OT17Lf	GCCGCACTAGTGATTATCAGG CGGGATTTGCAACCTACTTC	190	163–221	55	6-FAM	–
Pbra01Lf	ACAGTTCCTTTGCCTGTTGC CCACAAGGGGTTCACTCTAAA	181	161–185	55	NED	B
Pbra02Lf	GTCCTGAGCAGATGTTGTGC AGGGGCACACATACACATCC	114	88–127	55	6-FAM	A
Pbra24Lf	AAGTATTTTCCCTCCCTTCTTTT TGGTGAACTGAAATGTTACTTGAT	120	75–148	60	6-FAM	B
Rio11	TCTTCCACTTTTCAATTTAGGTA GCCCAAGGTTCACTATCAAG	NA	115–159	55	VIC	B
Rio13Lf	GCACATGGGCTTTTATGAAGA CACGTGGTAAGATGAGCATTG	139	103–139	60	NED	A
Rio18	TTCCATTGTCTCTTGGCTTG CCCTCTCCACACTTGTGCTC	NA	123–157	60	VIC	A

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.

Locus	N	Na	H_O	H_E	F_{IS}	P
O4OT17Lf	70	11	0.529	0.529	−0.006	0.100
Pbra01Lf	52	7	0.577	0.594	0.020	0.004
Pbra02Lf	79	10	0.392	0.387	−0.020	0.100
Pbra24Lf	78	9	0.449	0.454	0.006	0.670
Rio11	78	9	0.513	0.460	−0.121	0.160
Rio13Lf	80	10	0.788	0.651	−0.218	1.000
Rio18	70	10	0.586	0.742	0.205	0.001

N – number of individuals typed, Na – number of alleles, H_O – observed heterozygosity, H_E – expected heterozygosity, F_{IS} – 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.

Locus	Success rate	ADO	FA
O4OT17Lf	0.340	0.481	0.017
Pbra01Lf	0.195	0.228	0.002
Pbra02Lf	0.550	0.348	0.013
Pbra24Lf	0.495	0.400	0.015
Rio11	0.555	0.423	0.025
Rio13Lf	0.600	0.221	0.013
Rio18	0.410	0.272	0.022
Mean	0.450	0.339	0.015

dedicated AirClean® 600 PCR workstation. Negative controls were included in each PCR batch of 16 samples. PCR reactions were set up in 10 μl volumes containing 1 μl DNA, 0.5 μM primer, 1X Buffer with 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μl BSA and 0.04 μl *Taq* DNA Polymerase (Promega). Samples were run on an ABI 2720 thermal cycler (Life Technologies) using the PCR profile: 94 °C for 2 min, then 35 cycles of 94 °C for 30 s, 55 °C or 60 °C for 30 s, 72 °C for 1 min, and then a final extension at 72 °C for 10 min. Resulting PCR products were diluted in 50 μl of water. Six loci were pooled into two groups (A and B) and locus *04OT17Lf* was run singly for each sample (Table 3). One microliter of each coload was added to 10 μl of Hi-Di formamide and 0.1 μl of GS500 LIZ size standard (Life Technologies) and then electrophoresed on an ABI 3130XL Genetic Analyzer (Life Technologies). Genotypes were scored using GENEMAPPER 5.0 (Life Technologies).

We initially ran three replicates of all samples. We then used the comparative method (Frantz et al., 2003; Hansen et al., 2008; Hájková et al., 2009) to create consensus genotypes. A genotype at a locus was scored as a homozygote if a single allele occurred >2 times across replicates and a heterozygote was scored if more than one allele was scored ≥2 times across replicates. Consensus genotypes were constructed manually for each individual. If a sample had a consensus genotype with fewer than five loci we then ran three more replicates up to a total of nine replicates. Genotypes were then discarded if the consensus still had four or fewer loci. Genotypes were considered identical when they matched at all loci or all but one locus. We used the multilocus analysis in GenAlEx v 6.5 (Peakall and Smouse, 2012) to find genotyping matches.

