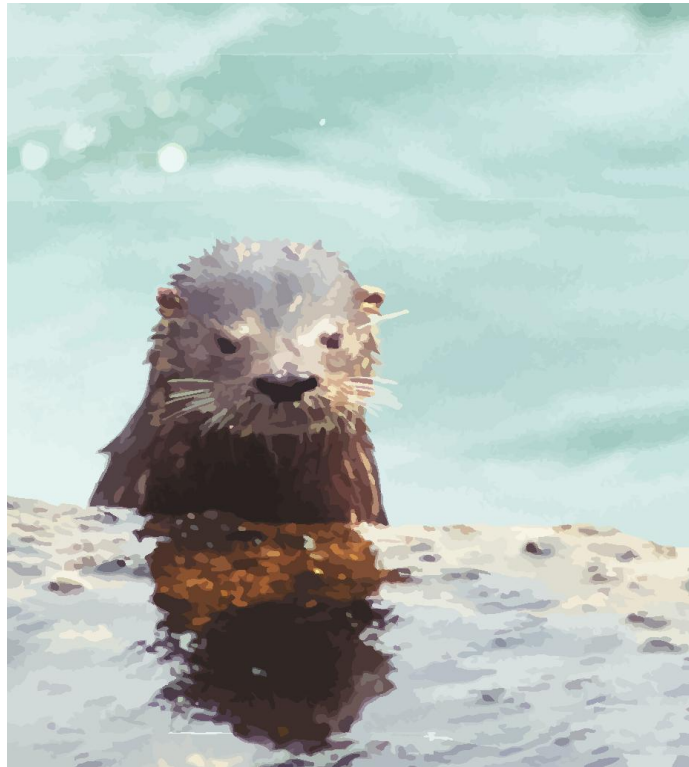


**Population Genetics and Conservation of the Marine
Otter (*Lontra felina*) at the Peruvian Coast**



Dissertation
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

vorgelegt von

Juan Valqui

Kiel, Juli 2012

Referent: Prof. Dr. Günther B. Hartl

Korreferent: Prof. Dr. Heinz Brendelberger

Tag der mündlichen Prüfung: 04.07.2012

Zum Druck genehmigt: _____ 2012

gez. _____

Der Dekan

To Maria...

SUMMARY

The marine otter *Lontra felina* is an endangered species living in the fragmented habitat of the Peruvian and Chilean Southern Pacific rocky shores. Little is known about the world's smallest marine mammal as population numbers, occurrence areas and limits of distribution are still under discussion. In the 20th century intensive hunting was the major threat to *L. felina* while today the effect of other human activities (urbanization, pollution and intensive fisheries) have a great impact on the marine otter population, principally through their habitat fragmentation forces. This thesis is the first step towards an assessment of the hitherto unknown genetic characteristics and population structure of the marine otter in Peru. In three field expeditions –consisting of over 10,000 km of travel covering 1,500 km of coastline, 143 localities were visited to collect 157 fresh scat samples. Due to the particular problematic of working with non-invasive samples containing low quality DNA, great caution was put in the laboratory work and data processing. Relatively high levels of genetic variability were found (for the control region (CR): haplotype diversity $h = 0,69$; nucleotide diversity $\pi = 0.0061$; for cytochrome b (cyt b): haplotype diversity $h = 0.848$; nucleotide diversity, $\pi = 0.0027$). Despite a disjunct population no isolation by distance – calculated as correlation of pairwise genetic and geographical distances between individuals – was found (no significance in the Mantel test for CR: $r = -0.013$, $p = 0.77$ and for cyt b: $r = -0.055$, $p = 0.68$), suggesting a moderate gene flow. Two subpopulations could be defined for the Peruvian area via cyt b analysis ($F_{ST} = 0.355$, $p = 0.016$), although this substructuring could not be confirmed by CR data (no F_{ST} value significantly different from zero, all $p > 0.12$). Demographic tests (mismatch analysis and Fu's F_s) did not show obvious signs of a bottleneck but rather some weak indication of a population expansion. With the available demographic and life-history data a Population Viability Analysis (PVA) explored a combination of scenarios with variable environmental and anthropogenic factors at the Peruvian and Chilean coast. A sensitivity testing showed that extinction of all populations is predominantly related to habitat reduction, while inbreeding, catastrophes and diseases are decisive factors for smaller, more isolated populations. Because the latter have reduced alternatives to respond to changes, extinction is more

likely to occur due to the stochasticity of genetic, environmental and demographic factors. This work provides a basis for developing conservation management plans of the Peruvian population of *Lontra felina*. Further studies based on alternative marker systems (microsatellites), behaviour (mating systems, social structure) and ecology (migration, population numbers) should be carried out to confirm population substructuring and patterns of gene flow. Environmental education work, law enforcement and management plans that include protection systems are recommended to compensate the loss of natural habitat and secure the survival of the species.

ZUSAMMENFASSUNG

Der Meeresotter (*Lontra felina*), der kleinsten Meeressäuger der Welt, lebt in dem fragmentierten Habitat der peruanischen und chilenischen Küste. Über diese bedrohte Art ist wenig bekannt und Ergebnisse von Populationszählungen sowie Angaben zu Vorkommen und zu den Grenzen des Verbreitungsgebietes sind stark umstritten. Die Wilderei stellte im 20. Jahrhundert die größte Bedrohung für *L. felina* dar, doch zurzeit ist der Einfluss einiger anderer menschlichen Aktivitäten (Urbanisierung, Verschmutzung und intensive Fischerei) besorgniserregend, da ihre Fragmentierungskräfte den Erhalt der Art gefährden. Mit dieser Arbeit ist ein erster wichtiger Schritt in der genetischen Charakterisierung und Erfassung der Populationsstruktur von *Lontra felina* gegeben. In drei Feldexpeditionen mit über 10 000 km zurückgelegtem Weg, wurden über 1 500 km der Küstenlinie erforscht; an 143 Orten wurden 157 frische Kotproben gesammelt. Bei der gewählten nicht invasiven Methode musste der niedrigen DNA Qualität durch eine besondere Vorsicht bei den Laboranalysen und der Datenauswertung begegnet werden. Durch das Auswerten von Sequenzen der Kontrollregion (CR) und Cytochrom b (cyt b), wurde eine relativ hohe Variabilität festgestellt (CR: Haplotypendiversität $h = 0,69$; Nukleotiddiversität $\pi = 0.0061$; cyt b: Haplotypendiversität $h = 0.85$; Nukleotiddiversität $\pi = 0.0027$). Trotz weit auseinanderliegender Populationen konnte keine Übereinstimmung zwischen genetischer und geographischer Distanz bestimmt werden (Mantel-Test für CR: $r = -0.013$, $p = 0.77$ und für cyt b: $r = -0.055$, $p = 0.68$), was auf einen moderaten Genfluss hindeutet. Zwei Subpopulationen innerhalb Perus konnten über eine Analyse der Differenzierung bei cyt b Sequenzen bestimmt werden ($F_{ST} = 0.355$, $p = 0.016$). Die Analyse der CR Sequenzen konnte diese Substrukturierung jedoch nicht bestätigen (kein F_{ST} Wert ungleich null, alle $p > 0.12$). Die demographische Analyse (mismatch und F_u 's F_s) zeigte keine Eindeutigkeit bezüglich eines Durchgangs durch einen Flaschenhals, sondern eher schwache Hinweise zu einer Expansion. Unter der Verwendung der von der Art verfügbaren demographischen Daten wurde mittels einer Population Viability Analysis (PVA) eine Vielfalt an möglichen Szenarien simuliert, bei denen der Einfluss von Umweltvariablen (Katastrophen und Krankheiten) und Faktoren menschlichen

Einflusses untersucht wurde. Ein Sensitivitätstest der PVA zeigte, dass das Aussterben hauptsächlich mit dem Habitatverlust stark in Verbindung steht, unabhängig von der Populationsgröße. Das Aussterben von kleineren, isolierten Populationen konnte eher auf den Einfluss der Inzucht, Katastrophen und Krankheiten zurückgeführt werden. Kleinere Populationen haben eingeschränkte Möglichkeiten um auf Veränderungen zu reagieren, deshalb wird das Aussterben durch die Stochastizität der Umwelt und genetischer Faktoren bestimmt. Diese Arbeit versteht sich als bedeutender Beitrag zum Natur- und Artenschutz in Peru, da sie wichtige Informationen zum Schutz von *Lontra felina* liefert. Die Durchführung von Studien, die das Wissen über Populationsgenetik und Ökologie von *L. felina* erweitern, wird empfohlen. Ebenso wird auf die Wichtigkeit der Umweltarbeit und die Strafverfolgung im Umweltrecht hingewiesen. Ein Schutzsystem, das den Ausgleich des Habitatverlusts in Betracht zieht, könnte den Erhalt der Art sichern.

CONTENT

Summary.....	i
Zusammenfassung.....	iii
Content.....	v
General introduction.....	1
CHAPTER I. The Marine Otter <i>Lontra felina</i> (Molina, 1782): A Review of its Present Status and Implications for Future Conservation.....	8
CHAPTER II. Genetic Variability and Population Structure of the Endangered South-American Marine Otter (<i>Lontra felina</i>) Based on Analysis of mtDNA Fragments of the Control Region and the Cytochrome b Gene.....	38
CHAPTER III. Population Viability Analysis of the Marine Otter (<i>Lontra felina</i>) Population Along the Pacific Coast of South America.....	58
General conclusions and outlook.....	74
Acknowledgements.....	77
References.....	78
Annex I. List of figures and tables.....	98
Annex II. Non-invasive Genetic Analysis Reveals High Levels of mtDNA Variability in the Endangered South-American Marine Otter (<i>Lontra felina</i>).....	102

Annex III. New Record of the Marine Otter <i>Lontra felina</i> (Molina, 1782) North to its Current Distribution (Abstract).....	113
Annex IV. The Order Carnivora (Mammalia) in Peru: Research Priorities for its Conservation (Abstract).....	114
List of publications.....	116
Contributions of authors.....	117
Curriculum vitae.....	118
Declaration by candidate.....	122

GENERAL INTRODUCTION

The rapid extinction of an unknown number of species on our planet due to anthropogenic factors and the reduction rates of several populations are alarming, as we face an unprecedented loss of biodiversity in the history of life (Ehrlich and Ehrlich 1991). Under the so called “sixth extinction” the rate of extinctions outruns the origins of new species, thus 50% of vertebrate animal species of the world and 12% of plants are considered vulnerable to near-term extinction (Leaky and Lewin 1995). Species loss is predicted to up to 50% of all species over the next 50 years (Koh et al. 2004) if no immediate action is taken to protect key habitats or biodiversity hotspots (Pimm and Raven 2000; Myers et al. 2000). In Peru, the intensive urbanization of the coast puts constantly increasing pressure on several species of the marine ecosystem. Habitat degradation and fragmentation, as well as a depletion of forage fishes have consistently reduced the abundance of birds and marine mammals (Majluf et al. 2002). Bycatch is at present killing several hundred penguins and thousands of small cetaceans (dusky dolphin *Lagenorhynchus obscurus*, bottlenose dolphin *Tursiops truncatus*, Burmeister’s porpoise *Phocaena spinnipinnis*, among others) per year (Majluf et al. 2002). Poaching presents a problem to several species at the East Southern Pacific coast that do not benefit either from their protection status or from governmentally protected areas – wildlife law enforcement does not have a higher priority neither for authorities nor for the principal actors in Peruvian and Chilean marine ecosystems (Miller et al. 1983; Red List of Threatened Species Version 2011.1; Ulloa et al. 2010). Heavy migrations by human populations to the coast have resulted in occupation of the littoral habitats by invasive species (dogs, cats and rats) that may be displacing local mammal fauna (Apaza et al. 2004; Medina-Vogel et al. 2008). Pollution is another critical problem in this habitat, as industries (fisheries, mining, among others) and domestic effluents of the big cities are constantly contaminating the ocean (Apaza et al. 2004; Hinrichsen 1998; Thiel et al. 2007; Thorne-Miller 1999). A scenario of increasing human impact on the Pacific coast of South America, with accelerating fragmentation of seashore patches and diminished productivity of marine life communities is given for the next decades at the Southern Pacific coast (Brownell 1978; Eisenberg and Redford 1989; Sielfield

and Castilla 1999; Majluf et al. 2002; Medina-Vogel et al. 2008; Vianna et al. 2010). Further, the impact of diseases on marine mammals is unclear and still very unpredictable in its periodicity and dimension, but events of almost thousand dolphins washed dead on northern Peruvian coasts do occur (recent event between November 2011 and April 2012, New York Times, May 7th 2012). At the same time the Peruvian coast, under the influence of the Humboldt Current System which is associated with exceptionally high marine productivity and huge populations of marine fauna (Majluf et al. 2002), presents a scenario of risk of extinction to at least nine carnivores, seven cetaceans, nine bird species and four marine turtle species (Cossíos (in press); Red List of Threatened Species Version 2011.1).

The population of the marine otter (Figure 1), whose total numbers are yet much discussed, has suffered the consequences of intensive hunting during the 20th century (Redford and Eisenberg 1992) while increasing habitat destruction and degradation, pollution, poaching, illegal fishing, bycatch and invasive species represent the main threats at the present (Red List of Threatened Species Version 2011.1). Therefore, it is considered an Endangered Species according to the International Union for Conservation of Nature and Natural Resources (IUCN) Red List, figures in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and in Appendix I of the Convention on Migratory Species (CMS). Additionally, it is protected by Peruvian and Chilean law.

Population Genetics performs analysis allowing to understand genealogical and evolutionary processes in populations (Avice 2004), assessing their phylogenetic relationships and structure, understanding their reproduction and mating systems and determining levels of dispersal and gene flow, inbreeding degrees and the loss of genetic diversity (expressed as the reduction of fecundity and increased mortality) (Waits and Paetkau 2005). Conservation Genetics, a branch more focused on extinction processes and survival of endangered species sets those population genetic factors in relation to environmental and anthropogenic factors. For example, habitat fragmentation due to human action may be found to be related to reduced gene flow, thus depicting a scenario of extinction risk for some threatened species. Collection of



Figure 1. The marine otter *Lontra felina* (Molina 1782).



Figure 2. Marine otter habitat with difficult access to samples.

DNA -needed for this kind of analysis- of elusive species represents a challenge when working with animals that are hard to see and even harder to capture. In the last two decades non-invasive techniques (e.g. DNA extraction from scat) have resulted as great alternative for the assessment of genetic information of wildlife populations when blood or tissue samples are not possible to collect (Kohn and Wayne 1997; Waits and Paetkau 2005). To test this method in the laboratory in beforehand, fresh samples from the American mink *Mustela vison* (a closely related species to the marine otter) were used as material for DNA extraction, amplification and genotyping, with positive results, thus the method was chosen to process the marine otter samples. Given the alternative, the marine otter is still a particularly problematic species in this sense, as individuals spend 80% of their time hidden in caves (Medina-Vogel et al. 2007) and prefer rocky shore and outcroppings that are often very difficult to access (Cabello 1983; Ostfeld et al. 1989; Villegas et al. 2006) (Figure 2). Thus, occurrence areas are hard to determine and sampling locations challenging to find. With a scouring-type field work to cover an area as big as possible, time is a limiting factor and scat samples are always found at a very low fresh to old ratio. A further problem to non-invasive sampling is the relatively low extraction, amplification and genotyping success in the laboratory work (Broquet et al. 2006) compared to high quality DNA obtaining techniques, as the ones based on blood or tissue (Bayes et al. 2000; Waits and Paetkau 2005).

To achieve population genetic analyses an affordable and not too time-consuming system of markers can be defined (Halliburton 2004). Carnivore DNA comprehends two to three millions of base pairs (bp) of which only three percent are coding for gene expression, and only one percent of the latter presenting variation among them. Ninety-seven of the resting genome consists of non-coding DNA (Campbell and Reece 2006), which is chosen for analysis of phylogenetic processes as mutations do not disappear because of their neutrality in selection processes.

Mitochondrial DNA (mtDNA) is a circular molecule present in the mitochondrion of eukaryotic cells, presenting several genes related to cell respiration. Due to its homoplasmic characteristics (one single sequence predominates in one individual), its rapid evolution due to inefficient repair mechanisms (Awise 2004) and its high concentrations in cells, mtDNA is one marker system particularly interesting for genealogy and phylogenetic relationships analysis. Being only transmitted through the maternal line, it provides an insight to matrilineal phylogeny (Birky 1995) and allows comparing groups or populations of individuals that have been separated long time ago and also those, who present a very recent separation (Awise 2004). The non-coding region of mtDNA (called D-Loop or control region, CR) presents two highly variable sequences of a few hundred bp (Madea 2003) and is thus considered a mutational hotspot and particularly interesting for these analyses (Stoneking 2000).

The power of statistical analyses is in general closely related to the representativeness of the sample. In population genetics several factors (animal behaviour, field work logistics and moderate to low genetic material extraction rates) may alter this representativeness. Thus, methods to compensate this loss of quality in the information have to be taken (e.g. bootstrapping) (Wu 1986). Although Population Viability Analysis (PVA) can be simulated under more or less rigorous statistics (McCarthy et al. 2001) a thorough knowledge of the species to be investigated is required. Yet, in a situation of uncertain input in the calculation models, the simulations may still show trends and allow formulating management actions based on the results of the simulation of deterministic and stochastic factors (McCarthy et al. 2001).

To reverse the above mentioned extinction process, the preservation of biodiversity and a better understanding of the wildlife populations via ecological and biological assessments has to be achieved. At the level of population genetics notorious variations in gene diversity, decrease of effective population size and the interruption of gene flow patterns are assessments necessary to provide solid arguments in the conservation management. In case environmental differentiated conditions are present for certain populations and a separate treatment is required, evolutionary significant units (ESU) should be defined (Avice 2004). Management plans should aim at increasing population numbers, reversing inbreeding levels and re-establishing the species in their original habitat (Frankham et al. 2007). Ultimately, to understand and maintain the ecological and evolutionary processes (genetic drift, gene flow, natural selection, sexual selection, speciation, among others) will help save as much as possible in the tree of life (Mace et al. 2003).

Between August 2008 and January 2011 three field expeditions were performed, completing a total of 62 field days. Within these, over 10,000 km of tour distance was covered to sample the 1,500 km of the distribution range of *Lontra felina* within the Peruvian coast. Typically, the terrain was rocky shore with dens and caves with difficult access (Figure 2 and Figure 3), but also beach resorts and fishing ports were surveyed (Figure 4). A total of 143 locations were visited to collect 239 scat samples in 49 of them. Samples were mostly collected in marine otter latrines but were sometimes found in boats or within the rocks of docks in fishing ports. After the field work, samples were stored under refrigeration in Lima until export permission was issued. The bureaucratic paperwork regarding wildlife research in Peru represents an important part of a project. To master the task of obtaining export permits for DNA samples requires knowledge of governmental agencies and policies which are extremely complex and highly time consuming. After a first relatively successful performance for the export of the first expedition sample set (35 days for permit obtainment) a new legislation was implemented in the area of access to genetic resources in Peru. From that moment on, permit obtainment was far more complicated than before, with critical implications to our work, as



Figure 3. Field work at the rocky shore coast of Peru.



Figure 4. Fishing port at the Peruvian coast.

sample storage time was determined by access to export permits. We can assume that this total storage time (two to four months) was considerably detrimental for the DNA genotyping success of the second and third sample set. Once these arrived in the laboratory of the Zoological Institute of the Christian-Albrechts-University of Kiel, samples were immediately processed in the following steps: DNA extraction, amplification, genotyping (see Materials and Methods in Chapter II for details on success rates of laboratory work).

From the samples collected during the first field expedition in 2008, a data set was published in March 2010 (*Conservation Genetics*) as the first study on the genetics of the marine otter published in a peer-reviewed journal, presenting an analysis of the control region (CR) of mtDNA. Parallel in time, a research group lead by the Chilean biologist Julianna Vianna, performed a similar study on marine otter at the coast of Chile (Vianna et al. 2010), publishing its results in August 2010. To enhance the power of the results of both studies, efforts to establish cooperation were made, but were not successful. Chapter II, the core of this thesis, presents an analysis of the genetic variability and population structure of the marine otter using part of the data set published in 2010 (Valqui

et al. 2010) combined with data generated in further laboratory work (adding cytochrome b and new CR sequences). This data was later combined with the published data set from Chilean populations (Vianna et al. 2010) for a metaanalysis, especially to test population substructuring between the two countries. Chapter I gives a broad overview about the knowledge and status of the marine otter while Chapter III is based on a PVA and investigates the most important environmental and anthropogenic factors as to their influence on *L. felina* populations in the short, medium and long term.



Mammalian Biology

Zeitschrift für Säugetierkunde



◆ www.elsevier.de/mambio

ISSN 1616-5047
Mamm. biol. - 77(2012)2
S. 75-152

2·2012

CHAPTER I

The Marine Otter *Lontra felina* (Molina 1782): A Review of its Present Status and Implications for Future Conservation

Juan Valqui^{1,2}

¹Centro de Ornitología y Biodiversidad (CORBIDI). Calle Santa Rita 105 Of. 202, Lima 33, Perú.

²Zoological Institute, Christian-Albrechts-University, Olshausenstrasse 40 24118 Kiel, Germany

Mammalian Biology 77: 75-83 (2012)

ABSTRACT

The marine otter *Lontra felina* is an endangered and little known species living in a fragmented habitat: the coast of Peru and Chile. The smallest marine mammal's presence is unclear in Argentina and southern Tierra del Fuego and the current limits of the distribution are still under discussion. Recent population genetic studies suggest certain levels of gene flow despite a disjunct population. In the 20th century intensive hunting was the major threat to *L. felina*. This top predator still faces an uncertain future due to the impact of human activities (urbanization, pollution, intensive fisheries). There is a need of further studies deepening the knowledge on population genetics, population numbers and migration behaviour. Environmental education work, law enforcement and monitoring of protected areas are suggested to secure the survival of the species.

Key words: *Lontra felina*, marine otter, Southeast Pacific, threats, conservation

INTRODUCTION

As observation of *Lontra felina* is challenging, information on the species has been very scarce. Studies on behavior, diet, reproduction and/or ecology were made in the second part of the 20th century. The first genetic studies were published in 2010 (Valqui et al. 2010; Vianna et al. 2010). However, further information on population numbers, migration and genetics are still needed to achieve a better understanding and to provide arguments for conservation measures of this top predator.

