USE OF NON-INVASIVE TECHNIQUES TO DETERMINE POPULATION SIZE OF MARINE OTTER *LONTRA FELINA* IN TWO LOCATIONS OF PERU

by

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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).

In Peru, marine otter populations are at risk due to habitat loss (Sielfeld & Castilla 1999) and artisanal fisheries (Pizarro 2008). Marine otters are considered Endangered by the Red List of the International Union for Conservation of Nature because of population declines due to habitat loss and exploitation (Alvarez & Medina-Vogel 2008). They are currently listed in Appendix I by the International Trade in Endangered Species of Wild Fauna and Flora (UNEP-WCMC 2013) and are considered Endangered by Peruvian law (MINAG 2004).

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

In 2002, the density of otters was estimated to be two individuals per kilometer (ind/km)(Apaza & Romero 2012). Between the Paracas peninsula (13°47' S) and Tacna (18° S) there is 310 km of suitable habitat and so the total population size was estimated to be 620 individuals (Apaza & Romero 2012). Later, in 2004, using

the mean density observed in seven localities (32.5 km of coastline), the density was adjusted to 2.21 ind/km and the total number of otters was estimated to be 690 ± 76 individuals (Apaza & Romero 2012). A year later, Apaza (Apaza & Romero 2012) censused 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 & Romero 2012). Valqui (2012), using research conducted between 2008 and 2011, estimated there were 789 to 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 – 2.7 ind/km are the more frequently reported densities in older studies (Valqui 2012).

Censusing 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) so the actual abundance of otters is probably higher than estimates based on sightings. In some locations, the only evidence of their presence is the spraints (otter feces) found 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 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 both 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. The methodology of Mowry et al. has not been tested on marine otters.

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 otter abundance.

Materials and methods

Study Sites

We searched for marine otter spraints 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. The southern region included the Departments of Moquegua and Tacna and encompassed nine localities (Fig. 1). Of the eleven sampling localities, two were artisanal fishing ports, seven were natural rocky shores, one was a concrete barrier at Enersur Thermic Central, and one was a yacht club where the spraints were located on recreational boats (Table 1). Anthropogenic pressure varies between localities. Artisanal fishing ports are the most impacted, due to the presence of humans, animals like dogs, cats and rats, waste and habitat modification. However, the otter populations in Pucusana, Puerto Grau and Vila

Vila's fishing ports, have been considered to be some of the largest along the Peruvian coast (Valqui 2004; Mangel et al. 2011).

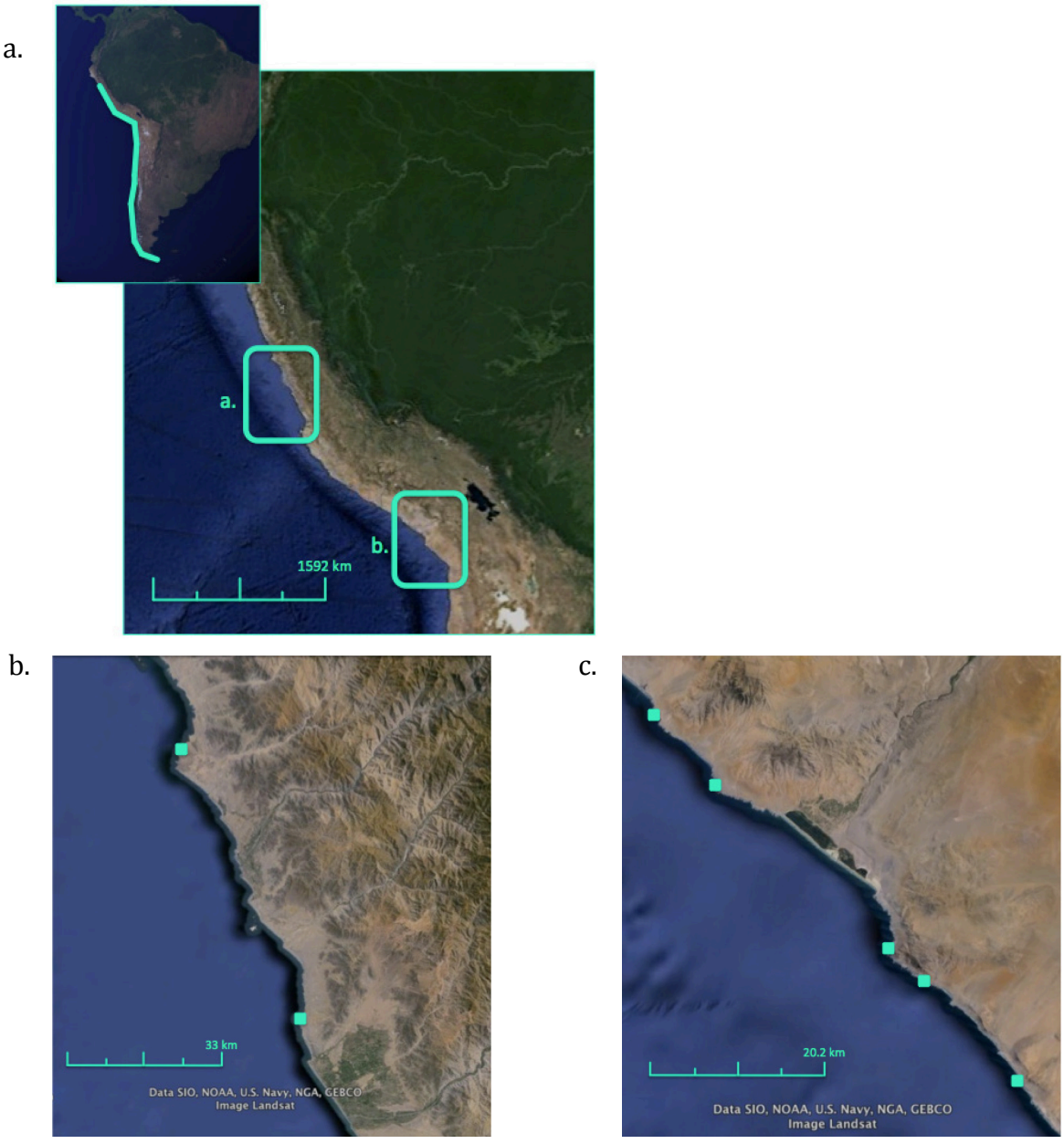


Figure 1. Study area sampled for marine otter scat between June and August 2012. a. Distribution of the marine otter in South America and Peru, b. Study sites in the central region, c. Study sites in the south region.

Initially we considered that marine otter feces were found in 11 different sampling locations, classifying them by their geopolitical limits, similar to what other authors have done (Valqui 2012; Apaza & Romero 2012). Medina-Vogel et al. (2007) determined the home ranges of marine otters were from 1.4 to 4.1 km of seashore, and so we merged sites that were closer than 5.0 km. This generated seven marine otter locations in total. The locations (and km of coastline searched at each site) were: Pucusana (0.14 km) and Punta Corrientes (0.10 km) in Lima; South Ilo (7.90 km) (Enersur, Acantilados, La Higuera and Chorrillos), in Moquegua; and Punta Picata (4.04 km), Puerto Grau (0.82 km), Quebrada Burros (2.94 km) and Vila Vila (2.2 km) (Vila Vila and Cánepa) in Tacna (Table 1). A total of 18.14 km of coastline was searched regularly for spraints.