We used GIMLET v. 1.3.2 (Valière, 2002) to determine error rates for each locus including the proportion of successful PCRs across all replicates, the proportion of allele dropout (ADO) which occurs when an allele of a heterozygous individual does not amplify in a PCR that is positive for the other allele, and the incidence of false alleles (FA) which occurs when a homozygote from the consensus genotype is typed as a heterozygote from repeated genotypes (Broquet and Petit, 2004). Using GIMLET v. 1.3.2, we also calculated the probability of identity (PI) and probability of identity for siblings (PI_{sibs}). PI is the probability that two individuals drawn at random from a population will have identical multilocus genotypes. The PI_{sibs} is the probability that a parent or offspring of a particular individual or their siblings would have the same genotype.

We calculated the number of alleles, observed heterozygosity, expected heterozygosity and the inbreeding coefficient F_{IS} using the unique consensus genotypes in GenAlEx v6.5. We tested for Hardy-Weinberg and genotypic linkage equilibrium using GENEPOP v4.2 (Rousset, 2008).

The number of otters at a site was estimated using the number of unique genotypes and using the program CAPWIRE that utilizes multiple observations of individuals within a sampling session to estimate population size (Miller et al., 2005). This program also accounts for potential capture heterogeneity (i.e. some individuals are captured more easily than others). We used the two innate rates model (TIRM) for all analyses. We analyzed data for each sampling session separately and across all sampling sessions. We then standardized all sites by the number of kilometers searched and used regression models (with intercept set at 0) to test whether the density of scats predicted the density of otters at a site estimated from the genetic data and from CAPWIRE.

Samples with consensus genotypes were sexed using the male specific SRY primers *SRY2F*: 5′-GAGAATCCCCAAATGCAAAA-3′ and *SRY2R*: 5′-CTGTATTCTCTGCGCCTCCT-3′ developed by Mowry et al. (2011). These primers were originally designed to amplify a 111-base pair fragment in male Nearctic river otters. Cloning of several PCR products (methods as above for microsatellite loci) indicated that these primers also amplify a portion of the SRY gene in marine otters. PCR conditions were the same as for the microsatellite loci except the annealing temperature was 55 °C and 40 cycles were used. We assigned samples as male when ≥3 amplifications had the PCR product and as female when ≥3 amplifications were blank.

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) ($X^2 = 7.98$, 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 SI (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 (PI_{sibs}) was not very high, averaging 0.024 (range 0.01–0.05) across the seven sites for genotypes with seven loci. For

Table 5
The probability of identity (PI) and probability of identity for full siblings (PI_{sibs}) for increasing number of typed loci at each sampling site (5 loci – *O4OT17Lf, Pbra01Lf, Pbra02Lf, Pbra24Lf, Rio11*, 6 loci previous five plus *Rio13Lf*, and 7 loci previous six plus *Rio18*).

Site	PI 5 loci	PI 6 loci	PI 7 loci	PI_{sibs} 5 loci	PI_{sibs} 6 loci	PI_{sibs} 7 loci
PU	0.021	0.0083	0.00160	0.153	0.099	0.046
PC	0.002	0.0004	0.00004	0.054	0.027	0.011
SI	0.011	0.0023	0.00020	0.117	0.058	0.022
PP	0.005	0.0007	0.00016	0.084	0.038	0.019
PG	0.013	0.0026	0.00077	0.130	0.064	0.035
QB	0.002	0.0004	0.00006	0.071	0.033	0.015
VV	0.003	0.0007	0.00018	0.068	0.035	0.018

Table 6
Comparison between marine otter abundances obtained either by visual counts (Valqui, 2012; Apaza and Romero, 2012) or by non-invasive genotyping (this study) and sex ratio. ND: no data, M: male, F: female.