Due to intensive hunting in the 20th century and the increasing human impact on marine ecosystems, the marine otter is listed as Endangered by the IUCN, in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), in Appendix I of the Convention on Migratory Species (CMS). Additionally, it is protected by Peruvian and Chilean law.

Considering implications for future conservation, this review summarizes the results of the most important peer-reviewed publications related to the marine otter and further presents important information from grey literature. Controversies created due to lack of information or poor documentation about the species are presented. Within the scope of “Proyecto *Lontra felina*” the author has executed exhaustive field trips along the Peruvian coast between 2008 and 2011, sampling non-invasively and evaluating the marine otter populations in over 80 locations. New observations and unpublished information on behavior gained in those field trips are also presented.

SPECIES DESCRIPTION

Otters are divided into seven genera and thirteen species (Wilson and Reeder 2005). The three primary lineages are: (i) a first lineage comprising the European, Asian and African river otters (*Aonyx*, *Hydrictris* *Lutra*, *Lutrogale*) and the sea otter (*Enhydra*), (ii) a second lineage composed of the American otters (*Lontra*, separated from *Lutra* by Van Zyll de Jong (1987)) and (iii) a third and basal lineage, the monotypic giant otter (*Pteronura*) (Koepfli and Wayne 1998; Koepfli et al. 2008). The genus *Lontra* is monophyletic and comprises four species, three of them occurring inside South America (Koepfli et al. 2008). *L.*

felina is the only species of its genus living in exclusively marine habitats and is presumably the most recently evolved mammal adapted to this habitat (Estes 1986). The smallest marine mammal of the world weighs 3.2-5.8 kg and measures 87-115 cm in total length. The fur is dark brown on the back and sides and slightly lighter on the underside. The species has large vibrissae and fully webbed feet and shows no sexual dimorphism in size or color (Larivière 1998 and references therein) (Figure 5). *Lontra felina* is relatively unknown and perhaps it is for that reason that it has several names. Spanish: chungungo, gato marino, lobito marino, nutria de mar (in all regions), chinchimén, chingungo, gatuna, huallaque (only in Chile and Argentina), anzumo, zorrillo, pejegato (only in Peru); English: South American Sea otter, marine otter, sea cat.

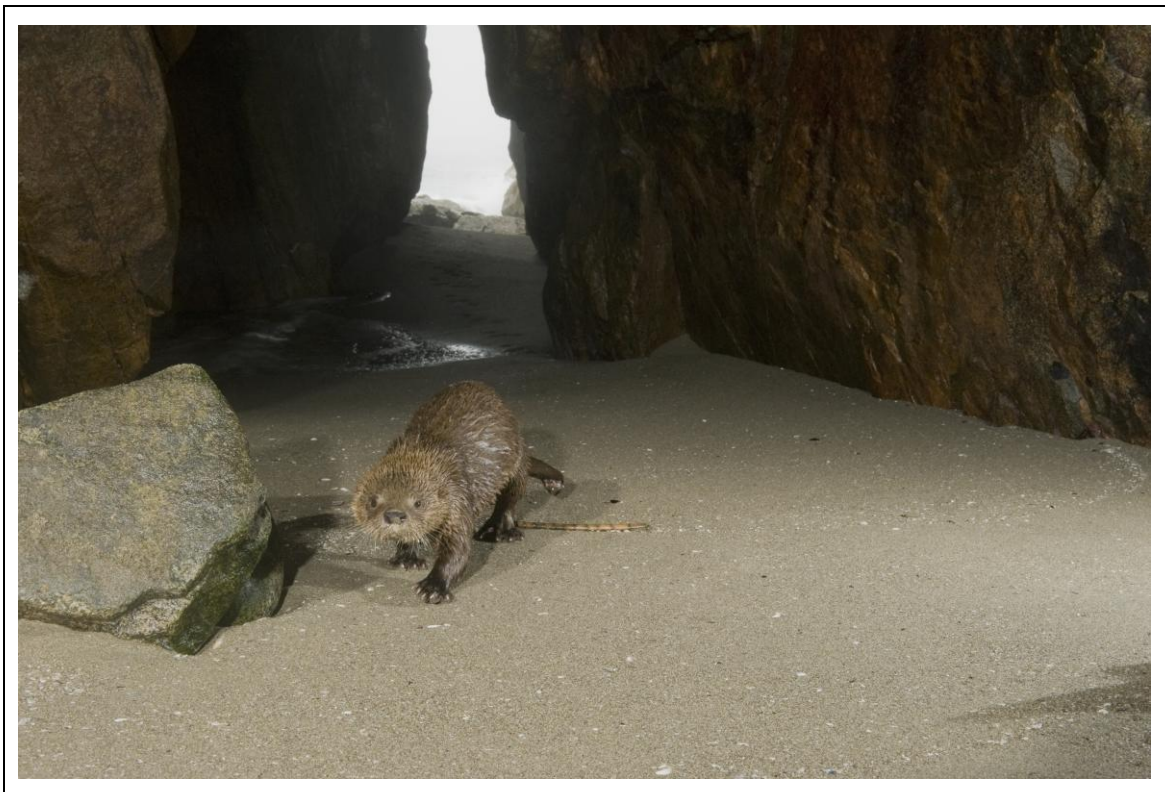


Figure 5. The marine otter *Lontra felina* in a cave. © www.kevinschafer.com

DISTRIBUTION AND ABUNDANCE

The marine otter *Lontra felina* was first described in 1782 in Chile by the Jesuit priest Juan Ignacio Molina who described many species to science (Molina 1782). Subsequent registers of the occurrence were scarce and few were published. In 1835, during his five-year journey on HMS Beagle, Charles Darwin recorded abundance of marine otters at the Chonos Archipelago, Chile (Darwin 1889). In 1844, the Swiss naturalist Johann Jakob Tschudi, who travelled through Peru from 1836 to 1842, reported very frequent sightings of *L. felina* in every coastal location, speculating that it could be present up to Ecuador (Tschudi 1844)—a fact that was never confirmed. Gay (1874) carried out descriptive studies on the species. In the mid 20th century, several authors documented reproduction, behavior and morphology (Housse 1953; Osgood 1943; Yañez 1948). In the 80s and 90s, the Chilean biologist Walter Sielfeld did research on conservation status, relative abundance, habitat characteristics, diet and also on niche overlap with *L. provocax* in southern Chile. These represent the most thorough studies on *L. felina* up to date (Sielfeld 1989; Sielfeld 1990a; Sielfeld 1990b; Sielfeld 1992; Sielfeld and Castilla 1999). Cabrera (1957) reported frequent sightings in scientific expeditions in Tierra del Fuego and Isla de los Estados. In 1959, the German naturalist Ernst Schweigger travelled along the Peruvian coast and documented marine otters up to Isla Lobos de Tierra (6°26'S), representing the northernmost record of *Lontra felina* (Schweigger 1964). Later studies registered the species only up to Chimbote (9°00'S), Peru (Brack 1978; Brownell 1978; Larivière 1998; Apaza et al. 2004; Sánchez and Ayala 2006; Valqui et al. 2010). Recent records of the marine otter in Salaverry (8°13'S) (Santillán and Caro 2007) and Huanchaco (8°04'S) (Alfaro-Shigueto et al. 2011), Peru, suggest that occasional events of colonization may occur north of 9°00'S, but several factors may hinder the establishment of populations in those areas: 1. rocky shore patches have been occupied by dogs, cats and rats; 2. lack of food availability exists due to a higher competition of the species with a more intensive fishery (Apaza et al. 2004); 3. the distances between suitable habitat patches may impede gene flow.

Between Chimbote and the limit to Chile, the Peruvian coast encompasses ~1600 km of about 100 rocky shore patches offering suitable conditions for marine otters alternated by patches of unsuitable habitat (e.g. sandy beaches and/or shoreline without caves). Interruptions average 9.1 km in length and are maximal 98.4 km long (calculation by the author). Some conspicuous groups of marine otters are present in Pucusana (12°28'S), Punta Corrientes (12°57'S), San Gayán Island (13°50'S), Morro Sama (17°59'S) and Vila Vila (18°07'S) (Sánchez 1992; Apaza et al. 2004; Valqui 2004; Mangel et al. 2010; Ruiz 2009; Valqui et al. 2010). See Table 1 (online appendix) for a complete list of locations with marine otter presence and abundance numbers.

Between its northern limit to Peru and Chiloé, the Chilean coast is fragmented similarly to the Peruvian coast and it encompasses ~3000 km of areas with marine otter presence alternated by marine otter free areas. In a larger scale, patches are separated increasingly, from northern Chile to central-south Chile, where in two shore line areas of 427 km between Algarrobo (33°22'S) and Peninsula Tumbes (36°36'S) and 273 km between Arauco Bay (37°10'S) and Queule (39°23'S) no marine otters were reported (Vianna et al. 2010 and references therein). Very little is known about the populations south of Chiloé Island (43°26'S) and the species has been nearly exterminated from the regions of Cape Horn and southern Tierra del Fuego, the southern limits of its distribution (Brownell 1978; Chehébar 1990). Conspicuous groups are reported for Soldado (26°10'S), Arrayán (29°43'S), Panul (30°03'S), Palo Colorado (32°01'S), Puquén (32°11'S), Papudo (32°26'S), Quintay (33°11'S), Colcura (37°07'S) and Punihuil (41°55'S) (Vianna et al. 2010) (Table 1).

In Argentina *Lontra felina* originally occurred in a 400 km segment of coast, but there are no recent studies or reliable records about distribution and population numbers. The last record was 25 years ago (Massoia and Chébez 1993 in: Cassini 2008; Parera 1996) (Figure 6). Interestingly, marine otters have sometimes been observed in rivers, feeding on shrimps in river farms of Camaná and Ocoña (Peru) up to 650 m above sea level (Hvidberg-Hansen 1970; Tello 1972; Viacava et al. 1978; Brownell 1978).



Figure 6. Historical, potential and current distribution of *Lontra felina* at the Pacific coast.

There is one report of a marine otter in the Majes River, 40 km away from the ocean (Grimwood 1968). The last report in a river was done in the river Cañete (13°07'S) in 2003 (Apaza et al. 2004). Presumably, the growing human presence and better guarding in aquaculture shrimp farms (Apaza et al. 2004) is causing the retrieval of the marine otter to solely oceanic waters.

Most studies assessing the marine otter population only covered limited regional areas, while the use of several different methodologies made it difficult to determine the total population and hinders an approximation today. Sielfield (1992) stated that the elusive behavior is an intrinsic obstacle for accurate population estimates and underlined the strong fluctuation of density values obtained in several studies done in past years. Population number for larger regions were proposed by Castilla and Bahamondes (1979), who estimated about 200-300 individuals for the Peruvian coast, without specifying how they obtained these numbers. Vaz Ferreira (1979) proposed the population of Peru, Chile and Argentina to consist of 1000 individuals, but Sielfield and Castilla

(1999) noted that no methodology was explained in that calculation. According to density numbers frequently found in the field, Sielfeld and Castilla (1999) stated that Vaz Ferreira's population number must be a sub-estimation. Along 1300 km of the Peruvian coast, from Lima (12°30' S) to the border of Chile (18°00' S), Apaza et al. (2004), proposed a total of 312 km of potential marine otter habitat. Multiplication with the average density of 2.21 ± 1.97 individuals per kilometer (ind/km) resulted in a population estimate of 691 ± 76 individuals for central and southern Peru. In a regionally more limited study, Mangel and Alfaro-Shigueto (2004) registered 22-28 individuals in 15.6 km of coast between Ilo (17°42' S) and Vila-Vila (18°07' S) in southern Peru. The population in 55 km of rocky shore was estimated at $88 (\pm 11)$ individuals.

The densities calculated in these and several other studies vary from 0.04 to 10.0 ind/km (12 studies, mean density = 3.3 ind/km), although densities between 1.0 and 2.7 ind/km are more consistently recorded (Apaza et al. 2004; Cabello 1983; Castilla 1982; Castilla and Bahamondes 1979; Ebensperger and Castilla 1991; Mangel and Alfaro-Shigueto 2004; Medina 1995; Medina-Vogel et al. 2006; Medina-Vogel et al. 2007; Rozzi and Torres-Mura 1990; Sánchez 1992; Sielfeld 1992). Based on the authors' research done between 2008 and 2011 in Peru, further areas with occurrence of otters were determined (via direct sightings, scat occurrence or references by other studies or by local fishermen), resulting in new calculations: 789 km (c.a. 50%) of a 1,800 km stretch of the Peruvian coast are proposed as habitat of *Lontra felina*. Considering the most frequently reported densities of 1.0-2.7 ind/km the population is estimated to range from 789 to 2,131 individuals at the Peruvian coast. Molecular analysis revealing high levels of mitochondrial DNA (mtDNA) variability (Valqui et al. 2010; Vianna et al. 2010) and further data on occurrence might support this data, indicating that the population numbers reported in the last years were underestimated. Medina-Vogel et al. (2006) discuss the uncertainty of the methodology in most surveys done in the last decades, while they propose minimum abundance indices rather than total survey numbers. Rarefaction analysis based on fecal genotyping (Kohn et al. 1999) could allow more accurate population size estimates.

ECOLOGY

Habitat

The Humboldt Current System (HCS) flows from southern Chile (~42°S) for over 4,000 km north-westward along the west coast of South America, up to Ecuador (Thiel et al. 2007). The cool upwelling system with coastal water temperatures ranging from 14°C to 20°C (Swartzman et al. 2008) shows high marine productivity and allows the presence of huge populations of seabirds, sea lions and cetaceans (Majluf et al. 2002). Paradoxically, this system creates desert conditions at the coast (Bertrand et al. 2004; Bertrand 2008). The nearest 150m of the cold and productive waters and the characteristic heterogeneous alternation of sandy beaches, rocky shores and islands of the littoral compose the habitat of the marine otter (Larivière 1998; Sánchez and Ayala 2006). They may inhabit islands that are several kilometers offshore. Sánchez and Ayala (2006) reported *L. felina* on Isla Pescadores (11°46'S), which is over 6 km away from the mainland but connected by several islets about 2 km away from each other.

Rocky shore patches (Figure 7) are preferred as they provide protected dens within caves with reproduction and resting places. Caves are above water at high tide and may be exposed to heavy seas (Cabello 1983; Ostfeld et al. 1989; Villegas et al. 2006) and present a variety of architecture (Medina-Vogel et al. 2006). Entrances, which may be several, are often well defined and do not have a submarine access, as they do have in other otter species (Sielfeld and Castilla 1999). If sandy ground is present in the inside, nest-shaped cavities may be built (10 cm deep and 50 cm in diameter) (pers. obs. by the author in 2009-2010). Human constructions like wharfs, ship wrecks, boats and artificial caves are used as part of their habitat (Medina-Vogel et al. 2007; Valqui 2004).

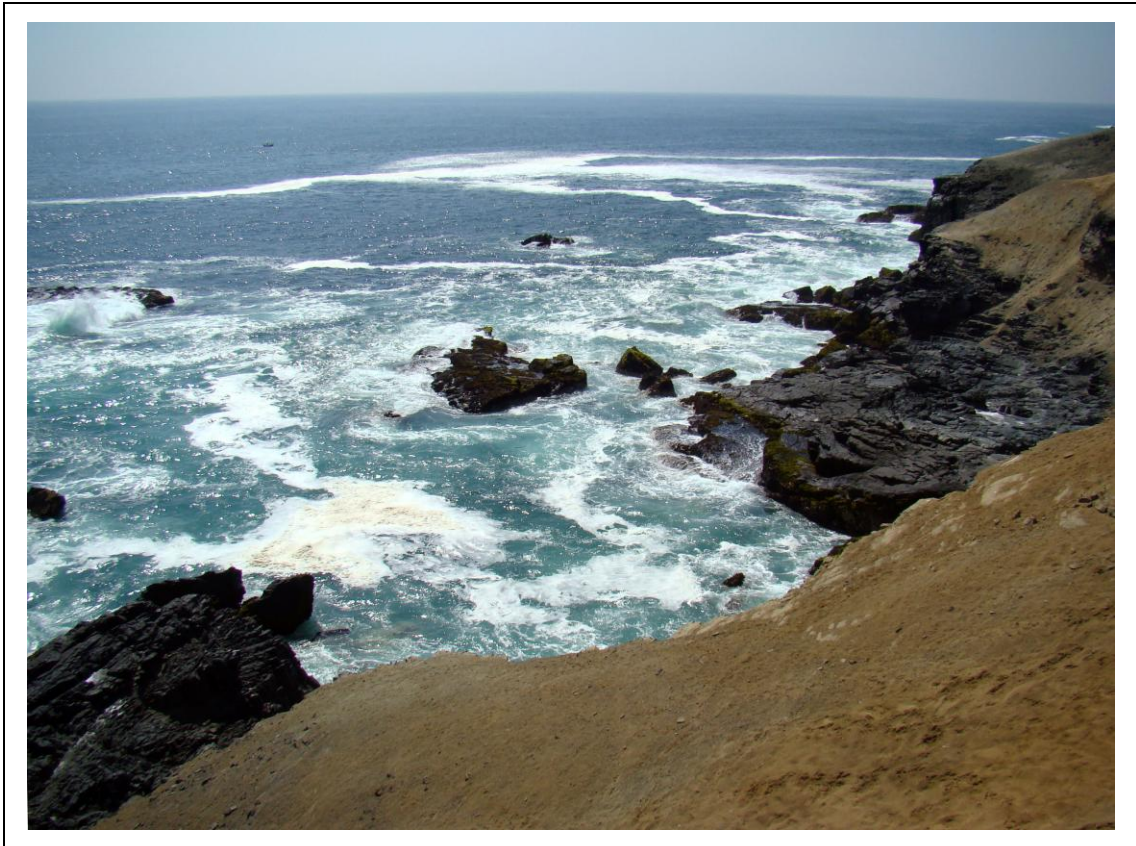


Figure 7. Rocky shore habitat of the marine otter.

Diet and interactions

Diet studies are commonly based on scat samples, which can be found relatively frequent at the littoral, while foraging behavior is difficult to monitor (Valqui 2004). These studies may be biased, under-representing soft-bodied prey and over-representing prey with spines and carapaces (Larivière 1998), which often remain in scat (pers. obs. by the author in 2008-2011). *L. felina* feeds on invertebrates (decapods, shrimps, crabs and mollusks) and vertebrates (mostly fish, but also birds and even small mammals) (Sielfeld 1990a; Larivière 1998) in very variable compositions (see Ostfeld et al. 1989; Sielfeld 1990a; Medina-Vogel et al. 2004; Valqui 2004; Medina-Vogel et al. 2008; Ruiz 2009 for numbers and percentages). According to research done by Sielfeld (1990a) south of 48°50'S, fish represents the main diet item (84.8%), followed by crustaceans (41.9%) and echinoderms (19.1%), which are preyed on seasonally, in the urchins' reproductive status. In contrast, urchins were found in 95% of the scat samples of the sea otter *Enhydra lutris* (Kenyon 1969).

Gasteropods were found in only 2.9% of the scat samples, but represented over half of the prey rests found in dens and therefore seem to be an important part of the diet. (Sielfeld 1990a). According to a study by Mangel et al. (2010), in which scat samples from nine study sites between 17°42'S and 39°40'S were analyzed, fish represents higher percentage of prey in the north, while it becomes less in more southern populations. In the south, crustaceans represent higher percentages of the diet (Mangel et al. 2010). Individuals preying on octopuses were observed in Pucusana (12°28'), Peru (Valqui 2004) and in Puñihuil (41°55'S), Chile, where in January 2010 an adult individual caught an octopus and fed its pup with it (Kevin Schafer pers. comm.). Birds are only preyed on occasionally and opportunistically. Sielfeld (1990a) found ten bird species contained in 2.9% of 105 scat samples and in 11.5% of 68 prey rests associated to dens. More than half of them (66.6%), were from a cormorant *Phalacrocorax sp.* (Sielfeld 1990a). On Isla La Vieja (14°16'S), Peru, marine otters are presumed to prey regularly on a colony of the Peruvian Diving Petrel (*Pelecanoides garnotii*) (Mattern et al. 2002). In January 2009 one individual was observed capturing a chick of a Magellanic flightless steamer duck (*Tachyeres pteneres*) in Puñihuil, Chile (Kevin Schafer pers. comm.). *L. felina* may even feed on small mammals (Sielfeld 1990a) and fruits (*Greigia sphacelata*, *Fascicularia bicolor*) (Brownell 1978; Cabello 1983), thus showing an overall opportunistic feeding behavior (Medina-Vogel et al. 2004).

Lontra felina may compete with gulls (*Larus sp.*) and the South American sea lion (*Otaria flavescens*) for prey (Cabello 1983). The latter was even shown to attack *L. felina* (Ruiz 2009). Allegedly, Orcas (*Orcinus orca*) and sharks represent predators (Cabello 1983 and Parera 1996 in Larivière 1998), but no direct observations of attacks have ever been reported. Notably, Orcas do prey regularly on another otter species, the Sea Otter (*Enhydra lutris*) in western Alaska (Estes et al. 1998).

Very little is known on parasites of *Lontra felina*. Nematodes and acantocephalans were identified as endoparasites (Cabello 1978). Villegas and Huamán (1989) detected undetermined hematophagous ectoparasites (acari).

Physiology

Marine otters are well adapted aquatic conditions due to their powerful tail and webbed feet (Larivière 1998). Due to a relatively small size and reduced body fat, heat loss is reduced by avoiding long-time periods in the water. They may swim 2 km in about 20 minutes (pers. obs. by the author in 2009), forage in shores with strong waves (Cabello 1983; Ostfeld et al. 1989; Villegas et al. 2006) and dive up to 77 seconds (Valqui 2004). They may even spend time swimming and feeding in the short period of time between two breaking waves (pers. obs. by the author in 2002, 2004, 2008, 2010 and 2011). Marine otters can move 50 m under water (Cabello 1978). As marine mammals are hypotonic, they regulate their salt balance by hormonal regulation of urine concentration and/or by the rate of urine concentration (Ortiz 2001). Costa (1982) showed that Sea otters (*Enhydra lutris*) create urine that is in higher concentration than found in their environment. This osmotic ability allows them to excrete excessive Na^+ and Cl^- . Thus, sea otters can actively consume sea water and gain free water (Ortiz 2001). Individuals of *L. felina* living close to rivers might directly drink freshwater, while those living far away from rivers and in areas with absence of rainfall –something common in the coastal desert of Peru and Chile– may further compensate loss of water by a osmoregulatory mechanism similar to the Sea otter.