The following locations were searched for otter spraints but none were found: Punta Negra (12°21’54’’S, 76°47’54’’W), San Bartolo (12°23’12’’S, 76°46’58’’W), La Tiza (12°26’25’’S, 76°46’33’’W), Palillos (12°54’11’’S, 76°30’40’’W) and Tollo Seco (12°56’50’’S, 76°30’32’’W) in Lima; Miraflores (17°24’36’’S, 71°23’50’’W) and Punta Icuy (17°49’16’’S, 71°08’31’’) in Moquegua; La Meca (17°51’11’’S, 70°54’29’’W), Morro Sama (18°00’15’’S, 70°53’18’’W), San Pedro (18°00’23’’S, 70°53’10’’W) and Claveles (18°31’34’’S, 70°47’19’’W) in Tacna. Finally, in the following sites, otter spraints were found but were not considered in the analysis since they were too old, or because faeces were only found on one visit: Cerro Azul (13°01’39’’S, 76°29’13’’W) in Lima; Jaboncillo (17°24’50’’S, 71°23’12’’W), Pocoma (17°24’90’’S, 71°23’14’’W) and Puerto Ilo (17°51’36’’S, 71 20’48’’W) in Moquegua.

Table 1. Localities where marine otter spraints were collected. Sites are those with localities separated by less than 5 km.

Region	Department	Sites	Localities	Type	Latitude	Longitude
Central	Lima	PU	Pucusana	Boats	12°28'42''	76°47'45''
Central	Lima	PC	Pta. Corrientes	Rocky Shore	12°57'17''	76°30'53''
South	Moquegua	IS	Enersur	Tetrapods	17°46'38''	71°11'32''
South	Moquegua	IS	Acantilados	Rocky Shore	17°47'04''	71°10'53''
South	Moquegua	IS	La Higuera	Rocky Shore	17°48'50''	71° 91'44''
South	Moquegua	IS	Chorrillos	Rocky Shore	17°49'04''	71°09'20''
South	Tacna	PP	Pta. Picata	Rocky Shore	17°51'59''	71°05'49''
South	Tacna	PG	Pto. Grau	Fishing Port	18°59'36''	70°53'40''
South	Tacna	QB	Qda. Burros	Rocky Shore	18°01'54''	70°50'26''
South	Tacna	VV	Cánepa	Rocky Shore	18°05'31''	70°45'10''
South	Tacna	VV	Vila Vila	Fishing Port	18°07'03''	70°43'42''

Sample Collection

Marine otter spraints can be easily found along the rocky shoreline. These feces usually contain conspicuous remains of prey-species like fish scales and bones, shards of crustacean exoskeletons, and occasionally otoliths and small mollusk shells. Marine otter spraints sometimes have a jelly-like substance in them which is called anal jelly and is used in scent marking, particularly by males (Kruuk 2006). This material can be white-yellowish or dark. We collected only fresh feces (~1 day old) and discarded older feces for every visit. GPS coordinates were taken for all feces collected (Appendices A-K). Two sampling periods were conducted in each region between June - August 2012.

Figure 2. Fresh scat (0 days old) found in a cave

Figure 3. Old scat (>3 days) found between the cracks of a breakwater

Table 2. Number of marine otter samples collected during each visit. Three days separated the 1st and 2nd and 3rd and 4th visits, respectively. *Days* indicate the number of days between the second and third collecting period. *Collected* - fresh scat (0-3 days) collected at a site, *worked* - the number of scat for which a consensus genotype was constructed.

Site	Days	1st collected	1st worked	2nd collected	2nd worked	3rd collected	3rd worked	4th collected	4th worked
PU	7	1	1	6	1	11	4	4	1
PC	15	8	7	16	15	12	8	1	0
IS	10	0	0	9	9	25	15	12	3
PP	9	3	3	5	5	4	1	9	9
PG	9	0	0	2	2	21	14	12	5
QB	10	4	3	7	7	12	8	13	6
VV	10	8	8	13	9	3	3	24	20

Each location was visited a first time and then a second time three days later and then again for a third time about 10 days after the 2nd visit and then again three days later for the 4th visit (Table 2). 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 8M Urea preservative buffer (10mM of Tris pH 7.5, 125mM of NaCl, 10mM of 0.5 M EDTA pH 8.0, 1% of 10% SDS and 8M of Urea). Fresh feces are easy to distinguish from older ones. Fresh spraints (Fig. 2) tend to have a darker color, are shiny in appearance and have a sticky texture. Old scats (Fig. 3) are a pale grey color and most of the time crumble when touched. Anal jellies are secretions that can be found next to spraints or alone. Fresh ones are moist and runny, while old ones are more dry and thick. Most of the samples collected (N = 258 samples) were feces (67%), followed by anal jelly samples (18%) and feces with anal jelly (15%). Two to three marine otters were spotted at six of the seven sites. We did not observe any otters at PU.

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.

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 & Piertney 1998; Huang et al. 2005) to test on 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, Rio13, Rio18* and *Rio11*. We designed new primers for 6 of the 7 loci to amplify shorter fragments of target DNA, amplifying less of the flanking region surrounding the repeat region (Table 3). PCR products for all six 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, edited, and contiged using Sequencher v. 5.0. We then used Primer3 (Rozen & Skaletsky 2000) to redesign primers flanking the microsatellite region in order to amplify fragments less than 200 bp.

Table 3. Characterization of the microsatellite loci used for marine otters.

Locus	Primer Sequence	Cloned PCR product (bp)	Size range in *L. felina* (bp)	Annealing temperature C°	Dye label	Coload multiplex
04OTLf17	GCCGCACTAGTGATTATCAGG CGGGATTTGCAACCTACTTC	190	163-221	55	Blue	-
Pbra01_Lf	ACAGTTCCTTTGCCTGTTGC CCACAAGGGGTTCACTCTAAA	181	161-185	55	Yellow	B
PbraLf02	GTCCTGAGCAGATGTTGTGC AGGGGCACACATACACATCC	114	88-127	55	Blue	A
PbraLF24	AAGTATTTTCCCTCCCTTCTTTT TGGTGAACTGAAATGTTACTTGAT	120	75-148	60	Blue	B
Rio11	TCTTCCACTTTTCAATTTAGGTA GCCCAAGGTTCACTATCAAG	NA	115-159	55	Green	B
Rio13Lf	GCACATGGGCTTTTATGAAGA CACGTGGTAAGATGAGCATTG	139	103-139	60	Yellow	A
Rio18	TTCCATTGTCTCTTGGCTTG CCCTCTCCACACTTGTGCTC	NA	123-157	60	Green	A

NA – primers were not redesign for these loci.

Genotyping

Sets of 16 samples were amplified at all loci with three to nine PCR replicates each (1,178 total PCR reactions). Negative controls were also included in each PCR batch of 16 samples. PCR reactions were set up in 10 µl volumes containing 1 µl DNA, 0.5 mM primer, 1X Buffer with 2.5 mM $MgCl_2$, 1X dNTPs 200 µm, 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 ^{0}C for 2 min, then 35 cycles of 94 ^{0}C for 30 sec, 55 ^{0}C or 60 ^{0}C for 30 sec, 72 ^{0}C for 1 min, and then a final extension at 72 ^{0}C for 10 min. Resulting PCR products were diluted in 50 µl of water. Each locus was fluorescently labeled with one of four dyes (Table 3). Each marker was amplified singly. Loci for each sample were coloaded into two groups (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 used the comparative method (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. Genotypes were discarded if the consensus had four or fewer loci. Genotypes were considered identical when they matched at all loci or all but one locus (regardless of whether they had 5, 6, or 7 loci). We used the multilocus analysis in GenAlEx v 6.5 to find genotyping matches.

We used GIMLET v. 1.3.2 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 & 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 (Hájková et al. 2009, Woods et al. 1999).

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 to test whether the density of scat predicted the density of otters at a site estimated from the genetic data and from CAPWIRE.