Visual counts			Noninvasive genotyping			
Site	Valqui	Apaza	Genotypes	CAPWIRE (95% CI)	Average recaptures	Sex M-F
PU	5	3	4	8 (4–21)	1.75	4–0
PC	5	2	16	39 (18–58)	1.56	9–7
SI	ND	7	14	35 (14–55)	1.64	6–8
PP	3	3	11	39 (13–200)	1.18	6–5
PG	9	8	7	11 (7–16)	2.57	4–3
QB	5	2	15	68 (25–200)	1.13	6–9
VV	8	6	13	31 (13–38)	2.31	6–7

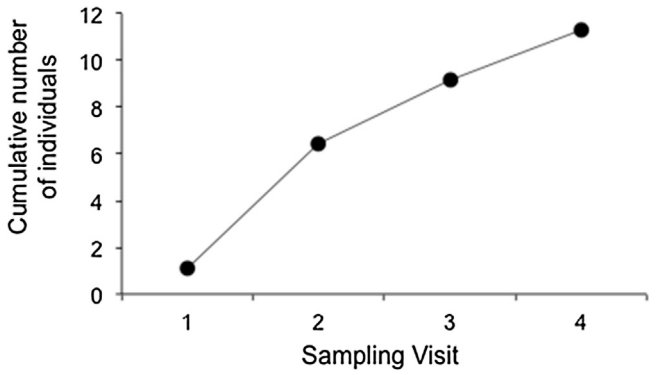

Fig. 2. Average cumulative addition of new individuals across sampling visits for all sites.

genotypes with only five loci there was no ability to differentiate siblings at a site with an average PI_{sibs} 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 ± 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 = four 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, $R^2 = 0.75$) 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

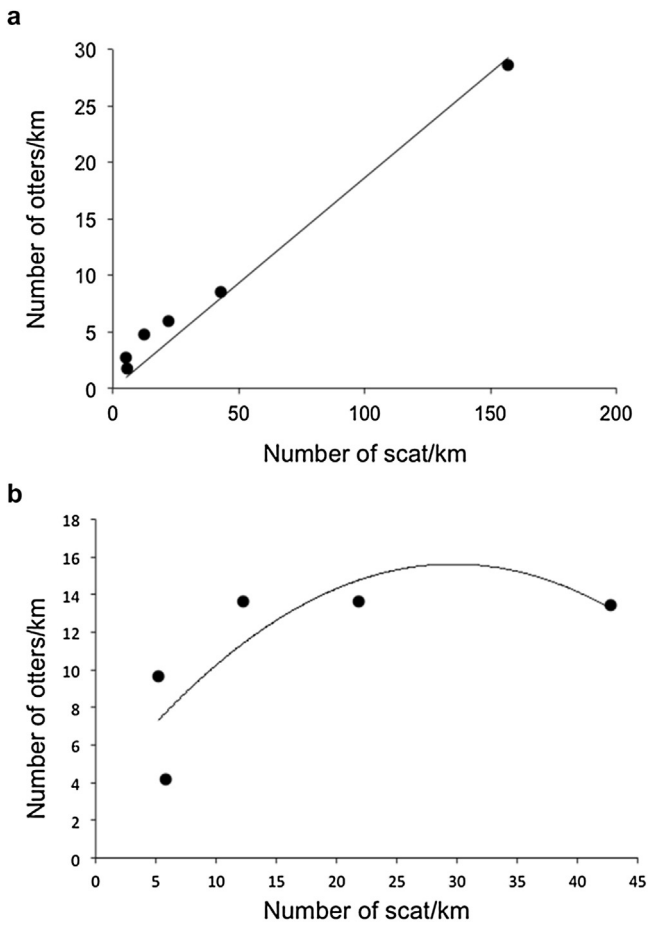

Fig. 3. Relationship between the density of otters (per km) and the density of scats (per km) for (a) minimum otter density from genetic data, (b) otter density estimated by CAPWIRE (see text).

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 spraint 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.

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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.

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