Behavior

Behavior studies of *Lontra felina* are problematic due to difficulties to follow them in the field: they spend more than 80% of their time hidden in caves. It is furthermore impossible to recognize individuals (Medina-Vogel et al. 2006 and references therein) as neither characteristic individual pigmentation nor sexual dimorphism exist. Several studies describe behavioral aspects, but there are a lot of contradictions concerning reproduction, social behavior, migration and territoriality. Several observational studies stated that the marine otter displays activity peaks at specific hours during the day (e.g. in the morning, in the afternoon or between 3 and 5 pm) (Ostfeld et al. 1989; Ruiz 2009; Sánchez 1992; Valqui 2004). On the contrary, a radio-tracking study showed no

significant preferences by the species for activity during the day, night, or any other period of time (Medina-Vogel et al. 2007). *L. felina* spends over 80% of its time on land, preferably resting in or nearby their dens (Medina-Vogel et al. 2007 and references therein). Nests are built and rebuilt repeatedly after high tide washed them away (pers. obs. by the author in 2009 and 2010). During long-distance swimming between rocky patches (pers. obs. by the author in 2002 and 2009) sandy beaches are eventually used for resting (Ebensperger 1992). To forage, they start to search for their prey once arrived at the bottom. Thus, dive length is correlated to water depth (Medina 1995). Foraging success ranges from 18% to 32% of the dives (Medina 1995; Ostfeld et al. 1989; Valqui 2004). Captured prey is carried in the mouth and may be put on belly while swimming on their back. Prey is then consumed with the help of the forepaws (pers. obs. by the author).

Although *L. felina* is reported as solitary and seldom to be found in groups (Cabello 1983; Castilla 1981; Larivière 1998; Sielfeld and Castilla 1999) there are several records of groups of five to eight individuals (Valqui 2004; Ruiz 2009; Apaza et al. 2002; Mangel and Alfaro-Shigueto 2004; pers. obs. by the author in 2009). In a radio-tracking study in Quintay (33°11'S) Chile, a group of individuals shared an area less than 100 m² in several occasions. Moreover, the study revealed a home range of <5 km for *L. felina*, with home-range overlap and no avoidances between individuals (Medina-Vogel et al. 2007).

Marine otters were formerly assumed to reproduce in austral summer (December-February) (Larivière 1998) but in recent years records of pups all year round have been made (Valqui 2004; Ruiz 2009; Medina-Vogel et al. 2006). Presumably, low seasonal variation of the cold waters of the HCS creates conditions that allow a continuous reproduction throughout the year. Parturition occurs after 60 days of gestation (Larivière 1998 and references therein). The litter of two to four pups stays with the mother for ten to twelve months (Parera 1996; Cabello 1983; Larivière 1998; Valqui 2004). During this time, adults carry pups in their mouths to move between dens (Valqui 2004) or let them rest on their bellies as they swim on their back (Castilla and Bahamondes 1979). Nursing and feeding may overlap: in Puñihuil (41°55'S),

Chile, a mother was observed breastfeeding a young individual while simultaneously feeding it with prey (pers. obs. by the author in 2010). Marine otters are presumed to be monogamous (Larivière 1998) but difficulties in assessing territoriality and spatial behavior impede to confirm patterns of reproductive behavior (Medina-Vogel et al. 2007).

Play behavior is observed in many taxons of mammals and interestingly the advantages of such behavior do not appear to carry an immediate biological benefit, such as access to mate or food or maintenance of a territory. Thus, the significance of this behavior is not yet clear (Pellis 1988). Individuals of *Lontra felina* have been observed displaying play behavior in several studies (Castilla and Bahamondes 1979; Ebensberger and Castilla 1991). Play behavior turning over into mating, fighting or separation has also been observed (Valqui 2004). High pitched vocalizations have been reported during fighting (Ostfeld et al. 1989). In Pucusana, Peru, several different vocalizations were registered during swims and while playing or foraging (Valqui 2004).

For scent marking, marine otters defecate in latrines or singular places at cave entrances (Valqui et al. 2010). Feces are regularly washed by high tides or high spring tides (Castilla and Bahamondes 1979). They may also defecate directly into the water while swimming (pers. obs. by the author in 2010).

POPULATION GENETICS

Since non-invasive molecular techniques have improved in the last decades, genetic analysis no longer relies on tissue samples that imply the capture of individuals. Genetic analysis can for example be based on scat samples (Kohn and Wayne 1997). This is especially relevant concerning species, that are elusive or on which ethical issues may arise due to animal handling (e.g. setting traps, supplying anesthesia).

In a first study along the entire distribution of *L. felina* at the Peruvian coast by Valqui et al. (2010), 87 scat samples were collected. 41 of those samples were sequenced at 265 bp of the control region of mtDNA, yielding 24 individuals with 11 haplotypes and 13 polymorphic sites. Considering a 5 km home range, pseudoreplication was avoided by excluding samples that came from within a

distance of 5 km or less. No population substructure (based on F-statistics) and no isolation by distance (based on a Mantel-test) could be detected. Considering the low population numbers reported, this study yielded surprisingly high values of haplotype diversity $h = 0.86$ and nucleotide diversity $\pi = 0.0017$, compared to variability values of the Eurasian otter (*Lutra lutra*), $h = 0.16$ and $\pi = 0.0006$ (Ferrando et al. 2004), which has considerably higher population numbers. h (varies between 0 and 1) is a measure of the uniqueness of a particular haplotype in a given population and is related to the relative haplotype frequency. A high value of h represents a high number of haplotypes. π (varies between 0 and 1) is a measure of the degree of polymorphism (diversity) in a given population (Nei and Tajima 1981). π represents the average number of nucleotide differences per site between two randomly chosen DNA sequences from the sample population. A high value of π represents a high number of differences within a sample (Nei and Li 1979). Based on geographic criteria the Peruvian groups can be separated into four groups (Figure 6, Table 1). This separation has to be inspected by further genetic analysis. A second study was carried out in Chile and Peru by Vianna et al. (2010), sequencing the control region of mtDNA in 168 out of 419 collected samples of blood (16), tissue (46) and scat (357). Analysis of 2261bp of mtDNA (control region, ND5 and Cyt-b sequences) yielded 26 haplotypes. Combining mtDNA haplotypes and microsatellite genotyping, 89 individuals were identified within the 126 scat samples processed. A significant population sub-structuring was detected ($F_{ST} = 0.86-0.77$, $P < 0.0001$) and the overall variability values were $h = 0.93$ and $\pi = 0.0047$. This is in the range of the study done in Peru (Valqui et al. 2010). Vianna et al. (2010) determined six groups (based on a spatial analysis of molecular variance, SAMOVA), with the Peruvian group separating from Chilean groups, while the two southernmost groups showed reduced genetic diversity (Chile Central-South: $h = 0$ and $\pi = 0$; Chile South: $h = 0.39$ and $\pi = 0.00069$) as it is often recorded in peripheral populations (Vianna et al. 2010; Wisely et al. 2004).

In an analysis covering data of about 3000 animal species Bazin et al. (2006) note that high levels of diversity are not always proportionally related to large

population numbers. In fact, maintenance of high levels of allelic variation in spite of a severe bottleneck in population size has been observed in some mammal populations, like the brown bear (*Ursus arctos*) in the Western Carpathians (Hartl and Hell 1994), the greater one-horned rhinoceros (*Rhinoceros unicornis*) (Dinerstein and McCracken 1990) and several gazelle species (Zachos et al. 2010 and references therein). According to Dinerstein and McCracken (1990) the erosion of genetic diversity attributed to bottlenecks may be overemphasized and Bazin et al. (2006) suggest data on nuclear markers to be more fitting in describing the relation between population size and genetic diversity. Although both genetic studies on *Lontra felina* have not yet detected if the population has actually gone through a bottleneck, thousands of otter pelts exported from Chile between 1910 and 1954 (Iriarte and Jaksic 1986) suggest a significant decrease in population numbers in the 20th century. The results obtained in the genetic studies of Valqui et al. (2010) and Vianna et al. (2010) suggest that gene flow occurs despite habitat fragmentation along the Peruvian and Chilean coast, which is in contradiction to the low dispersion capacity reported by Medina-Vogel et al. (2007). Young individuals of another otter (*Lutra lutra*) move several kilometers in search of new habitat (Koelewijn et al. 2010; Kruuk and Moorehouse 1991) and tracked Giant otters (*Pteronura brasiliensis*) have been recorded moving up to 160 km along a river in a period of 6 months (Staib 2002). A somehow similar behavior might cause remixing of genetic material in the marine otter population, neutralizing the effect of isolation by great distances between patches of rocky shore. Further studies on migration behavior of the species are needed to confirm this hypothesis.

THREATS - PAST AND PRESENT

Natural factors

Land refuges are decisive on the distribution of *L. felina* (Medina-Vogel et al. 2007) and due to natural fragmentation of the coastal habitat in Peru and Chile the population is disjunct (Redford and Eisenberg 1992; Valqui et al. 2010). Small habitat patches isolated from each other –a frequent scenario for *Lontra felina*–impede gene flow and present unfavorable conditions to support a marine otter population (Peltonen and Hanski 1991).

The El Niño Southern Oscillation (ENSO) is a periodic phenomenon causing more or less drastic climatic and oceanographic changes. Rise of temperature and reduction of nutrient supply of superficial waters considerably reduce the primary production, causing the mortality of several marine communities from fish to mammals (Apaza and Figari 1999; Wang and Fiedler 2006). Although very little information exists about concrete mortality events–five dead individuals were reported in a period of three months during a study on the mortality effects of the ENSO on marine fauna in Marcona, Peru (Apaza and Figari 1999)– ENSO events are considered to cause a decrease in population sizes of *Lontra felina* (Vianna et al. 2010).

Anthropogenic factors

In the last decades, heavy migrations from the Andes to the coast of western South America caused an intensive urbanization of the littoral. In Peru, the human population has increased from 7 million in 1940 to 28 million in 2007, with now 54% of the population living at the coast (INEI 2007). In Chile, the human population has increased from 5 million in 1940 to 15 million in 2005, with four of the five biggest Chilean cities at present located directly at the coast (INE 2002).

The pressure put on the coastal ecosystem by the increasing number of human settlements has accelerated habitat degradation and increased habitat fragmentation (Brownell 1978; Eisenberg and Redford 1989; Sielfield and Castilla 1999; Medina-Vogel et al. 2008; Vianna et al. 2010).

Industrial and artisan fishing ports have been established along the Pacific coast, affecting structure and productivity of marine life communities. Fishing has intensified natural declines in the abundance of many forage fishes, leading to reduced reproductive success and reduced abundance of birds and marine mammals (Majluf et al. 2002). Mariculture has increased dramatically in number and extraction volume in Peru (Apaza et al. 2004). Furthermore building of docks, dredges, dams, breakwaters, canalizations and sanding also produces enormous disturbances of coastal ecosystems (INRENA 2003).

Lontra felina may adapt to the fragmented habitat (Valqui 2004; Mangel and Alfaro-Shigueto 2004; Valqui et al. 2010) and shows certain capacity to coexist with humans, even in a severely modified environment. In Pucusana, Peru, a group of marine otters is present in a bay (400 m in diameter) where fishing, swimming, diving and jet skiing are regular human activities all year round (Valqui 2004; Ruiz 2009). In Chile, *L. felina* has been reported to live in fishing boats, ship wrecks and near highly populated areas (Medina-Vogel et al. 2007). Still, this coexistence implies competition for resources, that humans exploit for food, commerce and housing (Moreno et al. 1984; Ostfeld et al. 1989; Moreno 2001; Medina-Vogel et al. 2004; 2007; 2008).

Another threat for marine otters is accidental death by entanglement in fishing nets (Mangel and Alfaro-Shigueto 2004). 15 cases of bycatch or intoxication by rat poison were reported between February 2006 and October 2008 (Pizarro 2008), accidental death in crab pots occurred in Chile (Medina-Vogel et al. 2004). Dynamite fishing is a frequent problem in several localities of the Peruvian coast, such as Huarmey (Luis Rios pers. comm.) and Paracas (Apaza et al. 2004; pers. obs. by the author in 2010). Additionally, individuals that live close to mariculture areas are threatened to be killed by armed guards (Apaza et al. 2004).

Human presence has a side effect, whose consequences seem not to be directly harmful to the marine environment, but is definitely affecting the marine otter population. In and around human settlements, big dens with broad terrestrial entrances may be occupied or at least frequently visited by dogs, cats and rats. Several places with evidence of rats and cats inhabiting potential otter

caves (and where otters have been spotted nearby) have been sighted in northern Peru (Apaza et al. 2003; pers. obs. by the author in 2008 2010 and 2011). Dog attacks were increasingly reported in several locations of the distribution (Medina-Vogel et al. 2008; Mangel et al. 2010; Vianna et al. 2010). Dozens of beach resorts have been built, especially in the vicinity of big cities like Lima (Ludeña 2004), where it is common to observe jet skis and motor boats affecting the local fauna with oil spillage and extreme noise (Valqui 2004). Furthermore, several centers of industrial fishing activity spill their waste directly or indirectly into the ocean (Apaza et al. 2004). This is especially the case of Chimbote, probably the most important fishing port at the Peruvian coast corresponding to the northern distribution limit of the marine otter. In the mining cities Ite, Ilo and Marcona in Peru, tailings have been spilled directly into the ocean for over 40 years, altering several kilometers of the littoral (Apaza et al. 2004). Additionally, spills of domestic effluents reach the ocean directly or through rivers (Hinrichsen 1998; Thorne-Miller 1999). Thus, heavy metals and other toxic substances can be diffused through currents and progressively transmitted through the food chain (Valqui 2004; Apaza et al. 2004).

Hunting was the major threat on the marine otter in the 20th century (Red List of Threatened Species Version 2011.1; Sielfeld and Castilla 1999). However, there is a lack of specific numbers. In a report on Chilean fur trade it is documented that 38 000 otter pelts were exported from Chile between 1910 and 1954 (Iriarte and Jaksic 1986). Due to the fact that two otter species (*L. provocax* and *L. felina*) were subsumed as “otters”, no certain population estimate of each species can be inferred from this data. Indeed, it shows the magnitude of the pelt industry in the 20th century. Today, the lack of demand in the pelt market and fur trade prohibitions, have diminished the hunting threat considerably. Nonetheless, illegal hunting for fur and trophies still occurs in Samanco, (Sánchez and Ayala 2006), La Libertad (15°29´S) and Morro Sama (18°00´S) (Apaza et al. 2004), Peru and south of 39°S latitude in Chile. Killings do not implicate consequences for the hunters as law enforcement is not comprehensive (Red List of Threatened Species Version 2011.1).

A scenario of increasing human impact on the Pacific coast of South America is given for the next decades. In this context, Medina-Vogel et al. (2008) hypothesized that habitat fragmentation due to human impact might turn isolation into a cause of local extinction events. A level of interruption between habitat patches too big to be compensated by migration could be arrived, blocking gene flow. Considering the adaptability of the species, this hypothesis certainly remains under discussion.

Current rates of decline indicate that the population of *L. felina* will be reduced by at least 50% over the next three generations (30 years) (Red List of Threatened Species Version 2011.1). Although methods to obtain this estimate are not mentioned and recent results do not correspond to this prediction, the uncertainty about total population numbers, effective population size and population trends supports its classification as “Endangered” based on the precautionary principle. Further studies and monitoring have to clarify the reasons for the distribution reduction of the last decades to avoid further reduction or increased levels of threats.

From the early 1900s, Peruvian governmental agencies protected the terrestrial part of a system of islands and headlands along the Peruvian coast to secure the accumulation of guano in bird colonies, mainly of the Guano Cormorant (*Phalacrocorax bouganvillii*), the Peruvian Booby (*Sula variegata*) and the Pelican (*Pelecanus thagus*) (Muck and Pauly 1987). Although the marine otter was no subject of the plan, the species obviously benefited from the protection status of parts of its habitat. The Guano Administration Agency of Peru from the Ministry of Agriculture was focused in extracting the fertilizer from those areas. In December 2009, this production system was converted into a system of protected areas (Reserva Nacional Sistema de Islas, Islotes y Puntas Guaneras, RNSIIPG) of the Peruvian Natural Resources Agency (Dirección General Forestal y de Fauna Silvestre) and the surrounding oceanic waters were included. This increase of the protected area (1120 km² in total) presents a potential improvement of the protection of the coastal ecosystem. But certainly, several conflicts with the artisan and industrial fisheries were inherited from the former system (Apaza et al. 2004) and more will yet rise. The new

prohibition to fish in the nearby waters of those areas is in conflict with the ever growing pressure put on the marine ecosystem by more intensive fisheries. The ever increasing threat of displacement demands permanent solutions that allow the marine otter to evade introduced species occupying their habitat.

Although the marine otter is protected by law it has not been involved in any concrete conservation program by the Peruvian government. Environmental education work is a task of independent non-governmental organizations, both in Peru and Chile. Pro Delphinus (www.prodelphinus.org), an organization working at the Peruvian coast since 1995, focuses on the conservation of *Lontra felina* (especially in Pucusana, Puerto Grau and Vila Vila), providing workshops for both schoolchildren and fishermen. A better understanding of the ecological interactions of the marine environment and ideas to support conservation of marine otters and other threatened species are generated. The author has collaborated with Pro Delphinus for the last three years and created “Proyecto *Lontra felina*” (see facebook group under the same name) to unite the efforts regarding environmental education work as well as to promote scientific research. In Chile, the NGO “OBC Chinchimen” has established an environmental network in the region of Valparaíso in central Chile, since 2001. A marine otter rehabilitation and reintroduction program is accompanied by non-invasive behavioral studies (especially in Acantilados de la Quirilluca) assessing conservation priority areas (Ricardo Correa pers. comm.).

RECOMMENDATIONS

In Peru, the reasons for the retrieval of *Lontra felina* from the regions north of Chimbote ($9^{\circ}00'S$) have to be clarified and solutions to revert these conditions have to be proposed. Occasional events of occupation in those regions have to be monitored and conservation measures (bycatch reduction, total prohibition of dynamite fishing and hunting) have to ensure conditions for the species to return to its historical distribution. Given a favorable scenario, translocation and re-stocking programs should be taken into consideration. Another region to which special attention should be paid is the area around Morro Sama and Quebrada de los Burros ($17^{\circ}59'S$ to $18^{\circ}02'S$) in southern Peru, where

conspicuous groups of *L. felina* have been reported (Mangel and Alfaro-Shigueto 2004) in an area with a low density of human population (pers. obs. by the author). This area has never been protected nor is it comprised in the RNSIIPG. A proposal of an establishment of a regional conservation area exists (Ulloa et al. 2010) and has to be supported (Valqui et al. 2010). The area represents the southernmost group of otters in Peru which could act as a source to adjacent marine otter groups, especially to those over 300 km further south, in northern Chile. Mangel et al. (2010) suggest that habitat modified by humans and artificial habitats (shipwrecks, docks, etc.) can be used as stepping stones between rocky seashore patches widely separated from each other and that their management should be included in a strategy for marine otter conservation. The new system (RNSIIPG) in Peru, at least theoretically, points in that direction. Understanding population structures and dynamics as well as the species' migration and territorial behavior will allow compensation measures against the fragmenting effect of geographical barriers.

In Chile, to hinder the consequences of fragmenting forces acting upon *L. felina*, Medina-Vogel et al. (2008) suggested that conservation management should consider the need of establishing reserve networks including the protection of rocky seashore patches that would allow migration (gene flow) and secure the species survival. Domestic animals should be controlled within these areas (Medina-Vogel et al. 2008). Considering political and logistical implications, this idea seems very difficult to put into practice and will have to be supported by protection of other species. Studies in the southern part of Chile have to test the hypothesis of southern population extinction (Vianna et al. 2010). In Argentina a monitoring program should be established for Isla de los Estados and Mitre Peninsula to update the information on the species (Cassini 2008).

Further studies have to assess regional and total population numbers by molecular techniques (Kohn et al. 1999; Xu and Fu 2004) as well as by improvement of field techniques (Medina-Vogel et al. 2006), resulting in better estimates of the real population size. The effective population size has to be determined by genetic, behavioral and ecological studies describing reproduction ratios. Nuclear markers (microsatellites) should be used to deepen

the knowledge on population history and demography (Bazin et al. 2006). Studies on parasites, diseases, as well as the impact of pollutants on marine otters living in highly contaminated waters (Apaza et al. 2002), have to be continued. Physiological issues concerning the performance of the marine otter in its environment (e.g. osmoregulation) have to be addressed. Relevant information on reproductive ecology was found in studies of captive Giant otters (*Pteronura brasiliensis*) (Redford and Eisenberg 1992). Captive marine otters (at least 3 otters captive in Peruvian Zoos) could be used as reference or study subjects.

Lontra felina possesses a special status as flagship and umbrella species, which should be exploited to support educational campaigns along its distribution range. Sensitization of local people (especially fishermen) towards *L. felina* might lead to conservation programs potentially contributing to the conservation of marine ecosystems in Peru, Chile and Argentina. It is strongly recommended to coordinate efforts between research and environmental working groups, between and within these three countries.