Results

Genotyping

From a total of 245 samples, 138 (56%) were successfully amplified at five to seven loci. The remaining 107 samples were discarded, since after three replications they amplified at fewer than five loci. None of the negative controls for extractions (n = 29 blanks) or PCRs produced relevant results, indicating that extraction and PCR amplifications were uncontaminated. The genotyping success rate of anal jelly

was higher (74% of 43) than that of feces with jelly (61% of 36) and feces (51% of 166).

The seven loci were moderately polymorphic with an average of 9 alleles (range 7-10) and an average observed heterozygosity of 0.55 (range 0.359-0.772) (Table 4). All loci except *RIO18* were in Hardy-Weinberg equilibrium and none of the pairs of loci exhibited genotypic linkage disequilibrium when all sites are analyzed as a single population.

Table 4. Genetic diversity estimates at microsatellite loci for marine otters across all sites.

Locus	N	Na	H_o	H_e	F_{is}
O4Ot17	56	8	0.625	0.538	-0.173
Pbra01	49	7	0.571	0.570	-0.013
Pbra02	78	10	0.359	0.383	0.057
Pbra24	77	9	0.416	0.435	0.037
Rio11	77	9	0.519	0.465	-0.124
Rio13	79	10	0.772	0.647	-0.202
Rio18	68	9	0.588	0.748	0.208

N – number of individuals typed, Na – number of alleles, H_o – observed heterozygosity, H_e – expected heterozygosity, Fis – inbreeding coefficient

PCR success rate at a locus averaged 45% and ranged from a low of 19.5% for *Pbra01* to a high of 60% for *Rio13* (Table 5). Allelic dropout was high, averaging 34% and ranging from 22 to 48% while the incidence of false alleles was only 1.5% (Table 5). The probability of identity (PI) was 0.000054 for all sites combined and ranged from 0.00003 - 0.00086 across sites for genotypes with all seven loci and ranged from 0.015 – 0.0014 for genotypes with only five loci (Table 6). The probability of discerning full siblings from each other (PI_{sibs}) was not very high,

averaging 0.023 (range 0.01 – 0.035) 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 PI_{sibs} of 0.10.

Table 5. Success rate, proportion of allelic dropout (ADO) and false alleles (FA) by locus.

Locus	Success rate	ADO	FA
O4Ot17	0.340	0.481	0.017
Pbra01	0.195	0.228	0.002
Pbra02	0.550	0.348	0.013
Pbra24	0.495	0.400	0.015
Rio11	0.555	0.423	0.025
Rio13	0.600	0.221	0.013
Rio18	0.410	0.272	0.022
Mean	0.450	0.339	0.015

Table 6. The probability of identity (PI) and probability of identity for full siblings (PI_{sibs}) for increasing number of typed loci at each sampling site. N is the number of individuals at a site.

Site	N	PI 5 loci	PI 6 loci	PI 7 loci	PI_{sibs} 5 loci	PI_{sibs} 6 loci	PI_{sibs} 7 loci
SI	16	0.00138	0.00031	0.00003	0.052	0.026	0.010
PG	4	0.01146	0.00445	0.00086	0.117	0.075	0.035
PC	14	0.01424	0.00310	0.00026	0.130	0.064	0.025
PP	7	0.01480	0.00284	0.00085	0.127	0.063	0.035
PU	11	0.00464	0.00083	0.00018	0.084	0.040	0.020
QB	14	0.00307	0.00054	0.00009	0.076	0.035	0.017
VV	13	0.00430	0.00097	0.00025	0.081	0.041	0.022

Population Estimation

We identified individual otters in 24 of 28 site visits (four visits per site). Feces were either absent or not collected during the remaining four site visits. A total of 78 individuals were identified across all sites and visits, ranging from four otters in

PU to 16 otters in PC. Of the 78 individuals identified, 29 were typed at all seven loci, 31 were typed at six loci, and 18 were typed at five loci. New individuals were identified during every visit, with the second visit having the highest number of individuals being detected for the first time (Fig. 4). 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 5). Most individuals (N = 60) were sampled only a single time whereas eighteen individuals were sampled multiple times either during the same visit or across multiple visits. The average number of samples found for these individuals was 4.5 ± 0.74 SE (range 2 – 14). Samples that were categorized as having the same genotypes averaged 337 ± 0.035 SE meters apart and ranged from less than a meter up to 6.4 km apart (Fig. 6).

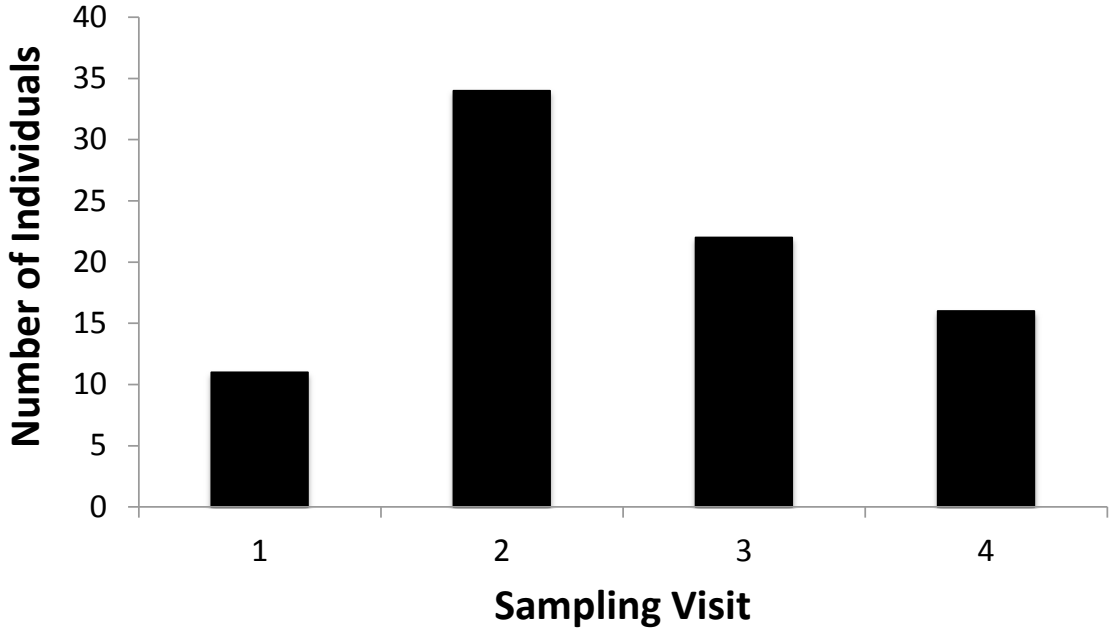


Figure 4. Number of individuals identified for the first time in each sampling visit.

