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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