ACKNOWLEDGEMENTS

Execution of field work of the “Proyecto *Lontra felina*” was authorized by permits N°98-2008-INRENA-IFFS-DCB, N°454-2009-AG-DGFFS-DGEFFS and N°0149-2011-AG-DGFFS-DGEFFS given by the Peruvian Natural Resources Agency from the Ministry of Agriculture of the Republic of Peru and permit N°19-2010-SERNANP-RNP, given by the Peruvian Natural Protected Areas Agency. Guano administration areas were accessed with authorization N° 037-2009-AG-AGRORURAL-DO given by the Peruvian guano governmental agency AGRORURAL. Sample export was authorized by permits N° 11378, N° 000582 and N° 1373 given by the Peruvian CITES authority and by Council Regulation (EC) No. 338/97 given by the German CITES authority. The author thanks researchers, fishermen and local people who provided unpublished information on marine otter sightings; Prof. Günther Hartl and Frank Zachos from the Zoological Institute at the Christian-Albrechts-University in Kiel for their great supporting and for providing the laboratory facilities; Natalia Ortiz, Jerico Solis,

Daniella Biffi, Elisa Ruiz, Ronald D´Brot, Enrique Hon, Karen Verde, Celia Cáceres, Alejandra Bussalleu, Carlos Calvo, Andrea Luna, Fernando Valdez, Julian Durán and Juan Yupanqui for their assistance in the field for the past four years; Yennifer Hernández for assisting in the laboratory work; Kevin Schafer for his comments on behavioral observations and the unpublished pictures of his photographic project on *Lontra felina*; Ricardo Correa from the Organization OBC Chinchimén for the information on marine otter programs in Chile; Luis Ríos for the data on dynamite fishing in Huarney; Agnes Hüttmann for assisting in the laboratory and for the thorough revision of this manuscript; William Ñañez, Luis Alza and Thomas Valqui from the Centro de Ornitología y Biodiversidad (CORBIDI) as well as Armando Valdés-Velasquez from the Laboratorio en Estudios de Biodiversidad (LEB) for institutional support in Peru; Ana Maria Haase for the sample storage facilities; Jeffrey Mangel and Joanna Alfaro from Pro Delphinus for the cooperation in the environmental education project; IDEAWILD for providing logistic material.

The Society for Marine Mammology (SMM) provided a grant in 2008; The Rufford Small Grants (RSG) provided a grant in 2010 (fund number: 52.11.09). Yaqu Pacha supports the “Proyecto *Lontra felina*” since 2009 with funding and ideas for developing the environmental education work.

ONLINE APPENDIX

Table 1. Occurrence and abundance of the marine otter *L. felina* along its distribution. Due to the one-dimensional distribution, locations are indicated with southern latitude (S. lat.) only. Type of record (Record) is specified as: REF (references by fishermen or locals), DS (direct sightings), or SBT (scat, blood or tissue samples collected). References⁽¹⁻⁸⁾ for type of records are listed. The least number of individuals is given for each location (Individuals). Locations in or close to protected areas are marked with *.

PERU NORTE

Location	S. lat.	Record	Individuals
Isla Monaque	9°00′	REF ¹	2
Isla Santa*	9°01′	DS ¹	5
Isla Blanca	9°06′	DS ¹	3
Isla Ferrol	9°08′	DS ¹	2
Peninsula Ferrol	9°12′	DS ¹	2
Vesique	9°13′	DS ²	4
Samanco	9°15′	DS ³	3
Los Chimus	9°19′	DS ³	1
Isla Los Chimus	9°20′	DS ³	4
Bahía Tortugas	9°22′	DS ⁴	2
Casma	9°27′	DS ²	1
La Gramita	9°44′	DS ²	1
Punta Culebras*	9°56′	DS ¹	4
Tuquillo	10°01′	DS ¹	1
Puerto Huarmey	10°05′	SBT ²	2
Playuelas	10°13′	SBT ²	1
Punta Colorado*	10°29′	DS ³	1
Punta Litera*	10°36′	DS ³	1
Playa Paraíso	11°12′	DS ⁴	1
Punta Salinas*	11°17′	DS ³	2

43

PERU CENTRO

Location	S. lat.	Record	Number
Isla Pescadores*	11°46'	DS ¹	1
Ancón	11°46'	DS ¹	4
Isla San Lorenzo*	12°04'	DS ⁴	1
Isla Pachacamac*	12°17'	DS ¹	5
San Bartolo	12°23'	DS ⁶	1
Cerro La Tiza	12°24'	DS ¹	3
Cerro Carbón	12°26'	DS ¹	1
Pucusana	12°28'	DS ²	5
Cerros Ya Ya	12°33'	DS ⁶	2
Pta de Cordero	12°35'	DS ⁶	1
León Dormido	12°37'	DS ⁶	1
La Ensenada	12°39'	DS ⁶	1
Cerro Salazar	12°41'	DS ⁶	2
Caleta de Bujama	12°44'	DS ⁶	1
Isla Asia*	12°46'	DS ³	1
Islote Los Leones	12°52'	DS ⁶	1
Puerto Fiel	12°56'	DS ³	2
Pta Corrientes	12°57'	DS ²	5
Cerro Azul	13°01'	DS ²	2

40

PERU CENTRO-SUR

Location	S. lat.	Record	Number
San Gallán	13°50´	DS ⁵	13
Pta. Culebra*	13°50´	DS ³	1
Arquillo*	13°54´	SBT ²	1
La Mina*	13°54´	SBT ²	1
Los Choros*	13°54´	SBT ²	1
Lagunilla*	13°54´	SBT ²	1
Yumaque*	13°54´	REF ⁷	2
Catedral*	13°56´	SBT ²	1
Supay*	13°57´	REF ⁴	2
Mendieta*	14°03´	SBT ²	1
Erizal*	14°06´	SBT ²	1
Sacasemita*	14°09´	SBT ²	1
El Ancla*	14°11´	DS ²	1
El Queso*	14°16´	SBT ²	1
Morro Quemado*	14°19´	SBT ²	1
El Negro*	14°23´	SBT ²	3
Punta Lomitas*	14°42´	SBT ²	1
Los Ingleses	15°03´	DS ²	1
San Fernando*	15°08´	SBT ²	3
San Nicolás*	15°15´	DS ⁶	1
San Juanito*	15°16´	REF ⁶	1
Pta. San Juan*	15°21´	REF ⁶	2
Tres Hermanas	15°26´	SBT ²	3
La Aguada	15°27´	DS ⁶	2
Yanyarina	15°27´	REF ⁶	2
El Cable	15°28´	REF ⁶	2
Playa la Libertad	15°29´	DS ⁶	5
El Submarino	15°30´	DS ⁶	2
El Cachucho	15°31´	DS ⁶	2
Playa de Chala	15°50´	DS ⁴	1

60

PERU SUR

Location	S. lat.	Record	Number
Punta Atico*	16°14´	DS ⁶	4
Punta Oscuyo	16°16´	REF ⁶	4
Punta Caleta	16°30´	REF ⁶	2
Punta El Uno	16°30´	REF ⁶	1
Pta. La Chira*	16°31´	DS ⁶	3
Quilca	16°42´	REF ⁶	1
Punta Arantas	16°48´	REF ⁶	1
Isla Hornillos*	16°52´	REF ⁶	1
Puerto Matarani	16°59´	DS ⁶	4
Matarani Faro	17°00´	DS ⁶	2
Catarindo	17°00´	DS ⁶	2
Mollendo	17°01´	REF ⁶	4
Pta. Corio	17°14´	DS ⁶	1
Pta. Jesús y Cocotea	17°16´	REF ⁶	1
Yerba Buena	17°19´	DS ⁶	3
Costanera	17°21´	REF ⁶	1
Punta El Carmen	17°23´	REF ⁶	4
Chuza	17°29´	REF ⁶	1
Ilo	17°38´	DS ⁶	1
Punta Coles*	17°42´	DS ⁶	1
Punta Picata	17°52´	DS ⁶	3
La Meca	17°56´	DS ⁷	3
Puerto Grau	17°59´	DS ⁷	8
Morro Sama	18°00´	DS ⁷	1
Quebrada de los Burros	18°01´	DS ²	5
Puerto Vila Vila	18°06´	DS ⁷	8

70

CHILE NORTE GRANDE

Location	S. lat.	Record	Number
Chanavaya	20°53´	SBT ⁸	4
Guanillos	21°13´	SBT ⁸	4
Tocopilla	22°05´	SBT ⁸	1
Caleta Fierro	22°38´	SBT ⁸	1
Punta Lobos	23°03´	SBT ⁸	1
Mejillones	23°28´	SBT ⁸	1
Punta Coloso	23°47´	SBT ⁸	3
Caleta El Cobre	24°20´	SBT ⁸	2

17

CHILE NORTE CHICO

Location	S. lat.	Record	Number
Pan de Azucar*	26°08´	SBT ⁸	3
Soldado	26°10´	SBT ⁸	11
C.Castillo	26°12´	SBT ⁸	4
C. Chanaral	26°16´	SBT ⁸	2
Punta Choros*	29°15´	SBT ⁸	4
Isla Dama	29°15´	SBT ⁸	5
Arrayan	29°43´	SBT ⁸	15
Panul	30°03´	SBT ⁸	10
Lagunillas	30°05´	SBT ⁸	1
Guanaqueros	30°11´	SBT ⁸	5
Tongoy	30°15´	SBT ⁸	1
Punta L. Vaca	30°17´	SBT ⁸	2
Talcaruca	30°28´	SBT ⁸	1
Limari	30°47´	SBT ⁸	1
C. Morrito	31°06´	SBT ⁸	1
C. Sierra	31°07´	SBT ⁸	1

67

CHILE CENTRO

Location	S. lat.	Record	Number
Norte L. Vilos	31°49′	SBT ⁸	1
Palo Colorado	32°01′	SBT ⁸	10
Puquen	32°11′	SBT ⁸	8
La Ballena	32°18′	SBT ⁸	1
Papudo	32°26′	SBT ⁸	16
El Pangue	32°31′	SBT ⁸	2
Isla Cachagua	32°35′	SBT ⁸	3
Quintero	32°46′	SBT ⁸	3
Quintay	33°11′	SBT ⁸	12

56

CHILE CENTRO-SUR

Location	S. lat.	Record	Number
Colcura	37°07′	SBT ⁸	7

7

CHILE SUR

Location	S. lat.	Record	Number
Mehuín	39°25′	SBT ⁸	3
Pilolcura	39°40′	SBT ⁸	2
Punihuil (Chiloe)*	41°55′	SBT ⁸	5
Ahuenco (Chiloe)	42°06′	SBT ⁸	1
Isla Guafo	43°36′	SBT ⁸	2

13

Table 1 references

- ¹ Sánchez and Ayala 2006
- ² Valqui et al. 2010
- ³ Apaza et al. 2003
- ⁴ Tovar and Rivera 2003
- ⁵ Sánchez 1992
- ⁶ Apaza et al. 2004
- ⁷ Mangel et al. 2010
- ⁸ Vianna et al. 2010

CHAPTER II

Genetic Variability and Population Structure of the Endangered South-American Marine Otter (*Lontra felina*) Based on Analysis of mtDNA Fragments of the Control Region and the Cytochrome b Gene

Juan Valqui^{1,2}

¹Zoological Institute, Christian-Albrechts-University, Olshausenstrasse 40, 24118 Kiel, Germany

²Centro de Ornitología y Biodiversidad (CORBIDI). Calle Santa Rita 105 Of. 202, Lima 33, Perú.

A partial data set of this Chapter was published in:

Conservation Genetics 11: 2067-2072 (2010). (See Annex II)

ABSTRACT

Between August 2008 and January 2011 three field expeditions covered the entire Peruvian distribution range of *Lontra felina* (1,500 km), visiting 143 locations to collect 157 fresh scats. Because of the particular problematic of working with non-invasive samples that contain low quality DNA, great caution was put in the laboratory work (sequencing in both directions and confirmation of results via double sequencing) and data analysis (pseudoreplication avoidance). Partial mtDNA control region (CR, 296 bp) and partial cytochrome b (cyt b, 690 bp) sequences were successfully amplified and analyzed in 29 CR and 21 cyt b samples. Based on spatial distribution and home range information of marine otters we assumed our final data set to represent at least 34 different individuals, yielding relatively high genetic variability (14 haplotypes, $h = 0.697$ and $\pi = 0.00612$ for CR; 6 haplotypes, $h = 0.848$ and $\pi = 0.00268$ for cyt b). Results on genetic substructuring within the Peruvian marine otter groups were not definitive, but suggest the existence of two subpopulations. Data from Chilean populations was combined to show a clear differentiation among populations of both countries. No bottleneck or isolation by distance could be

detected in Peru, suggesting at least moderate gene flow. This study is a consistent step to assess the genetic characteristics in this endangered species and highlights the significance of the Peruvian gene pool for the establishment of reserves, potential future expansion, recolonisation or translocations.

Keywords: *Lontra felina*, mitochondrial control region, cytochrome b, non-invasive genetic sampling, Peru

INTRODUCTION

The marine otter (*Lontra felina*) is the smallest marine mammal of the world and the only *Lontra* species confined solely to marine habitats. This monophyletic genus of otters comprises four species and is the sister taxon to all remaining otters (*Hydrictis*, *Enhydra*, *Lutra*, *Lutrogale* and *Aonyx*) except for the most basal species, the giant otter (*Pteronura brasiliensis*) (Koepli et al. 2008). The historical distribution of the marine otter has been reduced in the last decades due to anthropogenic factors such as habitat destruction, pollution and poaching, and in the southern part of their distribution range (Cape Horn and Southern Tierra del Fuego) marine otters are on the brink of extinction (Red List of Threatened Species Version 2011.1; Parera 1996; Apaza et al. 2004). It is likely that the population will decrease by at least 50% in the next 30 years (Red List of Threatened Species Version 2011.1). Therefore, the species is listed as Endangered by the IUCN, in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), in Appendix I of the Convention on Migratory Species (CMS), and it is protected by Peruvian law.

Marine otters occur along the Pacific coast of South America from 6° S (today more probably 8°S, Alfaro-Shigueto et al. 2011) to 56°S in Peru, Chile and Argentina, but because they depend upon habitats with rocky outcroppings and caves above the water at high tide, the distribution is disjunct (Larivière 1998; Red List of Threatened Species Version 2011.1). Chile holds the largest number of animals; studies on spatial distribution (Medina-Vogel et al. 2008) showed that strong predictors for marine otter presence are rocky seashore patches, and studies on spacing behaviour (Medina-Vogel et al. 2007) showed

home ranges of <5 km along coastlines and suggested availability of land refuges and food as decisive factors in the species' distribution.

Research on the status of *Lontra felina* in Peru has so far been limited few studies with divergent results: While earlier studies on population numbers reported numbers of about 200-300 (Castilla and Bahamondes 1979), a more recent survey yielded an estimate of c. 700 animals for the Peruvian coast (Apaza et al. 2004). In light of the bleak prospect for the coming decades, the species is in urgent need of help and knowledge on genetic variability and structure is of great relevance to conservation measures. The present study therefore aims at creating consistent genetic information, providing important molecular data for this endangered mustelid in the Peruvian part of the species' distribution range. We particularly want to quantify the amount of genetic diversity left in marine otters and also analyse its spatial distribution to find out if there are already clear signs of disrupted gene flow among the remnant populations along the coast.

While working with sample collection of an endangered species, several logistical and ethical issues arise. Due to the species characteristics and the difficult access in the field, trapping animals to obtain blood or tissue samples represent a considerable effort in cost and time, if at all achievable. Additionally, great care has to be put in the handling of animals: anesthesia could represent a mortality risk even in healthy animals (stress, drug overdose, drowning) (Arnemo et al. 2006). If the research group is working on environmental campaigns the negative image due to mortality cases may considerably work against the original purpose: cooperation with the communities to protect the species. Non-invasive sampling is in this sense a very good alternative, as samples can be taken without having contact to the animals and a relatively fair amount of samples can be taken, given the knowledge of the field conditions.

MATERIALS AND METHODS

Field work

Field work expeditions were performed at the Peruvian Pacific coast from Vesique (9°12'S, 78°29'W) to the port of Vila Vila (18°07'S, 70°43'W), covering more than 1500 km of coastline and the entire distribution range of marine otters in Peru (UICN-OSG 1998) (Figure 8) and touring over 10,000 km. In August 2008, at the first field expedition, a 4-person team travelled for 21 days along the Peruvian coast with a pick-up truck (Figure 9) and by foot, covering a touring route of 4,000 km (point-to-point distance: 1275 km), 47 locations were

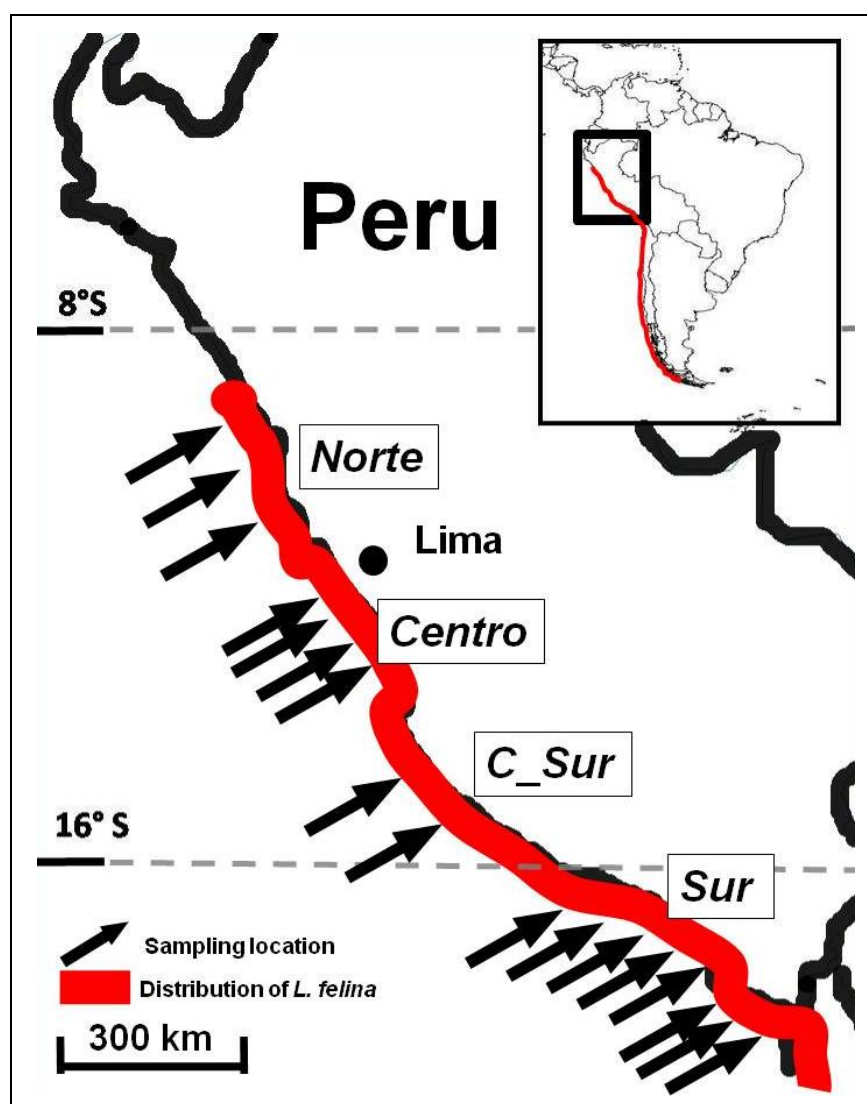


Figure 8. Geographic location of the sample sites of marine otter faeces along the Peruvian coast within four sampling groups.



Figure 9. 4x4 truck to access collection sites.

visited. A total of 92 samples (fresh and old) were found in 20 locations and 8 samples were collected from two individuals held in the Lima Zoo (Parque Zoológico de Huachipa). A second field expedition was performed under similar conditions in January and February 2010 for 25 days, touring a route of 3,800 km (point-to-point distance: 1,200 km). A total of 64 locations were visited and 95 scat samples were collected in 22 locations. A third field expedition of 15 days was realized in January 2011, touring a route of 2,255 km (point-to-point distance: 900 km) and visiting 32 locations. In 7 of them a total of 52 scat samples were collected (Table 2).

Table 2. Field work effort.

Date	Days	Total Km	Locations		Samples
			total	with scat	
Aug 2008	21	4,000	47	20	92
Dec 2009	25	3,800	64	22	95
Jan 2011	15	2,255	32	7	52
TOTAL	62	10,055	143	49	239

Typically, the terrain was rocky shore with dens and caves with difficult access (General Introduction: Figure 2 and Figure 3), where samples were primarily collected at latrines used for scent-marking. Beach resorts and fishing ports

were also surveyed (General Introduction: Figure 4), as samples were sometimes found in boats or at docks (Figure 10 and Figure 11). Non-invasive samples may generally contain low amounts of DNA (Bayes et al. 2000), so fresh scats were preferred as with the present laboratory techniques it has been shown that success rates of DNA amplification drop substantially after a few days or even hours (Murphy et al. 2007). In places where only older scats could be found, samples were taken for the case future techniques should allow DNA extraction from very low quality material. Samples were taken using surgical gloves (Figure 10), stored in 96% ethanol, sealed with Teflon tape and transferred to a refrigerator as quickly as possible. Based on geographic criteria, samples were assigned to 4 regions: *Norte*, *Centro*, *C_Sur* and *Sur* (Figure 8) which were separated respectively by 75 km, 98 km and 66 km of coastline in which either no marine otter habitat could be assumed or access was too difficult.



Figure 10. Sampling on a fishing boat.



Figure 11. Sampling on a dock.

Laboratory work

Preliminarily to this thesis, a test had shown that fresh samples from a closely related species (the American mink *Mustela vison*) could be used as material for DNA extraction, amplification with specific mtDNA primers, and genotyping. In the laboratory, only 157 fresh samples were used for the following procedures, as tests with some of the 82 older samples showed no positive results in neither of them. DNA was extracted using the QIAMP DNA stool mini kit. A blank extraction control was included to monitor potential contamination. A portion of the mitochondrial control region (CR) was amplified with the primer

DLH (5'-CCTGAAGTAAGAACCAGATG-3') and the primer ProL (5'-CACCAACACCCAAAGCT-3') at an annealing temperature of 57°C and 35 amplification cycles. A portion of the cytochrome b (cyt b) gene was amplified with the primers LfCYTB-1F (5'-ACCCACCCATTAGCCAAAAT-3') and LfCYTB-1R (5'-CCGACAGCTGGTATGAGGAT-3') (Vianna et al. 2010) at annealing temperature of 55°C and 39 amplifying cycles. We obtained successful extraction in 44 samples using CR primers and 52 samples using cyt b primers (see Table 3 for extraction and amplification success rates). Amplicons were then sequenced with an automated MegaBACE sequencer. Because we used faecal material, we used the following approach to validate our results: each sample was sequenced in both directions, and all haplotypes were confirmed by at least two independent PCR and sequencing runs, i. e. singletons were re-analysed, and in case of haplotypes occurring more than once, at least one sample was re-analysed. No differences were detected within any pair of runs for the same individual, which we interpreted as evidence of reliable sequences.