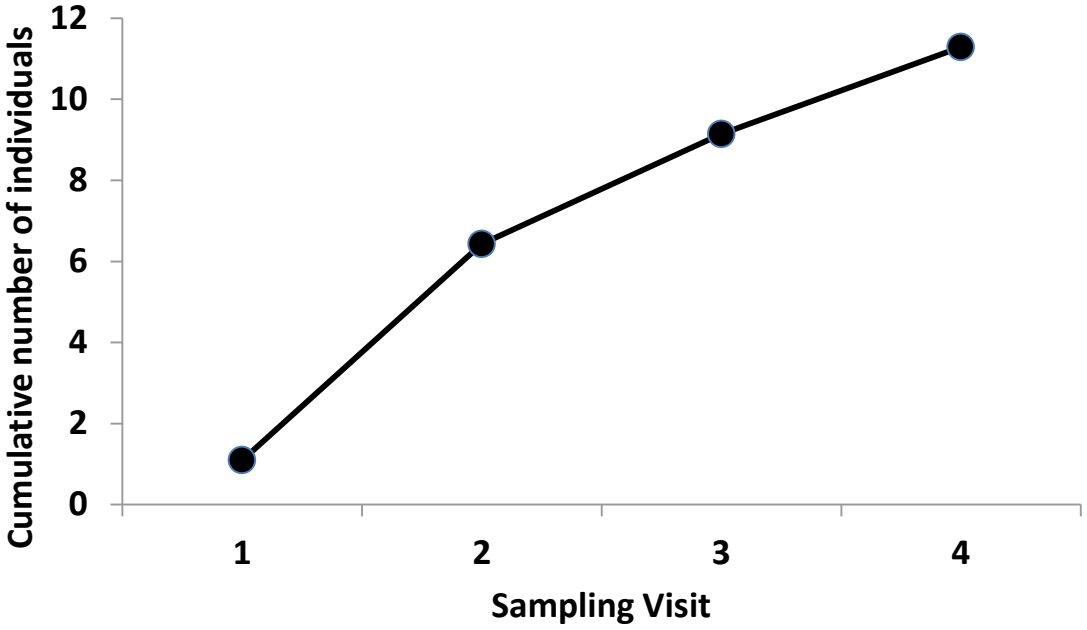


Figure 5. Average cumulative addition of new individuals across sampling visits for all sites.

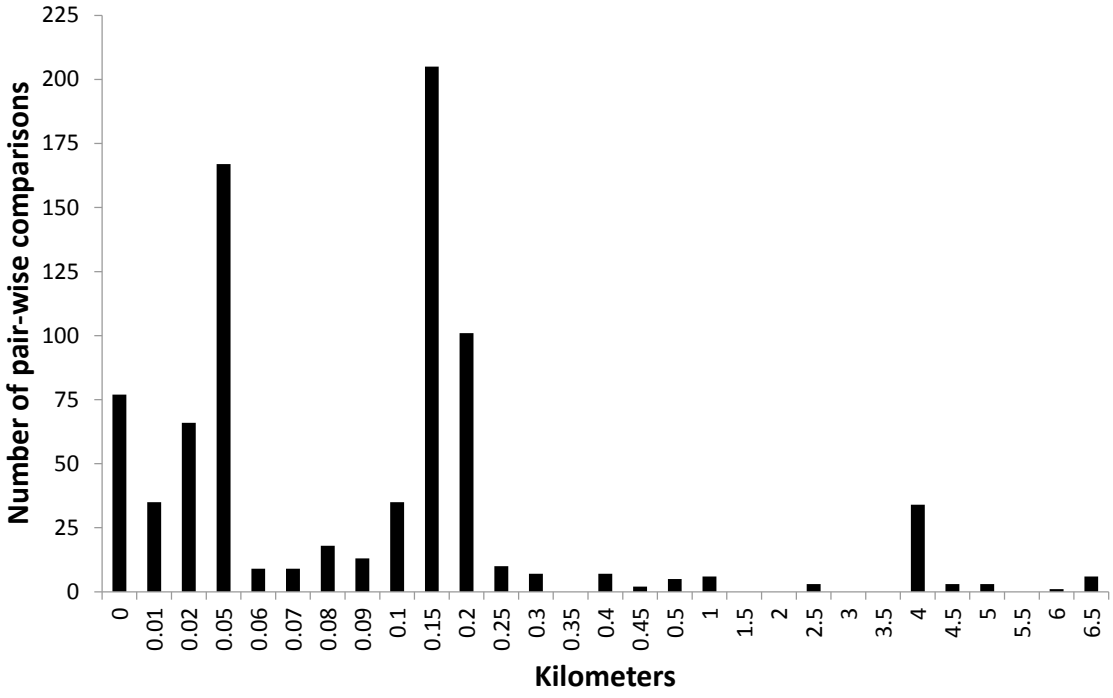


Figure 6. Distance between samples with identical genotypes.

We were able to estimate population size using CAPWIRE for 16 site visits that had recaptures. In our analysis, we considered a recapture when a unique genotype was found more than one time in the same visit. For the remaining 12 site visits, all individuals were sampled only a single time during a visit or no samples were collected. 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 & Romero 2012; Valqui 2012) (Table 7, Appendix L). The confidence intervals (95%) usually 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 (11 of 16 visits).

Both PU and especially PC had very high numbers of otters detected in a small area. 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 ($n = 5$, $y = 0.230x + 0$, $R^2 = 0.94$, $F_{1,4} = 60.55$, $P = 0.004$) (Fig. 7a). Adding PU into the analysis gives a similar relationship ($n = 6$, $y = 0.186x + 0$, $R^2 = 0.99$, $F_{1,5} = 338.29$, $P = 0.00005$) (Fig. 7a). The estimates from CAPWIRE across all four visits was non-linear when both PC and PU were removed ($n = 5$, $y = 0.014X^2 + 0.822X +$

3.42, $R^2 = 0.64$)(Fig. 7b) and strongly linear ($y = 0.371x + 0$, $R^2 = 0.90$) when PU was included although this data point has high leverage in the regression model.

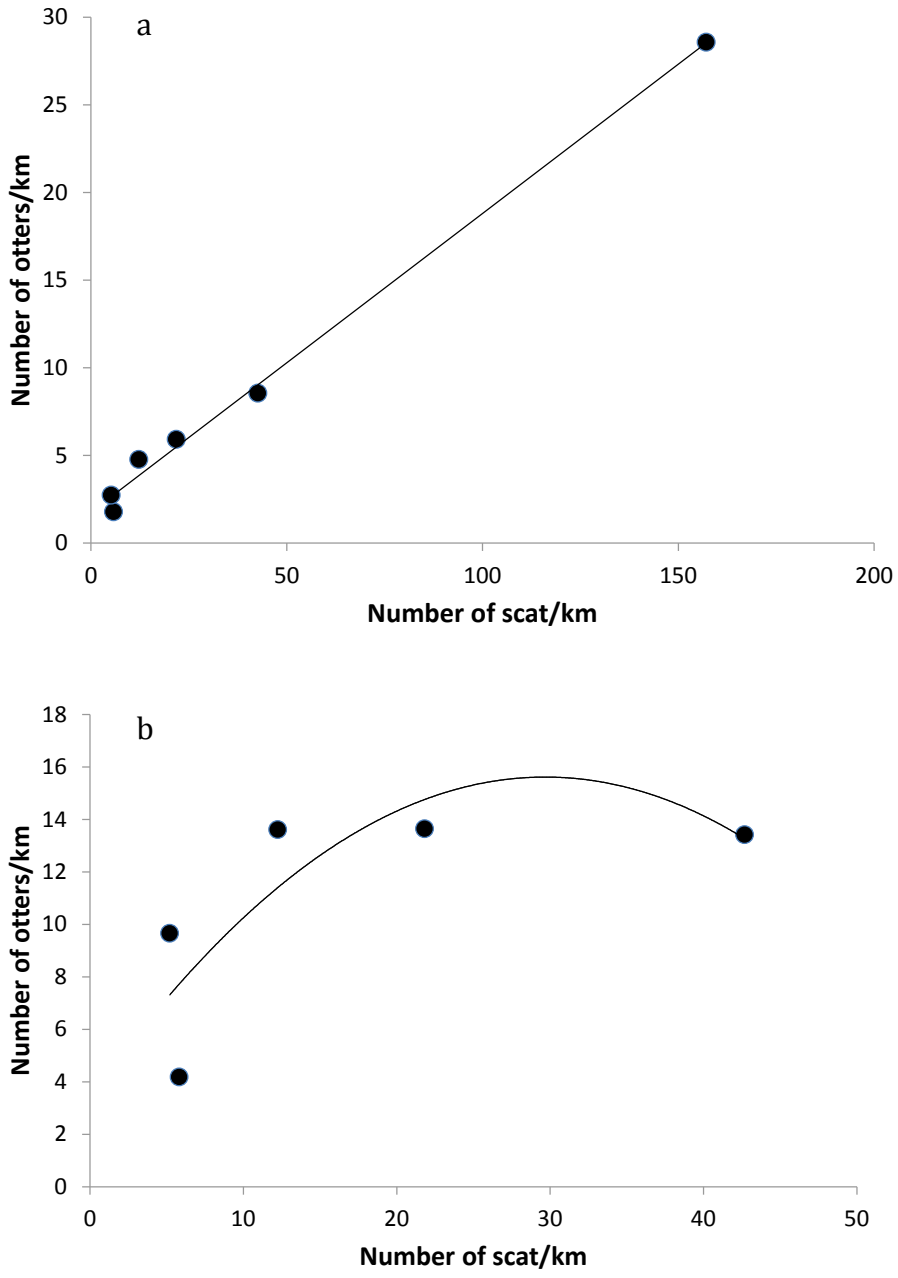


Figure 7. Relationship between the density of otters (per km) and the density of scat (per km) for a) minimum otter density from genetic data, b) otter density estimated by CAPWIRE (see text).

Table 7. Number of individuals (N) at each site and visit estimated from the genetic data and from CAPWIRE using TRIM (with upper and lower 95% CI and the average number of times an individual was resampled). Sample size is the number of feces for which a consensus genotype was obtained. For visit "All", the data is combined across visits at a site. Dashes indicate that individuals were not resampled during that visit (or no individuals were sampled) and so only the minimum genetic estimate is available.

Site	Visit	Sample Size	N (genetic)	N (CAPWIRE)	LowCI	UpCI	Avg Obs/Ind
IS	1	0	0	-	-	-	-
IS	2	9	7	23	7	200	1.29
IS	3	15	9	21	9	40	1.67
IS	4	3	1	1	1	1	3.00
IS	All	27	14	33	14	46	1.93
PG	1	0	0	-	-	-	-
PG	2	2	2	-	-	-	-
PG	3	11	6	12	6	26	1.83
PG	4	5	2	2	2	2	2.50
PG	All	18	7	11	7	16	2.57
PC	1	7	5	9	5	21	1.40
PC	2	11	10	56	16	200	1.10
PC	3	0	0	-	-	-	-
PC	4	7	4	6	4	13	1.75
PC	All	25	16	39	18	58	1.56
PP	1	1	1	-	-	-	-
PP	2	5	5	-	-	-	-
PP	3	0	0	-	-	-	-
PP	4	8	7	28	7	200	1.14
PP	All	14	11	39	13	200	1.18
PU	1	1	1	-	-	-	-
PU	2	1	1	-	-	-	-
PU	3	4	2	2	2	2	2.00
PU	4	1	1	-	-	-	-
PU	All	7	4	8	4	21	1.75
QB	1	3	3	-	-	-	-
QB	2	5	5	-	-	-	-
QB	3	6	5	15	5	200	1.20
QB	4	5	4	10	4	200	1.25
QB	All	19	14	40	16	86	1.36
VV	1	4	2	2	2	2	2.00
VV	2	7	6	21	6	200	1.17
VV	3	3	2	2	2	2	1.50
VV	4	16	6	12	6	20	2.67
VV	All	30	13	30	13	39	2.38

Estimates of density ranged from 4.4 otters per km using only the minimum genetic estimate (N = 78 otters) to 11 otters per km when using all CAPWIRE estimates for each site across all four visits (N = 200 total individuals).

Discussion

The presence and relative abundance of fresh water otters is often determined by the presence of scat and dens (e.g. Conroy and French 1987, Kruuk et al. 1989) and recent studies of fresh water otters utilizing genetic profiling of scat have revealed that the number of scat can be correlated with the number of otters in an area (e.g. Mowry et al. 2011). Some researchers have suggested that marine otter scat counts may not be an effective way to estimate marine otter abundance because, 1) some of the habitat is inaccessible to check for scat, 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. Studies based only on visual sightings have also been suggested to underestimate marine otter abundance because marine otters spend up to 80% of the day out of view and it is not possible to tell individuals apart visually (Medina-Vogel et al. 2006, Alvarez 2012). Some authors have suggested that marine otter abundance may be much higher than currently estimated by visual methods (Sielfeld & Castilla 1999). Our study revealed that 1) otter abundance is in fact higher than estimates based on sightings (Appendix L), 2) the number of fresh scat

is correlated with the number of otters detected at a site, and 3) a single genetic sampling period would underestimate the number of otters utilizing a single site. We discuss each of these findings below.

Otter abundance

Estimates of otter density using all combined visits and either the minimum genetic estimate or CAPWIRE estimate (4.4 - 11 otters per km) is 2-6 times higher than most 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. 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 few 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 sight. Given these uncertainties, we suggest that actual otter densities may be closer to the estimate derived from the total number of unique genotypes rather than the upper estimates derived from CAPWIRE.

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 ≥7 to 10 loci. Hansen et al. (2008), achieved a 56% success rate, however only 1 locus was used in the analysis, and it drops to 8% when 4 loci were considered in the analysis. More than half of the samples we collected (55%) amplified from 5 to 7 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). Valqui (2012) obtained a 43% (37 out of the 87) success rate amplifying the mtDNA control region in fecal samples of *L. felina* collected across the Peruvian coastline.

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. Hájkova et al. (2009) obtained a 2.9% false allele rate and Arrendal et al. (2007) reported 9% of false alleles.

We therefore believe that genotyping error is not responsible for our high estimate 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 actually underestimated the number of otters at some sites.

Estimating otter abundance using scat

The densities of fresh scat at a site were strongly correlated with the density of otters estimated from the number of unique genotypes when all four visits were combined. Our sampling curve indicates that probably more than four visits to a site will be necessary in order to capture all individuals utilizing that site. The CAPWIRE estimates appeared to level off at about 13-15 otters per km for the sites that did not have unusually high densities (i.e. PC and PU).

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 scat 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 sights over the course of this study by almost 50%.

Very little is known about individual movements in marine otters and will ultimately require more extensive radio tracking and non-invasive genetic studies to understand the full extent of individual' home ranges, how temporally stable they are, whether movement patterns differ by age, sex, or reproductive status, and how movement might be related to resource availability (Medina-Vogel et al. 2007). Our data suggest that home ranges are not very exclusive, similar to the single radio tracking study of six otters by Medina-Vogel et al. (2007) in Chile. In our study, the use of each site by individual otters may be in a constant state of flux, with a number of different individuals moving up and down the coast and possibly utilizing the

same sites at different times. 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. We did not find any identical genotypes between sites; however, the feces of four otters were found over 2.0 km (2.1 – 6.4 km) away from where they were originally sampled, suggesting that the otters are moving relatively long distances over short time periods. Some of these distances are further than those recorded by Medina-Vogel et al. 2007 (~1.3 – 4.1 km). 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 scat from at least 16 otters. It is unknown why this site contained so many different individuals. 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. (2006) for marine otters in Chile.

We only collected fresh scat which represented about 45% of all scat at a site and so if otters are not spending long periods of time at a single site then we may have missed sampling individuals during each visit. This may in part explain why the number of recaptures for individuals was so low. Mowry et al. (2011) found that older scat actually had higher genotyping success than fresher scat but this may have occurred because scat was first stored in plastic bags, which, with the wetter fresh samples may have accelerated DNA degradation. Our samples were placed immediately into preservative which should decrease this possibility. In the future,

researchers should collect all scat at a site in order to determine if individuals and recaptures are being missed when sampling only fresh scat.