Data analysis

Sequences were evaluated using BioEdit (Hall 1999) often showing unequal lengths. For samples yielding sequences too short to be included in the analyses, amplification and/or sequencing was repeated; if the length was within the range of the successfully sequenced samples, the new sequences were included. To obtain an optimal data set within the available material, a maximum number of sequences (individuals) in combination with maximum polymorphic loci within the evaluated sequences were considered. For example if, a small subset of samples was missing a fragment in which several polymorphic loci were contained in another (larger) subset, the shorter subset was excluded, resulting in a reduced final set. Otherwise, if such a shorter subset contained too many samples and the polymorphic sites were not in considerably high number, a loss of polymorphic sites was taken to keep the shorter samples in the final set. Thus, 29 CR and 21 cyt b sequences with 296 bp and 690 bp in length resulted after these considerations. Sequences were aligned by eye in Bioedit and collapsed into haplotypes using the *collapser*

function implemented in the FaBox package available at <http://www.birc.au.dk/fabox/> (Villesen 2007).

Marine otters are mostly solitary, and radio-tracking of animals in Chile revealed that home ranges comprised no more than 4,134 m of coastline (Larivière 1998; Larivière and Jennings 2009; Medina-Vogel et al. 2007). Therefore, identical haplotypes were only treated as different individuals if the sample locations were at least 5 km apart (possible pseudoreplication). Nine of both mtDNA region sequences were therefore excluded from further analyses, thus calculations were based on a final data set comprising 20 CR sequences and 12 cytb sequences (Table 3).

Table 3. Success rate in sampling and laboratory methods.

	#	Success
Total collected samples	239	
Appropriated for extraction (fresh)	157	66%

Out of 157 fresh samples...	#	Success (step by step)	Success (accumulated)
available for amplification	44-52	28-33%	28-33%
available for sequencing	21-29	48-56%	15-18%
discarded sequences	12-20	57-69%	8-13%

After analyzing CR and cyt b sequences separately, analysis of concatenated sequences was performed. For a comparative analysis of Peruvian and Chilean samples, additional CR and cyt b sequences were downloaded from GeneBank (accession numbers GQ843765 – GQ843795, Vianna et al. 2010)

Haplotype and nucleotide diversity were calculated with DnaSP (Rozas and Rozas 1999). Differentiation among the four sample regions was tested for with Arlequin (Excoffier et al. 2005) by means of an analysis of molecular variance (AMOVA) and pairwise F_{ST} -values under the Tamura and Nei model of

nucleotide substitution (this model was closest to the GTR model inferred for our data set with Findmodel, which is not implemented in Arlequin <http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>). To test for a pattern of isolation by distance we applied a Mantel test on matrices of the number of pairwise nucleotide differences and geographical distances between individuals.

Haplotype relationships were depicted by a median-joining network with the Network software (Bandelt et al. 1999). Networks are more appropriate for intraspecific phylogenies than tree algorithms because they explicitly allow for the co-existence of ancestral and descendant alleles in a sample, whereas trees treat all sequences as terminal taxa (see Posada and Crandall 2001 for a review). Demographic history was inferred by a mismatch analysis and calculation of Fu's F_s (Fu 1997) with the Arlequin software. Mismatch analyses depict the frequency distribution of pairwise differences between the sequences found in a sample. This distribution is dependent upon demographic processes. Unimodal distributions are typical of populations that experienced a recent expansion, while ragged and multimodal distributions are found in populations at demographic equilibrium (Rogers and Harpending 1992) and bimodal distributions often result from admixture of two previously separate lineages. Bottlenecked populations typically produce L-shaped distributions with a single peak at low pairwise differences. Arlequin tests for a mismatch distribution fitting the sudden expansion expectations. Fu's F_s statistic is a neutrality test but can also be used to infer demographic histories with large and statistically significant positive values of F_s being indicative of a deficit of rare haplotypes compared to expectations for a stable population and thus being a sign of a bottleneck, while large and significant negative values suggest an excess of recent mutations (many rare haplotypes), which is typical of recent expansions.

For the microsatellite analysis amplification was performed with 12 primers: Lut435, Lut457, Lut604, Lut615, Lut701, Lut715, Lut717, Lut733, Lut782, Lut818, Lut832, Lut833. After failure with the recommended annealing temperatures (Dallas and Piertney 1998) and reagent concentrations the following possibilities were explored: four alternative temperatures ($\pm 10^\circ\text{C}$), 4 different DNA concentrations (1-5 μl) and PCR-reagents (Mg^{++}) concentrations

by regular PCR, multiplex PCR (Henegariu et al. 1997) and touchdown PCR (Korbie and Mattick 2008). A total of about 15,000 PCR reactions were performed and no result could be obtained through genotyping. Therefore, microsatellite analysis was discarded.

RESULTS

Analysis of CR and cyt b from Peruvian samples

Eight polymorphic sites for cyt b and 11 polymorphic sites for CR defined 6 haplotypes for cyt b and 14 haplotypes for CR (accession numbers GU982296-GU982306). Overall haplotype diversity was 0.69 (± 0.083 SD) for CR and 0.85 (± 0.074 SD) for cyt b and nucleotide diversity was 0.00612 (± 0.00131 SD) for CR and 0.00268 (± 0.00055 SD) for cyt b. Regarding geographic distribution, CR haplotype A.pe was universal, C.pe showed in Centro and Sur and I.pe in C_Sur and Sur. For cyt b only pe.B was found in three locations: *Norte*, *Centro* and *C_Sur*. All other haplotypes (11 for CR and 5 for cyt b) were characteristic (private) for each geographic location (Table 4 and Table 5).

Table 4. Distribution and frequencies of the 14 CR haplotypes found in the present study within four sampling groups.

GROUP	HAPLOTYPES													
	A.pe	B.pe	C.pe	D.pe	E.pe	F.pe	G.pe	H.pe	I.pe	J.pe	K.pe	L.pe	M.pe	N.pe
<i>Norte</i>	3	1												
<i>Centro</i>	1		3	1	1	1	1							
<i>C_Sur</i>	1							1	1					
<i>Sur</i>	4		2						2	3	1	1	1	1
TOTAL	9	1	5	1	1	1	1	1	3	3	1	1	1	1

Table 5. Distribution and frequencies of 6 cyt b-haplotypes found in the present study within four sampling groups.

GROUP	HAPLOTYPES					
	pe.A	pe.B	pe.C	pe.D	pe.E	pe.F
<i>Norte</i>	2	1				
<i>Centro</i>		1	1			
<i>C_Sur</i>		1		1		
<i>Sur</i>					2	3
TOTAL	2	3	1	1	2	3

Only the Group *Sur* showed significant differentiation (Balloux and Lugon-Moulin 2002) from the three other Peruvian groups in the *cyt b* gene ($F_{ST} = 0.355$, $p = 0.016$). None other of the overall pairwise F_{ST} -values were significantly different from zero (all $p > 0.12$). The CR analysis was not in concordance with this separation. We found no significant correlation of pairwise genetic and geographical distances between individuals neither for CR (Mantel test, $r = -0.013$, $p = 0.770$) nor for *cyt b* (Mantel test, $r = -0.055$, $p = 0.676$). Network analysis (Figure 12 and Figure 13) revealed one universal central haplotype for CR (A.pe), two haplotypes that appeared in more than one location (C.pe and I.pe) and 11 relatively distant satellite haplotypes of low frequency (see also Table 5). *cyt b* haplotype network showed one central and several satellite haplotypes.

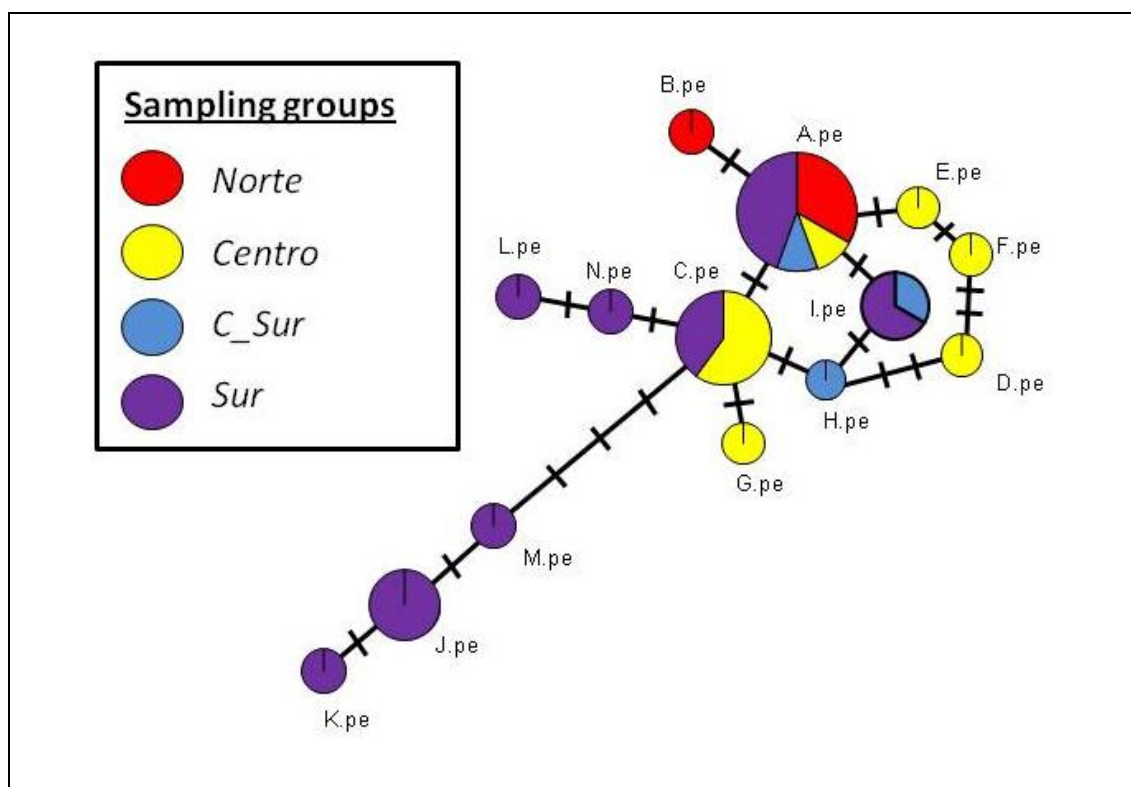


Figure 12. Median-joining network of the 14 marine otter mtDNA CR haplotypes found in this study. Circles are proportional to haplotype frequencies and show haplotype origin. Black dashes refer to mutational event.

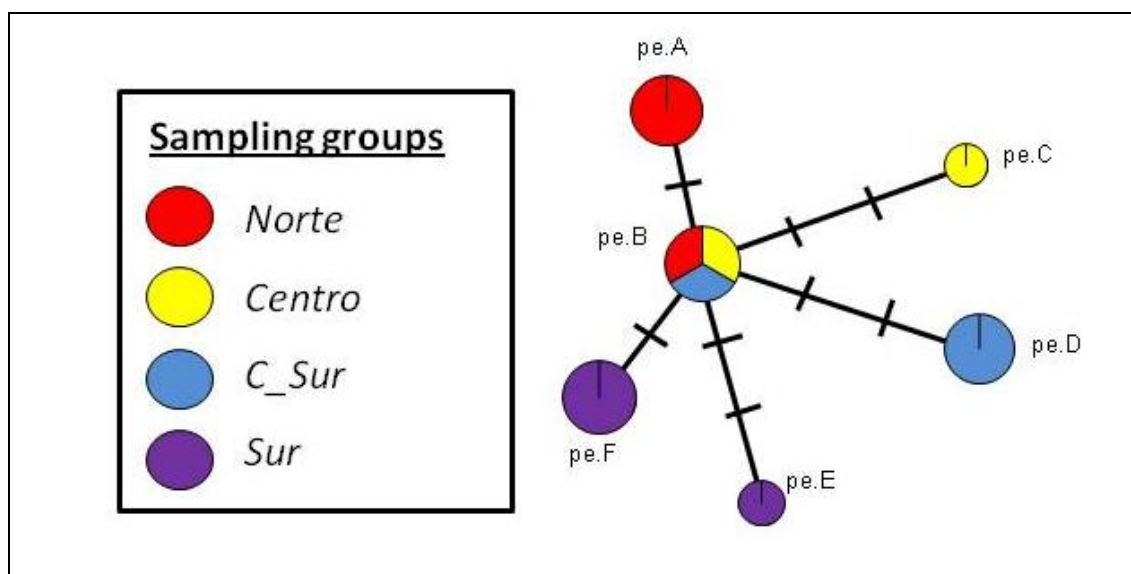


Figure 13. Median-joining network of the 6 marine otter mtDNA cyt b haplotypes found in this study. Circles are proportional to haplotype frequencies and show haplotype origin. Black dashes refer to mutational event.

In the mismatch analysis of CR, the sum of squared deviations (SSD) between observed and expected mismatch distribution showed significance (SSD = 0.013, $p = 0.01$) but the Harpending's raggedness index did not (0.034, $p = 0.99$). The Fu's F_s statistic was negative (-1.632) and not significant ($p > 0.01$). In the mismatch analysis of cyt b, neither SSD (0.010, $p = 0.52$) nor the Harpending's raggedness index (0.06, $p = 0.74$) showed significance. The Fu's F_s statistic was also negative (-1.540) and not significant ($p > 0.01$).

Analysis of concatenated CR and cyt b sequences

Analysis of the concatenated samples yielded consistent results with the separate calculated values although in this melted data sets information was lost due to the differences in the fragment each set was expressing. In this approach only 11 sequences (instead of 29 of CR and 21 of cyt b) with a total length of 992 bp could be used. 18 polymorphic sites defined 9 haplotypes.

Overall haplotype diversity was 0.86 (± 0.089 SD) and nucleotide diversity was 0.00306 (± 0.00105 SD). None of the calculated overall pairwise F_{ST} values were significantly different from zero (all $p > 0.37$). We found no significant correlation of pairwise genetic and geographical distances between individuals (Mantel test, $r = 0.054$, $p = 0.698$). In the mismatch analysis the sum of squared deviations (SSD) between observed and expected mismatch distribution







showed significance (SSD = 0.18, $p = 0.02$) but the Harpending's raggedness index did not (0.044, $p = 0.96$). The Fu's F_s statistic was negative (-0.519) and not significant ($p > 0.01$). Overall analysis of summarized sequences (CR, cyt b and concatenated sequences) showed that a relative high number of private haplotypes were present in all four sampling groups (Table 6).

The concatenated sequences from Peru were finally combined with similarly concatenated sequences from a study of Chilean samples (Vianna et al. 2010) resulting in 164 sequences with 992 bp of length and 17 haplotypes. Due to previous results and the loss of information, a reassignment of the originally assigned groups was performed in Peru: *Norte*, *Centro* and *C_Sur* were united to *PE_Nor3*; Peru *Sur* remained (*PE_Sur*). In Chile: *Central* and *Central South* were united to *CH_CS*. The data sets from these two different studies were concordant in 5 out of 15 polymorphic sites at the CR fragment and only for 2 loci at the cyt b fragment. Overall haplotype diversity and nucleotide diversity values were within the range of the separated data sets ($h = 0.86$, $\pi = 0.00334$). Demographic patterns did not differ from the data set from this study: mismatch analysis of the melted data set showed no significant difference from the sudden expansion model while Fu's F test was not significant ($p = 0.45$). Both Peruvian groups showed moderate differentiation ($F_{ST} = 0.17$), two Chilean groups *CH_Norch* and *CH_CS* showed moderate to high differentiation ($F_{ST} = 0.45$) while all other groups showed high differentiation ($F_{ST} = 0.59$ – 0.87) (Table 7).

Table 7. Pairwise F_{ST} values among all groups within Peru (*PE_*) and Chile (*CH_*). Groups are geographically listed from north to south.

	<i>PE_Nor3</i>	<i>PE_Sur</i>	<i>CH_NorG</i>	<i>CH_NorCh</i>	<i>CH_CS</i>	<i>CH_Sur</i>
<i>PE_Nor3</i>						
<i>PE_Sur</i>	0.17					
<i>CH_Norg</i>	0.75	0.70				
<i>CH_Norch</i>	0.67	0.62	0.59			
<i>CH_CS</i>	0.65	0.62	0.72	0.45		
<i>CH_Sur</i>	0.76	0.72	0.87	0.72	0.57	

Table 6. Distribution and frequencies of CR, cyt b and concatenated haplotypes found in the present study within four sampling groups. Haplotypes occurring in at least two location are represented by grey squares, private haplotypes by diagonally striped squares.

		mtDNA Haplotypes																private haplotypes																			
Location		Lat° S	N	CR	-	A	A	-	A	A	B	C	C	D	E	F	G	H	I	I	J	J	-	K	L	M	-	N	F								
				CytB	A	-	B	B	D	F	-	-	F	C	-	-		-	-	F	-	E	E	-	-	-	F	F									
<i>Norte</i>	Vesique-Samanco	9°13'	3(7)																																		
	La Gramita	9°44'	1																																		
	Punta Culebras	9°56'	1(2)																																		
	Huarmey	10°05'	1																																		
→340 km																																					
<i>Centro</i>	Pucusana	12°28'	3																																		
	Punta Corrientes	12°57'	3(4)																																		
	Cerro Azul	13°01'	2																																		
→365 km																																					
<i>C_Sur</i>	Tres Hermanas	15°26'	4																																		
→367 km																																					
<i>Sur</i>	Matarani	16°59'	1																																		
	Matarani Faro	17°00'	1																																		
	Catarindo	17°00'	1																																		
	Yerba Buena-LC	17°19'	3																																		
	Puerto Grau-M. Sama	17°59'	4(6)																																		
	Quebrada de Burros	18°01'	5(7)																																		
	Vila Vila	18°06'	3(4)																																		

The network analysis clearly depicted two groups within the complete data set: one Peruvian group and a Chilean group. Two haplotypes from the study of Vianna et al. (A.ch.A and B.ch.B) were correctly assigned by Network to the Peruvian group (Figure 14).

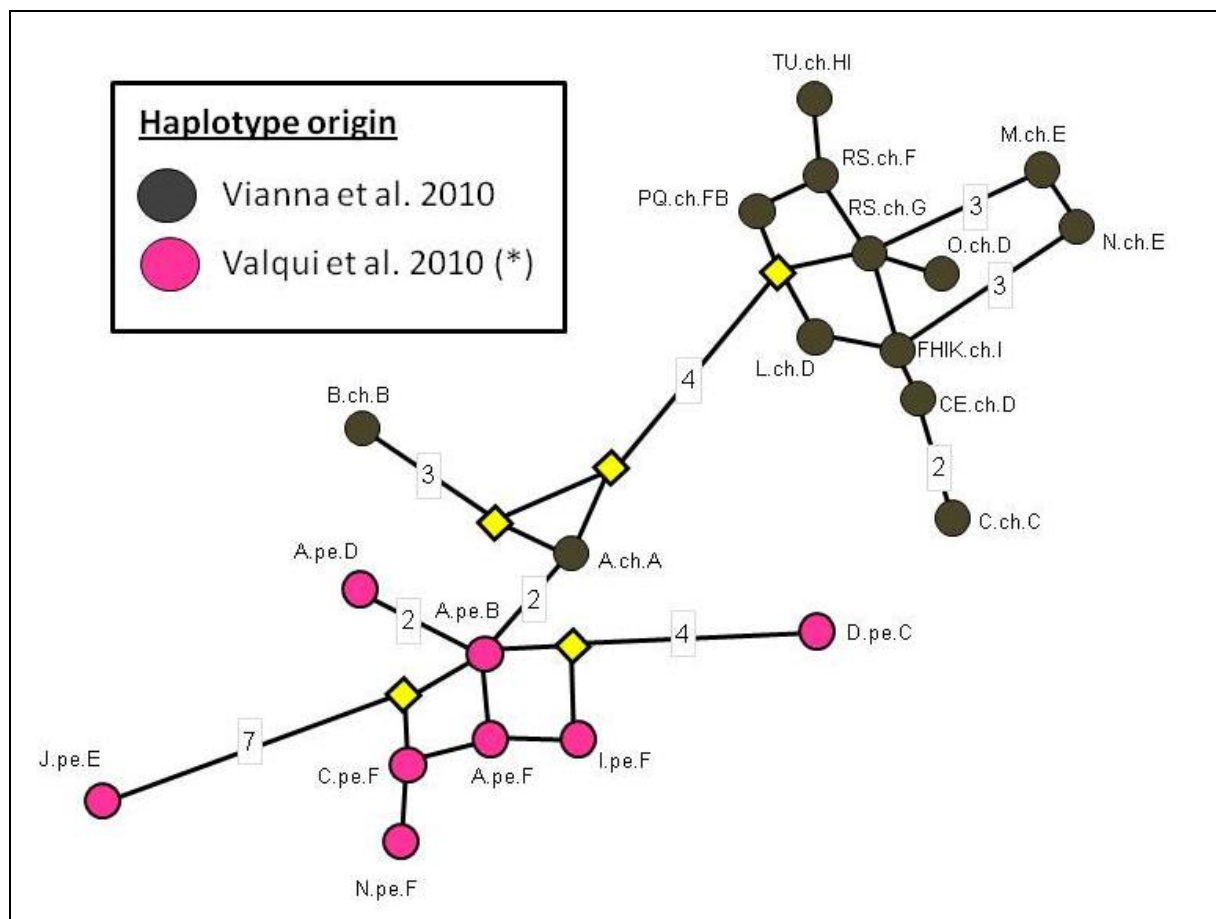


Figure 14. Median-joining network of the concatenated (CR and cyt b) marine otter mtDNA haplotypes from Vianna et al. (2010) and Valqui et al. (2010) (*) completed for this thesis. Haplotype frequencies are not shown. Yellow diamonds denote a median vector (hypothetical haplotype); mutational events are only shown if greater than one.

Scat samples from the only two individuals held in the Lima Zoo were collected. The origin of those individuals was unclear, as they were seized by the authorities from fishermen who had captured them and were holding them as pets. Within our analysis we could determine that one of the individuals shared a haplotype private for one determined location (Punta Corrientes). It can be assumed, that the individuals' origin is the named location or at least that it comes from the area. The other individual shared a universal haplotype; therefore its origin could not be determined.