Management implications

Non-invasive genotyping of marine otter feces could be a valuable tool for increasing our knowledge of this secretive species. Our data suggest that the number of fresh feces could be used to estimate otter abundance, although the methodology should be refined and tested in other areas of Peru and Chile to determine whether the relationship we found in this study between the abundance of scat and otters is geographically variable. Most of the concerns about utilizing scat to estimate marine otter abundance will still need to be taken into consideration when using non-invasive genotyping. Variable deposition rates over time by individuals are probably the most important consideration. Sites will need to be visited multiple times in order to sample all of the otters present. Determining the optimal time frame between visits would also be useful. For instance, should visits always be spaced 10 days apart and then how many times should a site be revisited to adequately sample individuals utilizing a site? Developing more marine-otter-specific loci will also be useful to increase the power to differentiate individuals, especially siblings. All feces should also be collected at a site and as many surrounding areas as possible to determine how many recaptures and extra genotypes one detects compared to just using fresh feces. These studies would increase our understanding of individual movement and what the relevant spatial and temporal scale would need to be in order to accurately determine the total number of otters utilizing a site by scat counts.

References

Alvarez R & Medina-Vogel G. 2008. *L. felina*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1. <www.iucnredlist.org>. Downloaded on 12 September 2013

Alvarez R. 2012. Estimating abundance of Marine otter populations (*L. felina*, Molina 1782) through Binomial N-Mixture Models from replicated counts in Southern Chile. M.Sc. Thesis. Imperial College London, UK

Apaza M & Romero L. 2012. Distribución y observaciones sobre la población de la nutria marina *L. felina* (Molina 1782) en el Perú. Rev. Perú. Biol. 3:285-298

Arrendal J, Villa C & Bjorklund M. 2007. Reliability of noninvasive genetic census of otters compared to field censuses. Conserv. Genet. 8:1097-1107

Beheler AS, Fike JA, Dharmarajan D, Rhodes OE Jr, & Serfass TL. 2005. Ten new polymorphic microsatellite loci for North American river otters (*L. canadensis*) and their utility in related mustelids. Molecular Ecology Notes 5, 602–604

Biffi D & Iannacone J. 2010. Variabilidad trófica de *Lontra. felina* (Molina 1782) (Carnivora: Mustelidae) en dos poblaciones de Tacna (Perú) entre agosto y diciembre de 2006. Mastozoología Neotropical, 1:11-17

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

Conroy JWH & French DD. 1987. The use of spraints to monitor populations of otters (*L. lutra l*). Symp. Zool. Soc. Lond 58:247-262

Creel S, Spong G, Sands JL, Rotella J, Zeigle J, Joe L, Murphy KM, & Smith D. 2003. Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. Molecular Ecology 12:2003-2009

Dallas JF & Piertney SB. 1998. Microstellite primers for the Eurasian otter. Mol. Ecol. 7:1248-1251

Hájková P, Zemanová B, Roche K & Hájek B. 2009. An evaluation of field and noninvasive genetic mothods for estimating Eurasian otter population size. Conserv. Genet. 10:1667-1681

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

Hung CM, Li SH & Lee LL. 2004. Fecal DNA typing to determine the abundance and spatial organization of otters (*L. lutra*) along two stream systems in Kinmen. Animal Conservation 7:301-311

Huang CC, Hsu TC, Lee LL & Li SH. 2005. Isolation and characterization of tetramicrosatellites DNA markers in the Eurasian otters (*L. lutra*). Molecular Ecology Notes 5:314-316

Kruuk H. 1989. An estimate of numbers and habitat preference of otters *L. lutra* in Shetland, UK. Biol. Conserv. 49:241-254

Kruuk H. 2006. Otters: Ecology, Behaviour and Conservation. Oxford University Press, USA

Mangel JC, Whitty T, Medina-Vogel G, Alfaro-Shigeto J, Cáceres C & Godley BJ. 2011. Latitudinal variation in diet and patterns of human interaction in the marine otter. Marine Mammal Science, 2:E14-E25

Medina-Vogel G, Bartheld JL, Alvarez Pacheco R & Delgado Rodriguez C. 2006. Population assessment and habitat use by marine otter *L. felina* in southern Chile. Wildl. Biol. 12:191-199

Medina-Vogel G, Boher F, Flores G, Santibañez A & Soto-Azat C. 2007. Spacing behavior of marine otters (*L. felina*) in relation to land refuges and fishery waste in central Chile. Journal of Mammalogy, 2:487-494

Miller CR, Joyce P & Waits LP. 2005. A new method for estimating the size of small populations from genetic mark-recapture data. Molecular Ecology 14:1991–2005

Mowry R, Gompper ME & Beringer J & Eggert LS. 2011. River otter population size estimation using noninvasive latrine surveys. The Journal of Wildlife Management, 7:1625–1636

Park HC, Han TY, Kim DC, Min MS, Han SY, Kim KS & Lee H. 2011. Individual identification and sex determination of Eurasian otters (*L. lutra*) in Daegu city based on genetic analysis of otter spraint. Gen & Genomics, 33:653-657

Pizarro J. 2008. Mortality of the marine otter (*L. felina*) in southern Peru. IUCN Otter Spec. Group Bull. 1:94-99

Peru, Ministerio de Agricultura. Decreto Supremo N° 034-2004-AG. Official Newspaper El Peruano. September 22, 2004:276853-276856

Prigioni C, Remonti L, Balestrieri A, Sgrosso S, Priore G, Mucci N & E Randi. 2006. Estimation of European otter (*L. lutra*) population size by faecal DNA typing in southern Italy. Journal of Mammology, 5:855-858

Ribas C, Vasconcellos AV, Mourao G, Magnusson W, Sole-Cava AM & Cunha HA. 2011. Polymorphic microsatellite loci from the endangered Giant Otter (*P. brasiliensis*). Conservation Genet. Resour. 3:769-771

Rousset F. 2008. GENEPOP'007: a Complete Re-implementation of the GENEPOP Software for Windows and Linux. Molecular Ecology Resources 8:103-106

Rozen S. & Skaletsky H.J. 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, pp 365-386.

Ruiz E. 2009. Estudio etológico de la nutria marina *L. felina* (Molina, 1782) en un ambiente antrópico, la bahía de Pucusana-Lima, Perú de junio a agosto del 2008. Tesis para optar el título de Biólogo. Universidad Nacional Agraria La Molina (UNALM), Lima, 55 p

Sielfeld W & Castilla JC. 1999. Estado de conservación y conocimiento de las nutrias en Chile. Estud. Oceanol. 18: 69-79

UNEP-WCMC. 22 August, 2013. UNEP-WCMC Species Database: CITES-Listed Species.

Valqui J. 2004. Comportamiento de la nutria marina *L. felina* (Molina 1782) en un ambiente antrópico, la bahía de Pucusana-Lima, Perú. Tesis para optar el título de Biólogo. UNALM, Lima, 58 p

Valqui J, Hartl GB & Zachos FE. 2010. Non-invasive genetic analysis reveals high levels of mtDNA variability in the endangered South-American marine otter (*L. felina*). Conserv. Genet. 11:2067-2072

Valqui J. 2012. The marine otter *L. felina* (Molina, 1782): A review of its present status and implications for future conservation. Mammal. Biol. 77:75-83

Vianna JA, Ayersi P, Medina-Vogel G, Mangel JC, Zeballos H, Apaza M & S Faugeron. 2010. Phylogeography of the marine otter (*L. felina*): historical and contemporary factors determining its distribution. J. Hered. 6:676-689

Wood JG, Paetkau D, Lewis D, McLellan BN, Proctor M & Strobeck C. 1999. Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin, 3:616-627

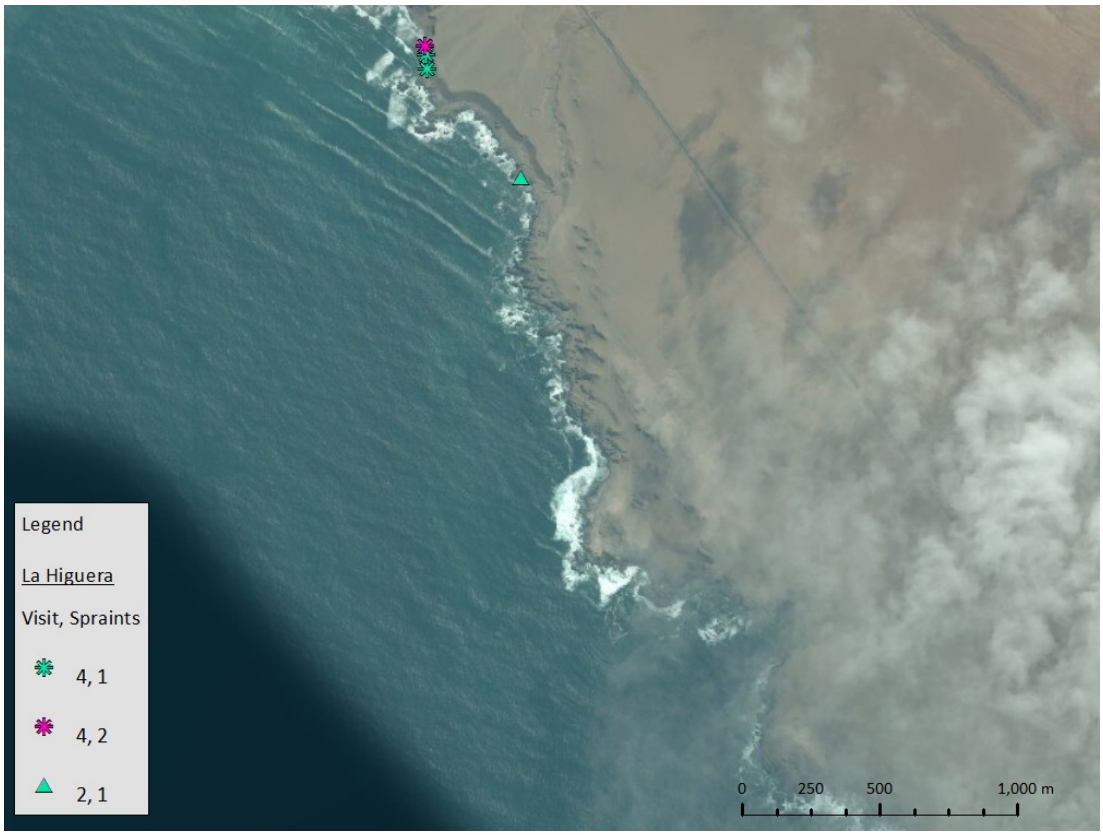
Appendix

Appendix A. Bahia de Pucusana (PU), Lima, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.

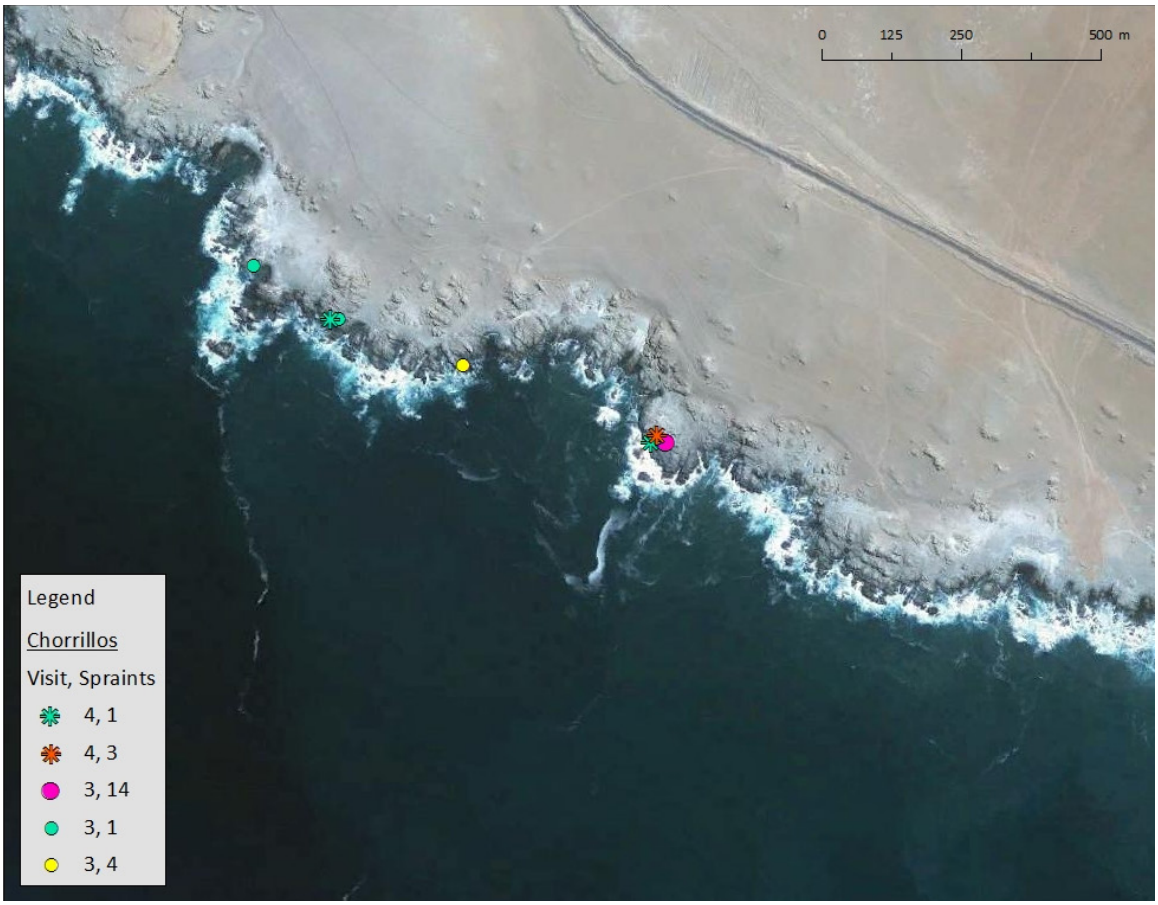
Appendix B. Punta Corrientes (PC), Lima, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.

Appendix C. Enersur pier (IS), Moquegua, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.