DISCUSSION

Although widespread and endangered, relatively little is known about marine otters in spite of a recent conservation focus on this species (e. g. Medina-Vogel et al. 2007; Medina-Vogel et al. 2008). This study is a consistent first step to assess the genetic characteristics of the Peruvian population and the results -yet giving a preliminary picture of their population structure- set a baseline upon which further studies should deepen its work. Handling of samples during the field work phase was critical, as field logistics and bureaucratic coordination (especially regarding sample export) not always allowed securing fresh sample material. Therefore a high amount of samples had to be discarded due to lacking DNA quality. Future development of techniques might find a way to extract DNA even from this low quality material (Beja-Pereira et al. 2009) but a good solution for the present time is processing the samples in situ, as fast as possible. DNA quality degrades within the first days and even hours of collection (Murphy et al. 2007), so at least the first step in the laboratory process (extraction) should be performed, if not in the field, at least within the first days of collection. DNA quality in extracted templates would not suffer considerable loss, even if stored for months during export permit transaction. If possible, all steps until final genotyping should be achieved. This would on one hand avoid time-consuming and expensive transactions, on the other it would simplify the sample/data handling ('samples' could be sent or transported electronically).

The strict considerations in the laboratory work (confirmatory amplification, sequencing in both directions) and further data processing (e.g. pseudoreplication criterion) could have been relaxed to yield a higher amount of samples and longer sequences, but importance to the sample characteristics and data quality were given, and an overall conservative approach was chosen. Considering only the good quality samples, amplification success (48-56%) yielded a value similar to those from other scat-based analyses (amplification: 27% in Centrón et al. 2008 and 50% in Sharma et al. 2009). After applying the pseudoreplication criterion -which forced us to eliminate further samples- the resulting sample size and fragment length (296 bp for CR and 690 bp for cyt b) is similar to previous genetic analyses of other *Lontra* species in South America: Centrón et al. (2008) analyzed 13 CR sequences and 34 cyt b sequences in their study of *Lontra provocax*, with lengths of 516 bp for CR and

671 bp for cyt b. Trinca et al. (2007) analyzed 24 individuals of *Lontra longicaudis* and sequence length was 491 bp for CR.

It is assumed that the marine otter population has gone through a bottleneck due to intensive hunting in the last decades. Additionally, the total number of individuals has always been estimated to be fewer than 1000 individuals for Peru or for Chile. Knowledge on migration is limited, but large distances between inhabited rocky shore patches suggest certain resistance to gene flow. Therefore, the most important of our results is the unexpectedly high genetic variability found via mtDNA ($h = 0.69-0.85$; $\pi = 0.00027-0.0061$). The 20 CR samples of the present study yielded more haplotypes at a short fragment of the CR than did the entire European population of *Lutra lutra* at a longer one. Overall haplotype diversity and mean nucleotide diversity in Eurasian otters were reported as 0.16 and 0.0006, respectively (Ferrando et al. 2004 and references therein), but recent studies have revealed higher diversity for Eurasian otters in the UK (0.73 and 0.003, Stanton et al. 2008) and Ireland (haplotype diversity of 0.75, Finnegan and Néill 2011). Still, marine otter haplotype diversity and nucleotide diversity were considerably higher, the latter at least by an order of magnitude, although population sizes of *Lutra lutra* are doubtless much larger. This genetic depletion is even more pronounced than that of the sea otter (*Enhydra lutris*), which suffered a loss of an estimated 99% of its population due to persecution but still yielded mean haplotype diversities of 0.41 (Larson et al. 2002). In closer related species, *Lontra longicaudis*, Trinca et al. (2007) obtained similar haplotype diversity levels ($h = 0.82$) and Centrón et al. (2008) had zero variability (only a single mtDNA CR haplotype). In *Lontra felina*, Vianna et al. (2010) found similar levels of diversity ($h = 0.39-0.83$ and $\pi = 0.00069-0.00340$) to this study. As it seems, the marine otter is genetically the most diverse South-American *Lontra* species.

Divergent results via CR and cyt b suggest that the population structure in Peru is not very pronounced; only the group *Peru Sur* ($F_{ST} = 0.35$ for cyt b) or *PE_Sur* ($F_{ST} = 0.17$ for concatenated sequences) differentiates moderately from the other Peruvian groups, while only 66 km of marine otter free coast separate these groups. This suggests that either there is regular gene flow among the sample localities or that a recent disruption of the meta-population has not yet resulted in significant differentiation through genetic drift. Presence of private haplotypes (Table 3, Table 4 and Table 5) in all locations also suggests certain differentiation, but larger sampling

sizes may discover haplotypes appearing in low frequencies (Avice 2004) (and therefore not yet found by us). In Chile, differentiation among groups is more conspicuous

($F_{ST} = 0.45-0.87$), which is concordance to geographic separation between rocky shore patches (77 to 400 km). Strong divergence between Peruvian and Chilean groups, though, is unequivocal. F_{ST} values are the highest in comparisons between groups of the two countries (Table 7) and the network of the metaanalysis shows clear separation among those two clades (Figure 14). This is in concordance with the results from Vianna et al. (2010), who evaluated a longer sequence of three regions of mtDNA (CR, cyt b and ND5), including only 9 Peruvian samples. Vianna et al. suggested the separation of Peruvian and Chilean groups in two Evolutionary Significant Units (ESUs). Although our results support this hypothesis, we suggest putting the attention in international cooperation programs covering the complete distribution range of *L. felina*, rather than those matching political separations.

The CR haplotype network with its various rare and divergent haplotypes and the demographic tests (mismatch analysis and Fu's F_s) did not show obvious signs of a bottleneck but rather some weak indication of a population expansion. The star-shaped cyt b haplotype network does suggest an expansion after a bottleneck with one central haplotype and several satellites, although the demographic test values were slightly controversial (close to the significance threshold). Interestingly, the cyt b sequences from a study of Chilean samples also showed a star-shaped structure, while the CR from that study did not (Vianna et al. 2010). Due to lower levels of purifying selection, non coding DNA accumulates mutations faster than coding DNA (Lynch 2010). Therefore the coding fragment cyt b is more conservative and may be showing traces of a bottleneck, which are not detectable with CR.

The fact that we did not find an isolation-by-distance pattern has to be viewed with caution given our approach: because we excluded identical sequences within the same five km, we actually might have considered whole maternal lineages as a single individual, which may bias correlations between geographical proximity and genetic similarity. In any case, disjunct occurrence is not only the result of human-caused habitat fragmentation; the specific habitat requirements of marine otters result in a naturally disjunct distribution pattern, and adaptations to dispersal may facilitate gene flow despite human pressure and mitigate the negative effects of fragmentation (long-distance swimming has been observed by one of us, JV, in the field).

Analysis of nuclear DNA that may allow a higher resolution view on phylogenetic relationships was intended but not possible to perform. Faeces are a very sensitive material to work with, especially regarding storage time and extraction success. In fact, freshness is crucial (Murphy et al. 2007) and due to bureaucracy, at least two months of storage lied between collection and DNA extraction. This might have lead to considerable loss of nuclear DNA material. Centron et al. (2008) reported similar experiences, managing to extract mtDNA from faeces of the Southern River Otter (*L. provocax*), but no nuclear material. Thus, sampling in large scale dimension on nuclear markers should provide data to elucidate the substructuring of the Peruvian subpopulations. To our knowledge there have not yet been studies on nuclear markers (microsatellites) in this species but they should include our recommendation of extracting the DNA from scat, if possible, within few hours or days of collection

The bleak prospect of future population reductions of 50% or more within the next 30 years (Red List of Threatened Species Version 2011.1) will certainly lead to a substantial loss of gene pool diversity. Our results are a first step towards a genetic underpinning for future conservation which should include information on the partitioning and distribution of genetic diversity. They are also interesting with respect to newly established reserve areas. Some twenty islands and ten mainland sites along the Peruvian coast were managed for decades by the government as guano harvesting areas. Based on the law *Decreto Supremo N°024-2009-MINAM*, these areas were officially transformed into a system of reserves (*Reserva Nacional Islas y Puntas Guaneras*) in December 2010, comprising a total area of more than 100,000 ha to protect threatened populations of seabirds (Humboldt penguin, Peruvian diving petrel, inka tern, Peruvian booby), marine mammals (South American fur seal, South American sea lion, marine otter) and reptiles (sea turtles) (Valqui 2011). Although this measure will help maintain meta-populations of marine otters and facilitate gene flow, areas with high levels of genetic diversity along the southern Peruvian coast are not included. The area around Morro Sama and Quebrada de los Burros (17°59'S to 18°02'S) (Ulloa et al. 2010, Valqui 2011) is a strong candidate for establishing a protected area based on information presented in this study, especially if the hypothesis of a Peruvian southern subpopulation is confirmed. The conservation programs could also benefit from the information of this and further studies to select appropriate individuals should translocations and re-stocking of abandoned areas become necessary. Further genetic studies based on larger sample sizes, nuclear

loci and, above all, covering also the Chilean and Argentinean part of the distribution range are what is now most urgently needed.

ACKNOWLEDGEMENTS

Execution of field work of the “Proyecto *Lontra felina*” was authorized by permits N°98-2008-INRENA-IFFS-DCB, N°454-2009-AG-DGFFS-DGEFFS and N°0149-2011-AG-DGFFS-DGEFFS given by the Peruvian Natural Resources Agency from the Ministry of Agriculture of the Republic of Peru and permit N°19-2010-SERNANP-RNP, given by the Peruvian Natural Protected Areas Agency. Guano administration areas were accessed with authorization N° 037-2009-AG-AGRORURAL-DO given by the Peruvian guano governmental agency AGRORURAL. Sample export was authorized by permits N° 11378, N° 000582 and N° 1373 given by the Peruvian CITES authority and by Council Regulation (EC) No. 338/97 given by the German CITES authority. Natalia Ortiz, Daniella Biffi and Jerico Solis assisted in the field. Yennifer Hernández assisted in the laboratory work. IDEAWILD provided logistic material through Elisa Ruiz. Financial support from The Society for Marine Mammology (SMM), the Rufford Small Grant and Yaqu Pacha is gratefully acknowledged.

CHAPTER III
Population Viability Analysis of the Endangered Marine Otter
(*Lontra felina*) Population Along the Pacific Coast of South
America

Juan Valqui^{1,2}

¹Zoological Institute, Christian-Albrechts-University, Olshausenstraße 40, 24118 Kiel, Germany

²CORBIDI. Calle Santa Rita 105 Of. 202, Lima 33, Peru.

ABSTRACT

The marine otter *Lontra felina* is the smallest marine mammal of the world and lives at the coast of Peru and Chile. Although this mustelid is still relatively unknown, it has potential as flagship species for environmental education campaigns and possesses an umbrella species character in its habitat, giving it a special status in conservation issues. Its populations is endangered due to anthropogenic factors (hunting until the end of the 20th century and habitat reduction in present time) thus, information of the species' biology is relevant for conservation management plans. This Population Viability Analysis (PVA) with available demographic and life-history data is performed to determine the impact of different environmental and anthropogenic factors on the species. The sensitivity analysis of this PVA revealed that habitat reduction had the most considerable impact on all populations, under all possible scenarios. Smaller and more isolated populations were additionally sensitive to inbreeding, catastrophes and diseases. This highlights the urge to stop the loss of the marine otter's habitat (via a heightened level of planning in urbanization and the control of invasive domestic species in rocky shore patches with marine otter presence) and take compensating measures (through a network of artificial structures or 'stepping stones'). Future studies should aim at a better understanding of the biology of the species and its habitat.

Key words: marine otter, *Lontra felina*, population viability analysis.

INTRODUCTION

Habitat reduction, catastrophes and diseases are common environmental factors affecting all wildlife populations. Small endangered populations are additionally affected by inbreeding while the environmental stochasticity and genetic drift present an increased risk of extinction (Frankham et al. 2007). The marine otter is a relatively unknown species inhabiting the southern coast of South America. Recent population estimates vary from 1,000 (Sielfeld and Castilla 1999) for the total distribution area, to about 700-2,000 only for Peru (Valqui 2011). The impact of the poorly documented but indeed significant pelt hunting activities across the 20th century is unclear -it is known that 38,000 otter pelts from *L. felina* and *L. provocax* were exported between 1910 and 1954 (Iriarte and Jaksic 1986) in Chile. These numbers could suggest a population bottleneck. The level of inbreeding in *L. felina* is not studied, but certain levels of inbreeding related to a bottleneck could mean a reduction in the marine otters' fitness as it has been observed in other small populations (e.g. red deer *Cervus elaphus* (Zachos et al. 2007)). A present threat is related to the forecasts of human demography in Peru and Chile (INE 2002; INEI 2007), which suggest an increased anthropogenic pressure at the marine otters' habitat in the short, medium and long term.

Population Viability Analysis (PVA) has been used to help assess population status as well as define the threats a certain population faces (Frankham et al. 2007). The simulation results can assist in the conservation planning, as it has been done with many species of mammals (Brook et al. 2000; Hosack et al. 2002; Wisdom and Mills 1997; Zachos et al. 2009). The VORTEX software, which simulates population development over years, allows understanding the effects of deterministic forces (e.g. birth and mortality rates) in combination with stochastic demographic, environmental and genetic events (Lacy et al. 2005). VORTEX first calculates the deterministic growth rate (det-GR) which is kept constant to simulate the dynamics of a certain population if stochastic effects are minimal. If det-GR is negative, the population will go extinct even without the influence of any other factors. Only if det-GR is positive, the influence of the stochastic factors might be recognized as extinction factors for that population

(Lacy et al. 2005) A varying stochastic growth rate (here merely GR) is then defined year-to-year based on the variation of stochastic factors.

This PVA has the aim of testing the factors impacting on the marine otter population in Peru and Chile. The results of this PVA may point out to the conservation actions taken in both countries, aimed at reducing the threats to the marine otter and securing the survival of the species. The flagship and umbrella species character of *L. felina* make this kind of analysis of particular interest as management of the marine otter will include and may favour several other species living at the coastal habitat of the south western Pacific.

METHODS

Within the VORTEX simulation, a sensitivity testing (ST) was performed to find the most important parameters in the simulation, to determine the critical threshold those parameters may conceal, and find possible synergic effects among them. The parameters to which special attention was given were inbreeding and catastrophes. Inbreeding is known to increasingly occur in species that have gone through population bottlenecks (Hartl and Pucek 1994); although no signs of inbreeding have yet been detected by the first genetic studies (Valqui et al. 2010; Vianna et al. 2010) the small isolated populations suggested by Vianna et al. (2010) and the increasing isolating pressure of anthropogenic action (Medina-Vogel et al. 2008) may cause a loss of genetic variability, affecting the viability of the species in the future. Catastrophes might originate out of environmental events such as El Niño Southern Oscillation (ENSO), a global warming of oceanic waters along the Peruvian and central and northern Chilean coast, which has severe mortality effects on various marine communities (Apaza and Figari 1999; Wang and Fiedler 2006). The dimension of the effects of ENSO events on marine otters are yet unknown (Valqui 2011; Vianna et al. 2010). The second catastrophe is diseases, which represent a high risk for smaller isolated populations (Hajji et al. 2007); iii) carrying capacity; the marine otter is dependent on the availability of caves and dens in the rocky shore (Valqui 2011), at the present a limiting factor. A loss in these available habitats can be simulated by a reduction of the carrying capacity in the simulation.

The demographic parameters were chosen after a very thorough revision of all of the studies done on the species over the last decades (see Larivière 1998; Valqui 2011). Missing information was completed with data on the closely related and the better known Eurasian otter *Lutra lutra* (Koelewijn et al. 2010). The population numbers were calculated indirectly via the available marine otter habitat. The latter was calculated based on the amount of rocky shore patches within the occurrence range of *L. felina*; field work notations were combined with an exhaustive analysis of maps using Google Earth (Google Earth Software 2009). Assuming a conservative density of 1.5 individuals per km (deduced from density parameters of 12 studies, see Valqui 2011), a metapopulation of 4473 individuals was defined. According to the results of the studies by Vianna et al. (2010) who defined 5 subpopulations for Chile, and Valqui et al. (2010), who defined 2 subpopulations for Peru, a 7 subpopulation scenario was selected. The populations from north to south (with initial population size given) were: *Peru NC* (1,229); *Peru S* (620); *Chile N* (600); *Chile NC* (879); *Chile C* (240); *Chile CS* (111) and *Chile S* (795). The human population is increasing the pressure on the coastal habitat, modifying the habitat and displacing the marine otters from their natural habitat via introduced species (dogs, cats, rats) (Valqui 2011). To show the effects of habitat loss on *L. felina* population (expressed as a reduction of K), low (-1%), moderate (-2%) and high (-5%) annual decrease rates were tested. K was defined based on the average density (1,5 individuals per km) multiplied by two, multiplied by the available marine otter habitat.

To test the effect of inbreeding a lethal:sub lethal ratio of 1:1 was assumed (Simmons and Crow 1977; Brook et al. 2000), that means 50% of the lethal equivalents (LE) were subject to the purging effect (lethal) and 50% were not affected by it (sub lethal). Three different levels of inbreeding depression were tested: none, moderate (3.14 LE and 12.3 LE) and high (30 LE). LE values were deducted from literature: the moderate value (3.14) was an average value of a summary of 40 studies by Ralls et al. (1983) while O'Grady et al. (2006) determined an average value of 12.3 LE based on 30 studies on mammals and birds.

As information on migration among populations is not documented for this species and also poorly documented for other otter species, a moderate dispersal scenario was chosen. Long distance swimming and relatively high genetic variability values suggest certain migration levels even among distant populations (Valqui et al. 2010). Dispersal rates were defined proportionally to the separation between two subpopulations. Dispersal started at age one, according to observations of pups staying with their mothers for about 10 to 12 months (Cabello 1983; Parera 1996; Larivière 1998; Valqui 2004).

A polygynous mating system (Ostfeldt et al. 1989) was assumed, with a sex ratio of 1:1. The age of first reproduction was 2 years for females and 4 years for males (Koelewijn et al. 2010). Both females and males reproduced until 10 years old (their maximum age), assuming that individuals can breed throughout their adult lifespan (Miller and Lacy 2005). Maximum number of progeny was 4, with 70% of the years containing one brood and 30% containing two broods (Valqui 2011). A density dependent reproduction was assumed, with 90% of reproduction occurring at low densities (when reproduction partners are harder to find) and 50% of reproduction occurring at carrying capacity (when the limiting factor are the available breeding caves). All scenarios were run with and without the density dependence feature. A stable age distribution was assigned and the proportion of breeding males was chosen as 34% (based on Koelewijn et al. 2010).

Annual survival rates were assumed as 90%, for all age classes except individuals 10 years or older, who had a survival rate of 60%. The first catastrophe simulated was an ENSO event, an environmental factor that affects the western coast of South America in cycles of two to seven years (Wang and Fiedler 2006). Assuming a stronger event occurs only every 10 years, a probability of 10% was given. Based on the considerable effects this has on other marine mammal and bird populations, a severity level of 75% survival rate was given. The more southern populations were given a higher survival rate (90%) as they do not suffer the effects from ENSO as strong as the more northern populations (Wang and Fiedler 2006). Diseases are a common cause of mortality in carnivore populations (Young 1994), so the second catastrophe simulated was diseases. A survival rate of 50% was determined, which is within

the range of survival rates of other species (e.g. harbour seals *Phoca vitulina* (Borkenhagen 1993) and lions *Panthera leo* (Packer et al. 1999)). Three catastrophe scenarios were run: one without catastrophes, two with only one of the catastrophes occurring and a last one with both catastrophes occurring simultaneously. All simulations were run for 100 years with 1,000 iterations.

Table 8. Input summary for the PVA simulation.

Demography		
Population	Initial N	K
<i>Peru NC</i>	1229	2703
<i>Peru S</i>	620	1362
<i>Chile NG</i>	600	1320
<i>Chile NC</i>	879	1934
<i>Chile C</i>	240	528
<i>Chile CS</i>	111	244
<i>Chile S</i>	795	1749
Metapopulation	4473	9841

Reproduction	
Age of first offspring for females	2
Age of first offspring for males	4
Maximum age of reproduction	10
One broods per year	70%
Two broods per year	30%
Maximum nr. of progeny per brood	4
Sex ratio at birth	1:1
% males in breeding pool	34%
% males successfully siring offspring	22.9%

Mortality rates			
Age class	Males /SD	Age class	Females / SD
0-1	10/1	0-1	10/1
1-2	10/1	1-2	10/1
2-3	10/1	After age 2	40/4
3-4	10/1	-	-
After age 4	40/4	-	-

Change in K (Sensitivity testing)				
Level	None	Low	Medium	High
Decrease	0%	-1%	-2%	-5%

Inbreeding (Sensitivity testing)			
Levels	None	Medium	High
Lethal equivalents LE	0	3.14 / 12.3	30
due to recessive lethals	50%	50%	50%

Catastrophes (Sensitivity testing)		
Parameter	ENSO	Diseases
Dimension	Global	Local
Frequency	10%	5%
Severity on reproduction	75%	50%
Severity on survival	75%	50%

RESULTS

Deterministic scenarios

All simulations in a deterministic scenario showed a positive growth rate (det-GR = 0.048), with populations growing until carrying capacity, where a stable number of individuals was maintained in time (Figure 15).