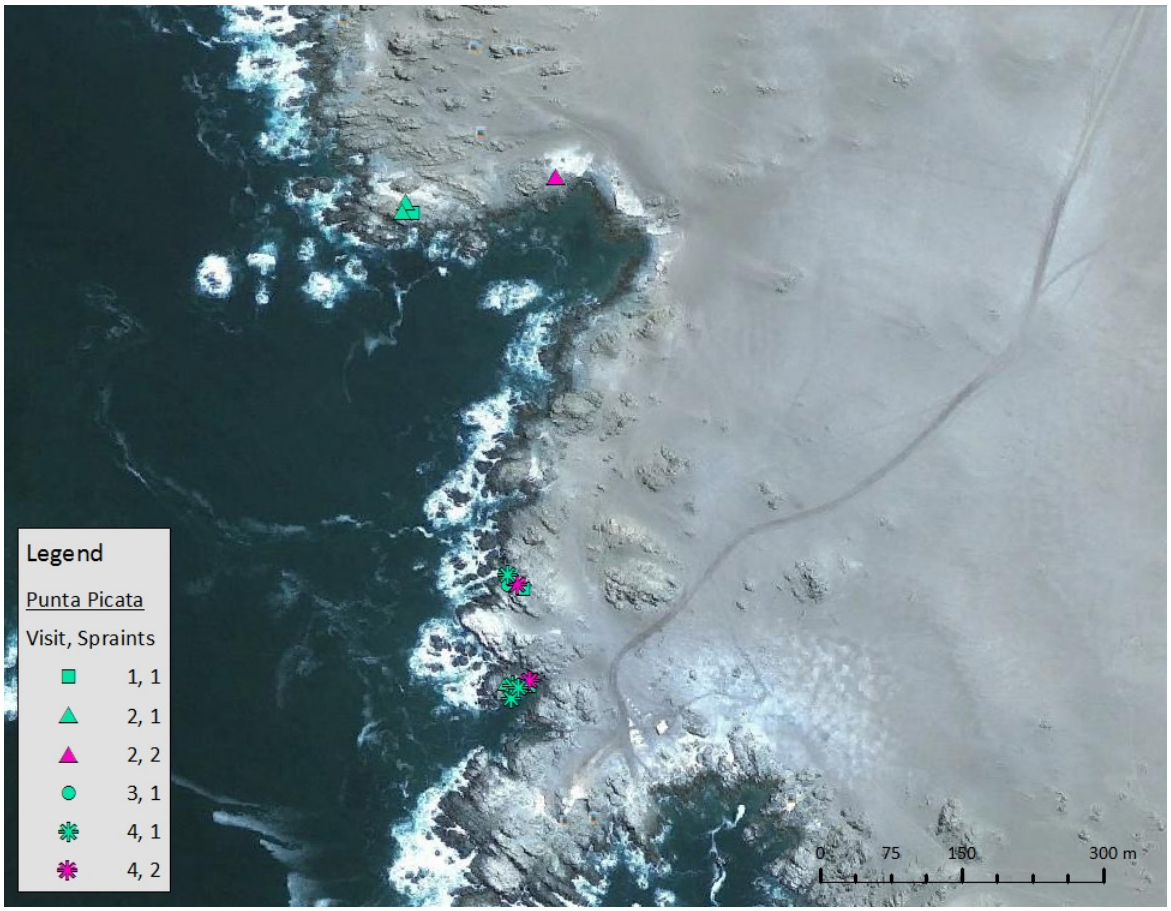
Appendix D. *Acantilados,* stretch of shoreline between Enersur pier and Playa La Higuera (IS), Moquegua, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.



Appendix E. La Higuera (IS), Moquegua, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.

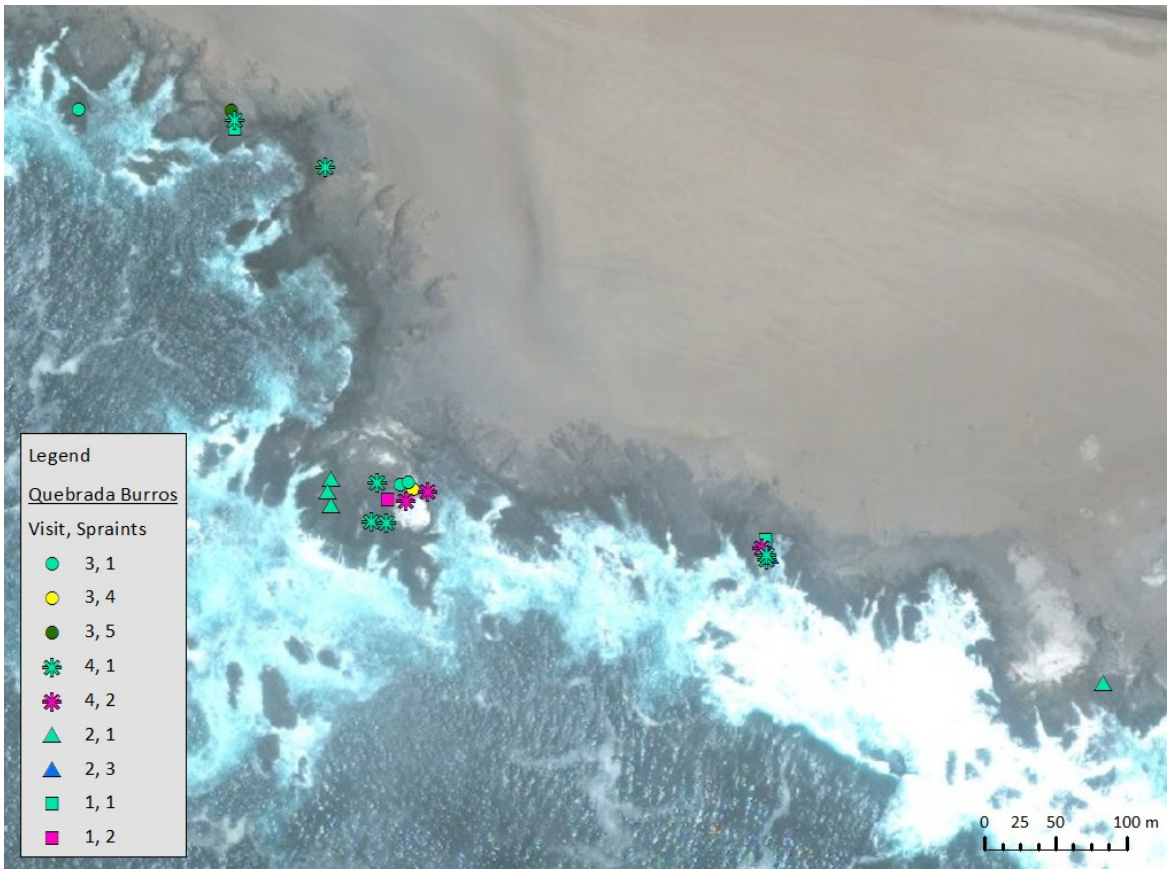


Appendix F. Chorrillos (PP), Moquegua, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.



Appendix G. Punta Picata (PP), Tacna, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.

Appendix H. Puerto Grau artisanal fishing port (PG), Tacna, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.



Appendix I. Quebrada Burros (QB), Tacna, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.

Appendix J. Cánepa (VV), Tacna, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.

Appendix K. Vila Vila artisanal fishing port (VV), Tacna, Peru. The shape of the symbol indicates the visit number. The color denotes the number of feces collected at a point.

Appendix L. Comparison of marine otter abundance in seven sites of Peru done by visual censusing and by noninvasive genotyping techniques. Valqui and Apaza estimates obtained from from Valqui 2012 and Apaza & Romero 2012, respectively. ND indicates no data.

	Visual censusing		Noninvasive genotyping	
Site	Valqui	Apaza	Genotypes	CAPWIRE
IS	ND	7	15	34
PC	5	2	16	39
PG	9	8	8	12
PP	3	3	11	39
PU	5	3	4	8
QB	5	2	16	57
VV	8	6	13	31

VITA

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ABSTRACT

USE OF NON-INVASIVE TECHNIQUES TO DETERMINE POPULATION SIZE OF MARINE OTTER *LONTRA FELINA* IN TWO LOCATIONS OF PERU

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Marine otters (*Lontra felina*) are very difficult to observe and census. Currently, there is no reliable information on the population size of marine otters. Between June and August 2012 we collected 258 samples of marine otter feces from 7 localities in Peru. All locations were visited four times. 141 samples (55%) were successfully amplified at five to seven loci. We identified a minimum of 79 individuals across all locations from the genetic data for a density estimate of 4.4 otters per km. CAPWIRE estimates of N were greater than the minimum estimated from the genetic data (11 otters/km) and were almost 2-6X higher than previous estimates based on sightings. There was a strong relationship between the number of fresh scat and the number of detected otters, suggesting scat counts might be used to estimate the number of otters at a site.