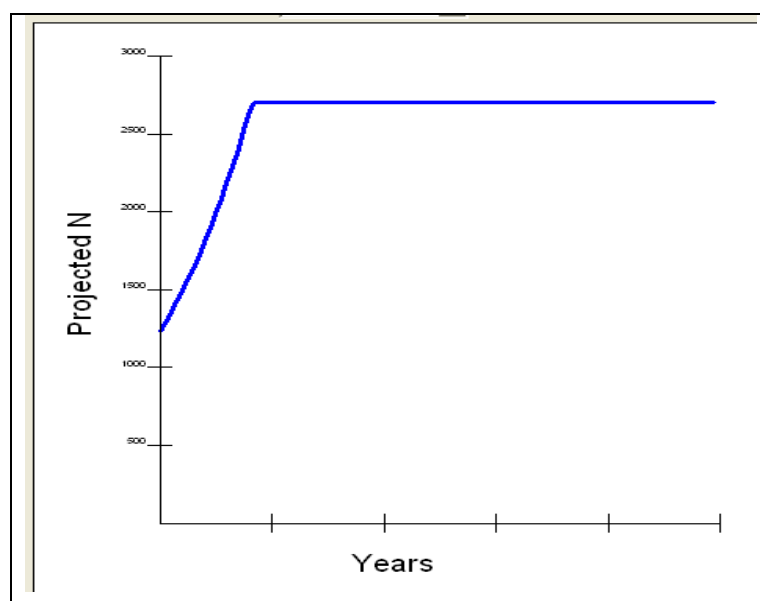


Figure 15. Population numbers in a deterministic scenario with growth rate det-GR = 0.0048

Inbreeding sensitivity

In a scenario without decreasing K and no catastrophes occurring, a simulation run of 100 years yielded the following results: the mean number of surviving individuals in the metapopulation (containing all 7 subpopulations) with zero LE was 7,735; 4,359 individuals survived with 3.14 LE; 4,359 with 12.3 LE and 4,350 with 30 LE. The growth rate (GR) showed a similar pattern in the alternatives scenarios: an overall rate of 0.0029 for no inbreeding, moderate inbreeding (3.14 LE and 12.3 LE) both within half of the high inbreeding rates (0.0017 and 0.0018), but for the first time within the alternatives a negative rate (-0.0004) appears for populations with high inbreeding (30 LE). A clear pattern of decrease was also observed for the final expected heterozygosity (H_E) (Table 9, Figure 16).

Table 9. Mean number of surviving individuals (N), growth rate (GR) and expected heterozygosity (H_E) in a metapopulation at different inbreeding scenarios.

	Inbreeding level			
	0 LE	3.14 LE	12.3 LE	30 LE
N	7,735	4,359	4,359	4,350
GR	0.0029	0.0017	0.0018	-0.0004
H_E	0.995	0.851	0.843	0.837

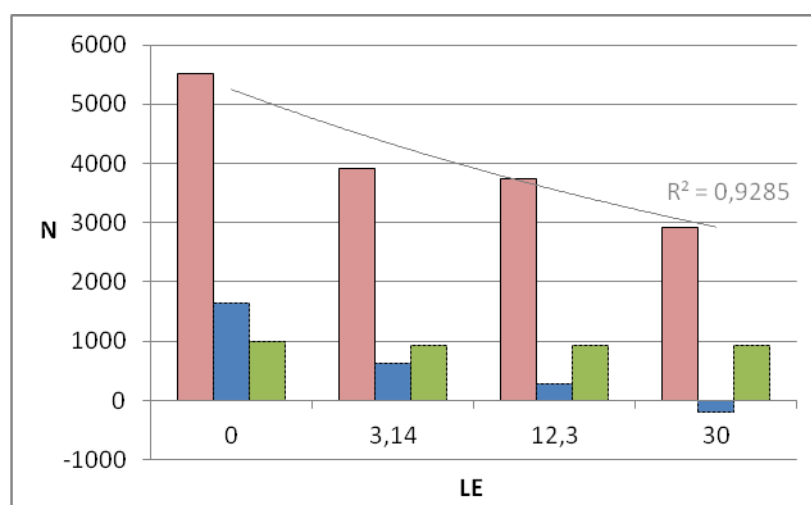


Figure 16. Mean N, GR and H_E for different inbreeding scenarios

(data from Table 9).

Catastrophe sensitivity

In a scenario without decreasing K and no inbreeding, after 100 years the mean numbers of surviving individuals of the metapopulations were: 7,729 with absence of catastrophes; 6,839 with only the ENSO effect occurring; 6,372 with diseases occurring (both scenarios presenting equal growth rates) and 5,544 with both catastrophes combined. The growth rate (GR) showed a similar pattern in the alternatives scenarios: a higher rate (0.027) for no catastrophes, both of the catastrophes each with an equal rate (0.021) and a lower rate when both catastrophes occurred simultaneously (0.014). The final expected heterozygosity (H_E) followed a similar pattern (Table 10, Figure 17).

Table 10. Mean number of surviving individuals (N), growth rate (GR) and expected heterozygosity (H_E) in a metapopulation at different catastrophe scenarios.

	Catastrophes			
	No event	ENSO	Disease	ENSO + Disease
N	7,729	6,839	6,372	5,544
GR	0.027	0.021	0.021	0.014
H_E	0.995	0.994	0.992	0.991

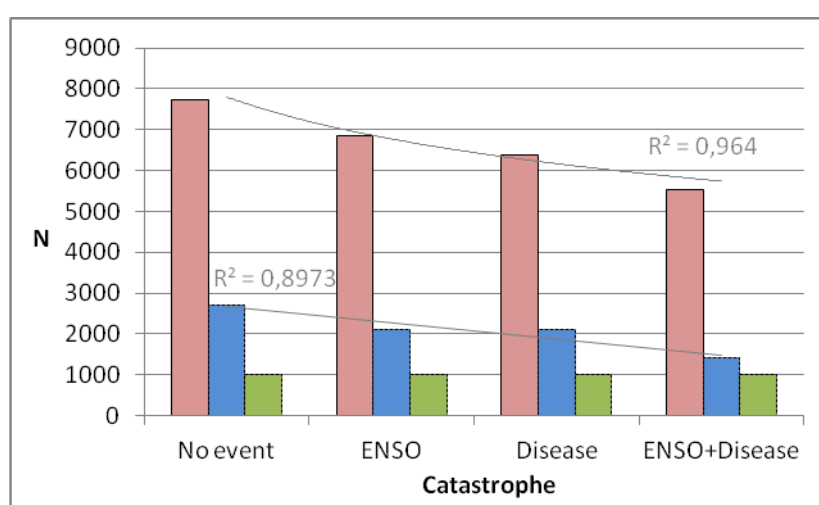


Figure 17. Mean N, GR and H_E for different catastrophe scenarios

(data from Table 10).

A focus in the analysis of smaller populations showed that only *Chile CS*, with 111 initial individuals went extinct immediately after 10 years, under every possible catastrophe scenario. All other populations remained stable, regardless of catastrophes occurring or not.

Sensitivity to a change in K

First, to test the effects of changing the level of decrease in K, a scenario with no inbreeding and no catastrophes was simulated. With constant K, population grew above 7,000 individuals and maintained its size along the 100 years of simulation. 1% decrease in K meant almost a reduction to half the original size after 50 years, ending in extinction after 100 years. 2% decrease in K, meant extinction after 60 years and in the scenario with 5% decrease in K, the metapopulation went extinct after only 40 years (Figure 18).

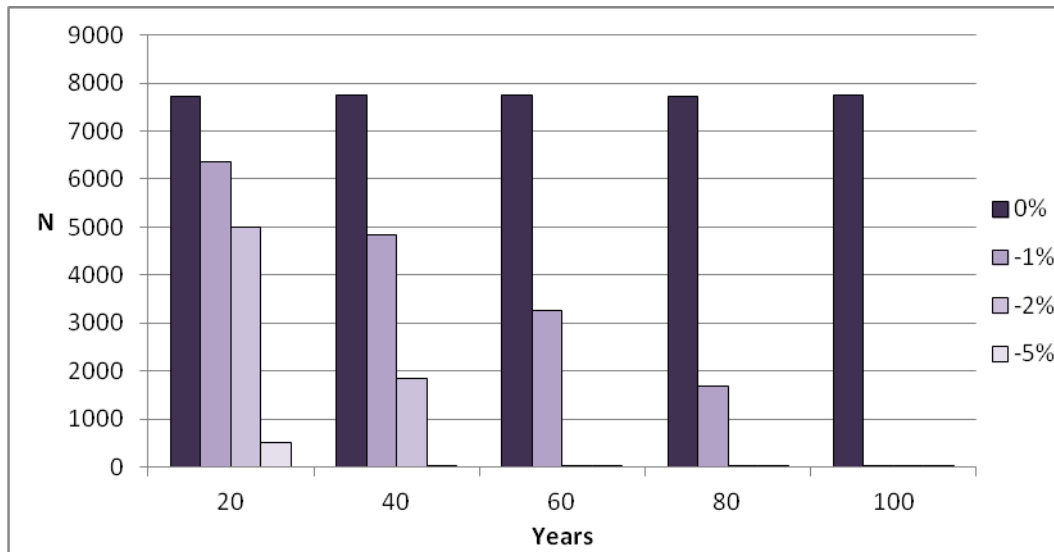


Figure 18. Mean N for different decrease levels in K, in a scenario with no inbreeding and no catastrophes.

Later, a scenario without inbreeding and all alternatives of two possible catastrophes were analyzed to see the effect of a change in K in combination with environmental events. All possible catastrophe scenarios showed similar patterns regarding the sensitivity to K and are shown in Table 11, which is a picture after 40 years. Populations with constant K could afford the effect of both events occurring simultaneously, but 1% decrease in K, caused the population to half its size when under the influence of both events. A level of decrease of 2% in K resulted in considerable reduction in population size and showed, for the first time in these scenarios, a negative growth rate for all possible combinations (underlined values of Table 11). A 5% decrease in K meant extinction regardless the influence of events.

Table 11. Effect of different levels in decrease of K (columns, 0%, -1%, -2% and -5%) and type of catastrophes (rows) on number of surviving individuals (N), growth rate (GR) and expected heterozygosity (H_E) for the marine otter metapopulation, after 40 years and an initial population size of 4,473 individuals. Each run was performed with 1,000 iterations.

		Level of change in K			
		0%	-1%	-2%	-5%.
no event	N	7,740	4,829	1,851	0
	GR	0.0273	0.0193	<u>-0.0267</u>	0
	H_E	0.998	0.997	0.996	0
ENSO	N	6,883	4,653	1,712	0
	GR	0.0235	0.0116	<u>-0.0338</u>	0
	H_E	0.997	0.997	0.995	0
Disease	N	6,639	4,522	1,681	0
	GR	0.0276	0.0126	<u>-0.0339</u>	0
	H_E	0.997	0.997	0.995	0
ENSO + Disease	N	5,471	3,875	1,499	0
	GR	0.0174	0.0080	<u>-0.0421</u>	0
	H_E	0.996	0.996	0.994	0

After 100 years, all possible scenarios containing at least 1% decrease in K resulted in extinction, even those with positive growth rates and initial increasing number of individuals (Table 12).

Table 12. Survival after 100 years under different levels of decrease in K.

		Level of change in K			
		0%	-1%	-2%	-5%.
no event	N	7,734	0	0	0
	GR	0.0304	0	0	0
ENSO	N	6,837	0	0	0
	GR	0.0214	0.0122	0	0
Disease	N	6,657	0	0	0
	GR	0.0216	0.0285	0	0
ENSO + Disease	N	5,518	0	0	0
	GR	0.0187	0.0286	0	0

DISCUSSION

Even though several facts about the marine otter are yet under discussion (population numbers and carrying capacity are still gross estimates), the strength of a PVA sensitivity analysis lies in the analysis of variation of one or more certain parameters in a population, regardless of its inaccuracy (Miller and Lacy 2005; Zachos et al. 2009). It is in this sense that the results of this study may result relevant for conservation management of the *L. felina* population.

Spielman et al. (2004) found a direct relation among inbreeding and extinction in 170 threatened taxa, while Bailey et al. (2007) and Frankham (2005) discussed if that relation could be conclusive, asserting that it could be that species usually already are on their way to extinction before genetic factors impact them. For the marine otter population simulation, inbreeding has shown an overall moderate effect on the mortality of the metapopulation and the bigger subpopulations. Small populations are indeed more sensitive to this factor, as the extinction of *Chile* CS (with 111 initial individuals) after 10 years and *Chile* S (with 240 initial individuals) after 20 years show under scenarios of moderate to high inbreeding. Management of endangered population implies the maintenance of 90% of their genetic diversity throughout 100 years (Soulé 1980; Frankham et al. 2007). It is then possible to say that some marine otter subpopulations could be at a 'point of no return', as formulated by Bailey et al. (2007) for the Mexican Goodeid fish *Zoogoneticus tequila* which presents strong signs of inbreeding. In our simulations, no inbreeding (0 LE) resulted in all populations –including the smaller ones– to survive the 100 years, but as soon as inbreeding was included, some populations start to go extinct (3.14 LE, 12.3 LE and 30 LE) (Figure 19). In fact, when a scenario of high inbreeding was simulated, negative growth rates first started to appear (GR = -0.004 for 30 LE) (Table 9).

The effect of catastrophes, either the more frequent but less intense ENSO event or a less frequent but more severe disease event resulted in an overall population reduction of about 10-20% in 100 years. These two catastrophes occurring together resulted in a reduction of about 30% in the same time frame. Notoriously, the growth rate lowered moderately with either of the catastrophes occurring alone, but halved with both of the catastrophes in simultaneous

occurrence (Table 10). Interestingly, Young (1994) suggested that the estimation of mortality rates due to diseases may be too optimistic, as mortality rates in the range of 70-90% showed in his comprehensive review of 96 die-offs in mammal populations.

Interestingly, inbreeding, catastrophes and other factors as density dependence or mating system (data not shown) were of secondary importance compared to the effect of a change in K . Any scenario of decreasing K showed a considerable impact to the population numbers and probability of extinction, regardless any other possible combination of scenarios. As shown by this PVA, this was especially the case for the two smaller marine otter subpopulations, which were always the first to go extinct under any increase of pressure from the different alternatives (inbreeding, catastrophes and decrease of K). This result is of particular importance as in fact, increasing human population (INE 2002; INEI 2007) will affect the marine otter and its habitat through urbanization, pollution and introduction of invasive species (Red List of Threatened Species Version 2011.1; Valqui 2011). Additionally, the discussion on global warming suggests that ENSO events may be stronger in the future (Fedorov and Philander 2000; Zhang et al. 2008). For sure, the levels of fragmentation due to the natural distribution of rocky shore patches (Valqui 2011) will be enhanced one or the other way by human action, thus generating more isolated and smaller marine otter populations (Medina-Vogel et al. 2008). A worse-case scenario could be inducted, generating a spiral of extinction (Miller and Lacy 2005). To counteract this, a combination of actions should be taken to secure the survival of the species. Research priorities should be set to gain a better understanding of the biology of the species (migrating behaviour, reproduction, mortality rates, among others) and the assessment of its habitat and demographic parameters (population numbers, effective population size and densities). Further, additional genetic analysis with intensive sampling should refine the picture on phylogeographic characteristics and population substructuring. Management policies should aim at stopping the habitat loss, through the controlling of human activities (e.g. sustainable urbanization plans and feral animal management) especially in marine otter population hot spots, like Morro Sama in Southern Peru (Ulloa et al. 2010, Valqui 2011). If the

threshold, in which isolation of populations is avoiding gene flow is trespassed, stepping stone model via artificial structures (shipwrecks, docks, among others) could be planned to allow exchange of individuals among subpopulations (Mangel et al. 2010). If genetic studies further show that inbreeding has reached levels in which the existence of a certain subpopulation is in danger, translocation and/or re-colonization events (artificial gene flow) should be considered in those management plans at realistic and affordable levels.

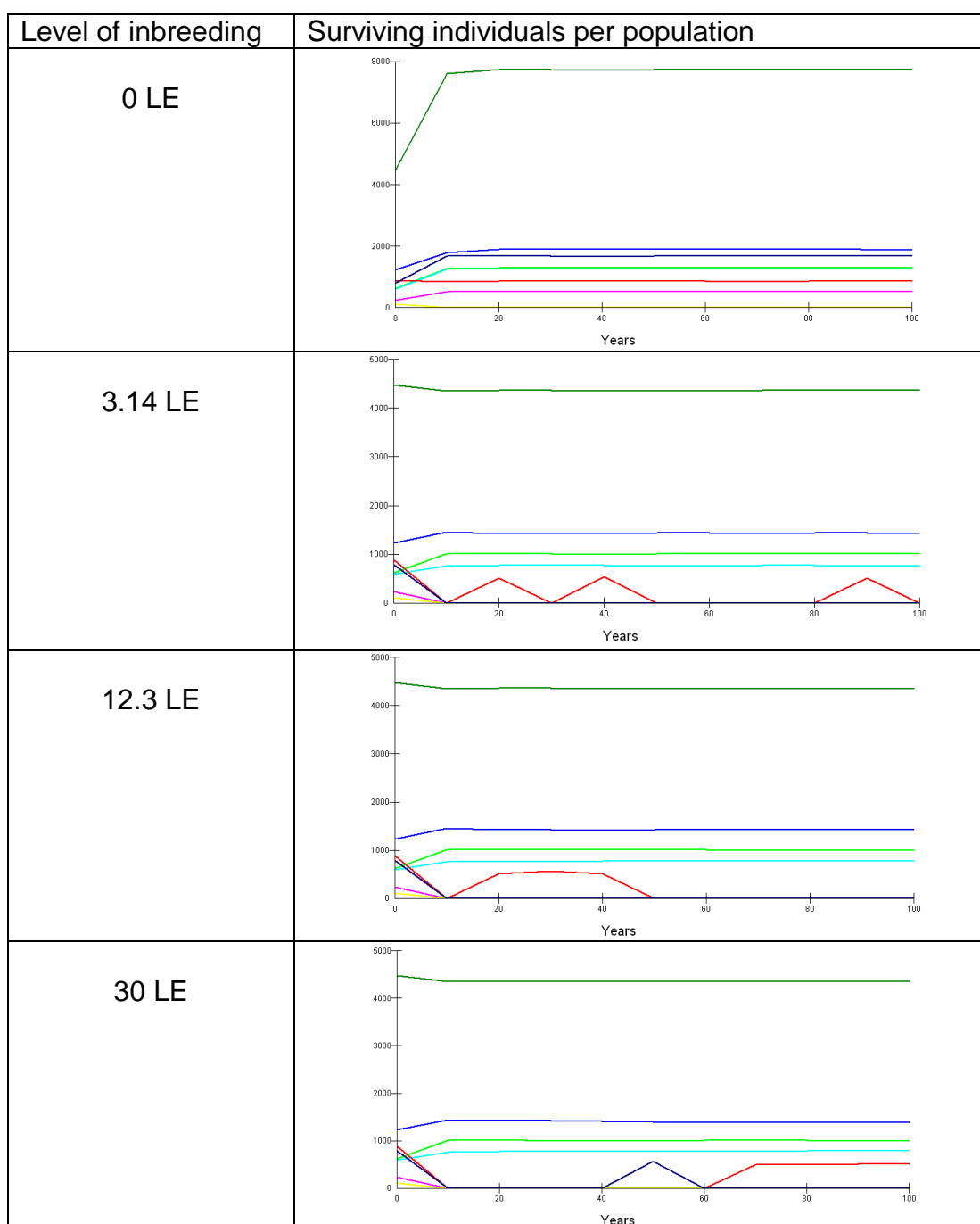


Figure 19. Development of subpopulations in a scenario without decrease in K , two catastrophes and different levels of inbreeding, expressed as number of lethal equivalents (LE). The y-axis represents the number of extant individuals, the upper line (green) represents the metapopulation, all lower lines are the subpopulations. Extinct populations may reappear as the simulations keep running, but do not count in the final surviving numbers.

GENERAL CONCLUSIONS AND OUTLOOK

The marine otter lives in a naturally fragmented habitat at the coast of Peru and Chile (Redford and Eisenberg 1992) and is dependent on availability of rocky shore patches (Medina-Vogel et al. 2007). Even after a considerable decrease in population size over several decades due to hunting in the 20th century (Valqui 2011), the species shows relatively high levels of genetic variability in its mtDNA. Although the dispersal ability for the species is unknown (Vianna et al. 2010), the isolating effect of large distances between subpopulations may have been compensated by certain levels of gene flow, which is why the Peruvian population only presents a moderate substructuring. At present, resilient larger populations may be balancing out the detrimental effects of inbreeding and catastrophes, but smaller isolated ones are indeed threatened by genetic depletion due to inbreeding and extinction due to environmental events and diseases.

Even if marine otters may be able to co-exist with humans in highly perturbed habitats, anthropogenic factors are considerably altering the marine ecosystem of Peru and Chile, presenting a worrying scenario for the marine otter population. Mining and the big-scale fisheries (overfishing) are strong factors of impact at regional level, with industrial spillings and heavy metals directly polluting the coastal waters in still unknown dimensions (Apaza et al 2004; Majluf et al. 2002). Urbanization, or human presence in general, has further indirect -but not necessarily minor- effects: invasive species (dogs, cats and rats) accompanying the development of settlements are displacing the marine otter from its natural habitat (Valqui 2011). At a more local level the species is affected by illegal fishing methods (dynamite fishing) and bycatch (Apaza et al. 2004). A combination of all anthropogenic factors may be causing a considerable decrease in habitat, increasing isolation of marine otter populations. Thus, at a certain moment a threshold that impedes gene flow may be reached, presenting unfavorable conditions to support the *L. felina* population (Peltonen and Hanski 1991) in the future. In this sense the Red List reports rates of decline population of at least 50% over the next three generations (30 years) (Red List of Threatened Species Version 2011.1). It is

therefore urgent to take action before the threats to the marine otter initiate extinction processes that cannot be reversed.

The overall aim of conservation plans on the species should be to avoid further reduction of habitat and to counter the threats related to human activities at the Pacific coast of Peru and Chile. Peruvian and Chilean groups may be separated in two management units (MU), even though cooperation in conservation efforts of these two countries is strongly recommended. Within Peru, at least two MUs may be defined: the first ranging from the limits of the northern distribution (around Chimbote, 9° S) to the locality of Chala (15°50' S) and the second from the protected area of Atico (16°14' S) to Puerto Vila Vila (18°06' S), the last locality with marine otter occurrence before Chile (Figure 8, Table 1). Within the northern MU of Peru, reasons for the decrease in area of occupancy north of Chimbote (the northern limit of distribution) have to be investigated and the re-establishing of the population should be planned. Re-stocking programs may be an option in this case, but they must be accompanied by genetic management to guarantee that the population is reestablished for the long-term (Howes et al. 2009). One particularly interesting recolonization event north of Chimbote should be taken into consideration (Alfaro et al. 2011) as a sign that natural reestablishment of abandoned areas is possible. Within the southern MU of Peru, around Morro Sama (18°00' S) and Vila Vila (18°06' S) unusual abundance of individuals has been observed (Mangel and Alfaro-Shigueto 2004). In that same region, Ulloa et al. (2010) proposed a new protected area, based on high levels of diversity of flora and fauna. The existence of a marine otter 'hotspot' that may function as a source to adjacent populations may support the establishment of this new reserve. Taking advantage of the one-dimensional distribution of the marine otter (Vianna et al. 2010) conservation work should aim towards the establishment reserve networks (Medina et al. 2008), as the recently implemented system of coastal protected areas in Peru. The systems should be designed to contain as many rocky shore patches as possible in a 'stepping stone' model. If it turns out that these measures do not guarantee the species survival, an alternative solution to secure gene flow may be the implementation of a network of artificial structures (boats, shipwrecks and docks) (Mangel et al. 2010; Medina-Vogel et al. 2007). To minimize further

habitat fragmentation, sustainable urbanization plans and feral and domestic animal management (Medina-Vogel et al. 2008) especially in the vicinity of urbanized areas have to be implemented. Not only is displacement of the marine otter by invasive species a problem, but also diseases may affect individuals coming in contact with infected domestic animals.

Law enforcement of all regulations around protected areas and endangered species is an issue to be looked upon in the future. Regulations do not bring any result if comprehensive control and penalizations do not support those regulations, which is why illegal fishing and other human activities have been affecting the marine ecosystems in Peru in the last decades. Studies have to be performed on ecology, physiology, infectious diseases as well as the impact of pollutants on marine otters living in highly contaminated waters (Apaza 2002). In the few genetic studies done to the present, only matrilineal (mtDNA) markers have been used to analyze the marine otter populations. Genetic assessment has to be enhanced with biparental (nuclear) markers to achieve a higher resolution view on phylogenetic relationships (Awise 2004). Non-invasive methods represent the most realistic approach for genetic assessment; therefore the complications of working with low quality DNA should be considered to choose an appropriate methodical and logistic approach. Sampling sizes should be maximized to compensate the loss of information due to the usual low success of DNA extraction and amplification while an immediate processing of fresh samples is recommended. To avoid excessive delays in research and sample export permits, logistic and bureaucratic planning should be comprehensive.

Environmental education programs should aim at making the species better known while exploiting its potential as a flagship to achieve sensitization of local people (especially fishermen and schoolchildren in coastal settlements). Conservation actions in favor of the marine otter also promise to have a broad umbrella effect: protection of the species and establishing of a network of protected areas will directly benefit several other species (endangered or not) in the coastal marine ecosystem of Peru and Chile.

ACKNOWLEDGEMENTS

The realization of this thesis was only possible thanks to the funding, research authorizations and valuable help of several persons and institutions. These are acknowledged specifically in each Chapter. Further, I would like to express my gratitude to Professor Günther B. Hartl, who always believed in this project, supporting it as my PhD assessor and as a private person; Dr. Dorit Feddersen-Petersen and Stefan Bootsman of the Tiergarten at Kiel University, for providing stool samples of mustelids during the methodology testing phase; Frank Zachos for the assistance in statistic analysis; Stephan Göbel and Haresh Bhojwani for the comments on this manuscript and Lorenzo von Fersen (Yaqu Pacha) for the promising cooperation prospect within the “Proyecto Lontra felina”.

REFERENCES

- Alfaro-Shigueto, J., Valqui, J., Mangel, J.C. 2011. Nuevo registro de la nutria marina *Lontra felina* (Molina, 1782) al norte de su distribución actual. *Ecología Aplicada* 10 (2): 87-91.
- Arnemo, J.M., Ahlqvist, P., Andersen, R., Berntsen, F., Ericsson, G., Odden, J., Brunberg, S., Segerström, P., Swenson, J.E. 2006. Risk of capture-related mortality in large free-ranging mammals: experiences from Scandinavia. *Wildlife Biology* 12 (1): 109-113.
- Apaza, M., Figari, A. 1999. Mortandad de aves marinas durante “El Niño 1997-1998” en el litoral sur de San Juan de Marcona, Ica-Peru. *Revista Peruana de Biología* Vol. Extraordinario: 110-117.
- Apaza, M., Lleellish, M., Valqui, J., Céspedes, C., Roca, M., Alfaro J., Munemura, G. 2002. Estado de Conservación de las Poblaciones de *Spheniscus humboldti* y *Lontra felina* en la Costa Sur del Perú. Informe no publicado. Instituto Nacional de Recursos Naturales (INRENA). Lima. 37p.
- Apaza, M., Valqui, J., Castañeda, C. 2003. Estado de Conservación de las Poblaciones de *Spheniscus humboldti* y *Lontra felina* en la Costa Norte del Perú. Informe no publicado. Instituto Nacional de Recursos Naturales (INRENA). Lima. 30p.
- Apaza, M., Valqui, J., Mangel, J., Roca, M., Alfaro, J., Santillan, L., Perret, J.P., Onton, G., Castaneda, C., Munemura, G., Tovar, A. 2004. Estado de Conservación de *Lontra felina* (Molina, 1782) en la Costa Peruana. Reporte para la Comisión Permanente del Pacífico Sur, Lima. 23p.
- Avise, J.C. 2004. *Molecular Markers, Natural History and Evolution*. Sinauer Associates Inc. U.S.

- Bandelt, H.-J., Forster, P., Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37-48.
- Bailey, N.W., Macias Garcia, C., Ritchie, M.G. 2007. Beyond the point of no return? A comparison of genetic diversity in captive and wild populations of two nearly extinct species of Goodeid fish reveals that one is inbred in the wild. *Heredity* 98: 360-367.
- Balloux, F., Lugon-Moulin, N. 2002. The estimation of population differentiation with microsatellite markers. *Molecular Ecology* (11): 155-165.
- Bayes, M.K., Smith, K.L., Alberts, S.C., Altmann, J., Bruford, M.W. 2000. Testing the reliability of microsatellite typing from faecal DNA in the savannah baboon. *Conservation Genetics* 1: 173-176.
- Bazin, E., Glémin, S., Galtier, N. 2006. Population Size Does not influence Mitochondrial Genetic Diversity in Animals. *Science* 312 (5773): 570-572.
- Beja-Pereira, A. Oliveira, R., Alves, P., Schwartz, M., Luikart, G. 2009. Advancing understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources* 9: 1279-1301.
- Bertrand, A., Segura, M., Gutierrez, M., Vasquez, L. 2004. From small-scale habitat loopholes to decadal cycles: a habitat-based hypothesis explaining fluctuation in pelagic fish populations off Peru. *Fish and Fisheries* 5: 296-316.
- Bertrand, A. 2008. The northern Humboldt current System: Brief history, present status and a view towards the future. *Progress in Oceanography* 79: 95-105.
- Birky, J.C. 1995. Uniparental inheritance of mitochondrial and chloroplast genes: Mechanisms and evolution. *Proceedings of the National Academy of Sciences* 92 (25): 11331-11338.

Borkenhagen, P. 1993. Atlas der Säugetiere Schleswig-Holsteins. - Landesamt für Naturschutz und Landschaftspflege Schleswig-Holstein, Kiel, 131 pp. (In German).

Brack, E. A. 1978. Situación actual de las nutrias (Lutrinae, Mustelidae) en el Perú. En: Duplaix, N. (Ed.) Otters – Proceedings of the First Working Meeting of the IUCN/SSC Otter Specialist Group. Paramaribo, Suriname: 76-84.

Brook, B.W., O'Grady, J.J., Chapman, A.P., Burgman, M.A., Akcakaya, H.R., Frankham, R. 2000: Predictive accuracy of population viability analysis in conservation biology. *Nature* 404: 385-387.

Broquet, T., Ménard, N., Petit, E. 2006. Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. *Conservation Genetics* 8: 249-260.

Brownell, R.L. 1978. Ecology and conservation of the Marine Otter (*Lutra felina*). En: Duplaix, N. (Ed.) Otters – Proceedings of the First Working Meeting of the IUCN/SSC Otter Specialist Group. Paramaribo, Suriname: 104-106.

Cabello, C.C. 1978. La nutria de Mar *L. felina* en la Isla de Chiloé. Proceedings of the first International Otter Colloquium (1): 118-119.

Cabello, C.C. 1983. La Nutria de Mar en la Isla de Chiloé. CONAF. Boletín Técnico 6.

Cabrera, A. 1957. Catálogo de los mamíferos de América del Sur. Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". Ciencias Zoológicas 4(1): iv+308p.

Campbell, J. N., Reece, J. 2006. Biología. Pearson Studium.

Cassini, M.H. 2008. Present status of *Lontra felina* in Argentina. *Endangered Species Update* 25 (2): 57-60.

Castilla, J.C. 1981. La nutria de mar chilena, especie en extinción. *Creces* 2: 1-37.

Castilla, J.C. 1982. Nuevas observaciones sobre conducta, ecología y densidad de *Lutra felina* (Molina, 1782) (Carnívora: Mustelidae) en Chile. *Publicación Ocasional Museo Nacional de Historia Natural* 38: 197-206.

Castilla, J.C., Bahamondes, I. 1979. Observaciones conductuales y ecológicas sobre *Lutra felina* (Molina) 1782 (Carnívora: Mustelidae) en las zonas central y centro-norte de Chile. *Archivos de Biología y Medicina Experimental* 12: 119-132.

Centrón D., Ramirez, B., Fasola, L., Macdonald, D.W., Chehébar, C., Schiavini, A., Cassini, M.H. 2008. Diversity of mtDNA in Southern river otter (*Lontra provocax*) from Argentinean Patagonia. *Journal of Heredity* 99:198-201.

Chéhebar, C. 1990. Action plan for Latin American otters. *Otters: An action plan for their conservation*. P. Foster-Turley, P. S. MacDonald and C. Mason. Brookfield, Illinois, Chicago Zoological Society: 64-73.

Cossíos D., Alcázar P., Fajardo U., Chávez K., Alfaro J., Cárdenas S., Valqui J., Montero F., Lescano J., Quevedo M., Vivar E., Leite R., Ledesma K., Medina C., Maffei L., Amanzo J., Chávez C., Enciso M., García A., Mangel J., Mendoza J., Rojas G., Silva L., Villegas J., Williams R., Zúñiga A., Cruz A., Ruiz E. 2012. The order Carnívora (Mammalia) in Peru: research priorities for its conservation. *Revista Peruana de Biología* 19(1) (in press).

- Costa, D. 1982. Energy, Nitrogen, and Electrolyte Flux and Sea Water Drinking in the Sea Otter *Enhydra lutris*. *Physiological Zoology*. 55(1): 35-44.
- Dallas, J.F., Piertney, S.B. 1998. Microsatellite primers for the Eurasian otter. *Molecular Ecology* 7: 1248-1251.
- Darwin, C. 1889. *Viaje de un naturalista alrededor del Mundo*, Tomo II. Madrid.
- Dinerstein, E., McCracken, G.F. 1990. Endangered greater one-horned Rhinoceros carry high levels of genetic variation. *Conservation Biology* 4: 417-22.
- Ebensperger, L.A., Castilla, J.C. 1991. Conducta y densidad poblacional de *Lutra felina* en Isla Pan de Azúcar (III Región), Chile. *Medio Ambiente* 11: 79-83.
- Ebensperger, L. A. 1992. Selección de hábitat en tierra por la nutria marina, *Lutra felina*, en Isla Pan de Azúcar, Chile. *Revista Chilena de Historia Natural* 65: 429-434.
- Ehrlich, P.R., Ehrlich, A.H. 1991. *Healing the Planet*. Addison-Wesley Publishing Company, New York.
- Eisenberg, J.F., Redford, K.H. 1989. *Mammals of the Neotropics: the Central Neotropics*. Ecuador, Peru, Bolivia, Brazil, The University of Chicago Press. 3: 294.
- Estes, J.A., 1986. Marine otters and their environments. *Ambio*. 15: 181-183.
- Estes, J.A., Tinker, M.T., Williams, T.M., Doak, D.F., 1998. Killer Whale Predation on Sea Otters Linking Oceanic and Nearshore Ecosystems. *Science* 282: 473.

- Excoffier, L., Laval, G., Schneider, S. 2005. ARLEQUIN Ver. 3.1: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* 1: 47-50.
- Fedorov, A.V., Philander, S. G. 2000. Is El Niño Changing? *Science* 288 (5473): 1997–2002.
- Ferrando, A., Ponsà, M., Marmi, J., Domingo-Roura, X. 2004. Eurasian otters, *Lutra lutra*, have a dominant mtDNA haplotype from the Iberian Peninsula to Scandinavia. *Journal of Heredity* 95: 430-435.
- Finnegan, L.A., Néill, L.O. 2011. Mitochondrial DNA diversity of the Irish otter, *Lutra lutra*, population. *Conservation Genetics* 11:1573–1577.
- Frankham, R. 2005. Genetics and extinction. *Biological Conservation* 126: 131–140.
- Frankham, R., Ballou, J.D., Briscoe, D.A. 2007. *Introduction to Conservation Genetics*. Cambridge University Press, New York.
- Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915-925
- Gay, C. 1874. *Historia Física y Política de Chile. Zoología Tomo I*. Maulde y Renou, Paris.
- Google Earth Software - Version 5.1.3533.1731. 2009 Mountain View, CA: Google Inc. (2009). Available from <http://www.google.de/intl/de/earth/index.html>
- Grimwood, I.R. 1968. Notes on the distribution and status of some Peruvian mammals. *New York Zoological Society Special Publication* 21: 86p.

- Hajji, G.M., Zachos, F.E., Charfi-Cheikrouha, F., Hartl, G.B. 2007: Conservation genetics of the imperilled Barbary red deer in Tunisia. *Animal Conservation* 10: 229-235
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series* 41: 95-98.
- Halliburton, R. 2004. *Introduction to Population Genetics*, Pearson Education International.
- Hartl, G.B., Hell, P. 1994. Maintenance of high levels of genetic variation in spite of a severe bottleneck in population size: the brown bear (*Ursus arctos*) in the Western Carpathians. *Biodiversity and Conservation* 3: 546-554.
- Hartl, G.B., Pucek, Z. 1994. Genetic depletion in the European bison (*Bison bonaus*) and the significance of electrophoretic heterozygosity for conservation. *Conservation Biology* 8: 167-174.
- Henegariu, O., Heerema, N.A., Dlouhy, S.R., Vance, G.H., Vogt, P.H. 1997. Multiplex PCR: Critical Parameters and Step-by-Step Protocol. *BioTechniques* 23: 504-511.
- Hinrichsen, D., 1998. *Coastal waters of the world: trends threats and strategies*. Washington DC.
- Hosack, D.A., Miller, P.S., Hervert, J.J., Lacy, R.C. 2002: A population viability analysis for the endangered Sonoran pronghorn, *Antilocapra americana sonoriensis*. *Mammalia* 66: 207-229.

- Housse, R. 1953. Animales Salvajes de Chile. Universidad de Chile, Santiago. 189p.
- Howes, B.J., Pither, R., Prior, K. 2009. Conservation implications should guide the application of conservation genetic research. *Endangered Species Research* 8: 193-199.
- Hvidberg-Hansen, H. 1970. A survey of the coast otter (*Lutra felina*) Molina in the Peruvian rivers of Camana and Ocoña. FAO – UNDP/SP. 116.
- Instituto Nacional de Estadística e Informática (INEI). 2007. Resumen de Censos realizados entre 1940 y 2007. Lima. <http://www.inei.gob.pe/>.
- Instituto Nacional de Estadísticas (INE). 2002. Síntesis de Resultados del Censo 2002. Santiago. <http://www.ine.cl/>
- Instituto Nacional de Recursos Naturales (INRENA). 2003. Expediente Técnico de la Reserva Nacional Islas y Punta Guaneras y Ampliación de la Reserva Nacional de Paracas. Lima. 71 p.
- Iriarte, J.A., Jaksic F.M. 1986. The fur trade in Chile: an overview of seventy-five years of export data (1910-1984). *Biological Conservation* 38: 243-253.
- Kenyon, K.N. 1969. The Sea-otter in the Eastern Pacific Ocean. US Department of the Interior, Bureau of Sport Fisheries and Wildlife 60: 1-357.
- Koelewijn, H. P., Pérez-Haro, M., Jansman, H.A.H., Boerwinkel, M.C., Bovenschen, J., Lammertsma, D.R., Niewold, F.J.J., Kuiters, A.T. 2010. The reintroduction of the Eurasian otter (*Lutra lutra*) into the Netherlands: hidden life revealed by noninvasive genetic monitoring. *Conservation Genetics* 11(2): 601-614.

- Koepfli, K.P., Wayne, R.K. 1998. Phylogenetic relationships of otters (Carnivora: Mustelidae) based on mitochondrial cytochrome b sequences. *Journal of Zoology* 246: 401–416.
- Koepfli, K.-P., Deere, K.A., Slater, G.J., Begg, C., Begg, K., Grassman, L., Lucherini, M., Veron, G., Wayne, R.K. 2008. Multigene phylogeny of the Mustelidae: resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. *BMC Biology* 6: 10.
- Koh, L.P., Dunn, R.R., Sodhi, N.S., Colwell, R.K., Proctor, H.C., Smith, V.S. 2004. Species coextinctions and the biodiversity crisis. *Science* 305: 1632-1634.
- Kohn, M.H., Wayne, R.K. 1997. Facts from feces revisited. *Trends in Ecology and Evolution*. 12 (6): 223-227.
- Kohn, M.H., York, E.C., Kamradt, D.A., Haught, G., Sauvajot, R.M., Wayne, R.K. 1999. Estimating population size by genotyping faeces. *Proceedings of the Royal Society of London B Biological Sciences*. 266: 657-663.
- Korbie, D.J., Mattick, J.S. 2008. Touchdown PCR for increased specificity and sensitivity in PCR amplification. *Nature Protocols* 3: 1452-1456.
- Kruuk, H., Moorehouse, A. 1991. The spatial organisation of otters (*Lutra lutra*) in Shetland. *Journal of Zoology* 224: 41–57.
- Lacy, R.C., Borbat, M., Pollak, J.P. 2005. VORTEX: A stochastic simulation of the extinction process. Version 9.50. - Chicago Zoological Society, Brookfield, Illinois, USA. Available at: <http://www.vortex9.org/vortex.html>.
- Larivière, S. 1998 *Lontra felina*. *Mammalian Species* 575: 1-5.

- Larivière, S., Jennings, A.P. 2009. Family Mustelidae (weasels and relatives). In: Wilson DE, Mittermeier RA (eds) Handbook of the Mammals of the World. Vol. 1. Carnivores, Lynx Edicions, Barcelona.
- Larson, S., Jameson, R., Etnier, M., Fleming, M., Bentzen, P. 2002. Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. *Molecular Ecology* 11: 1899-1903.
- Leakey, R., Lewin, R. 1995. *The Sixth Extinction: Biodiversity and its Survival*. Doubleday, New York.
- Ludeña, W. 2004. Lima: Con-cierto de-sierto barroco. *Arquitectura* 57: 10-13.
- Lynch, M. 2010. Evolution of the mutation rate. *Trends in Genetics* 26 (8): 345–352.
- Mace G.M., Gittleman J.L., Purvis A. 2003. Preserving the Tree of Life. *Science* 300: 1707.
- Madea, B. 2003. *Praxis Rechtsmedizin*. Springer-Verlag, Berlin.
- Majluf, P., Babock, E., Riveros J.C., Arias, M., Alderete, W., 2002. Catch and Bycatch of Sea Birds and Marine Mammals in the Small-Scale Fishery of Punta San Juan, Peru. *Conservation Biology* 16(5): 1333-1343.
- Mangel, J.C., Alfaro-Shigueto, J. 2004. Comunidades pesqueras y la conservación de la nutria marina (*Lontra felina*) en el sur de Peru. *Revista de Conservación Regional* 2: 5-10.
- Mangel J.C., Whitty, T., Medina-Vogel, G., Alfaro-Shigueto, J., Caceres, C., Godley, B.J. 2010. Latitudinal variation in diet and patterns of human interaction in the marine otter. *Marine Mammal Science* 27(2): 14-25.

- Mattern, T., Ellenberg, U., Luna-Jorquera, G. 2002. A South American Marine Otter *Lontra felina* preys upon chicks of the Peruvian Diving Petrel *Pelecanoides garrnotii*. *Marine Ornithology* 30: 95-96.
- McCarthy, M.A., Lindenmayer, D.B., Possingham, H.P. 2001. Assessing spatial PVA models of arboreal marsupials using significance tests and Bayesian statistics. *Biological Conservation* 98: 191-200.
- Medina, G. 1995. Feeding habits of the marine otter (*Lutra felina*) in southern Chile. *Proceedings of the International Otter Colloquium* 6: 65-68.
- Medina-Vogel, G., Delgado, C., Alvarez, R.E. 2004. Feeding ecology of the marine otter (*Lutra felina*) in a rocky Seashore of the south of Chile. *Marine Mammal Science* 20(1): 134-144.
- Medina-Vogel, G., Bartheld, J.L., Alvarez, R., Delgado, C. 2006. Population assesment and habitat use of marine otter (*Lontra felina*) in southern Chile. *Wildlife Biology* 12 (2): 191-199.
- Medina-Vogel, G., Boher, F., Flores, G., Santibanez, A., Soto-Azat, C. 2007. Spacing behavior of marine otters (*Lontra felina*) in relation to land refuges and fishery wastes in Central Chile. *Journal of Mammalogy* 88: 487-494.
- Medina-Vogel, G., Merino, L.O., Monsalve Alarcón, R., Vianna, J. de A. 2008. Coastal-marine discontinuities, critical patch size and isolation: implications for marine otter conservation. *Animal Conservation* 11: 57-64.
- Miller, S.D., Rottmann, J., Raedke, K.J., Taber, R.D. 1983. Endangered Mammals of Chile: Status and Conservation. *Biological Conservation* 25: 335-352.

- Miller, P.S., Lacy, R.C. 2005. VORTEX: A Stochastic Simulation of the Extinction Process. Version 9.50 User's Manual. Apple Valley, MN: Conservation Breeding Specialist Group (SSC/IUCN).
- Molina, J. I. 1782. Saggio sulla storia naturale del Chili, del signor abate Giovanni Ignazio Molina. Bologna, Stamperia del S. Tomaso d'Aquino. Bologna.
- Moreno, C., Sutherland, J., Jara, F. 1984. Man as predator in the intertidal zone of Southern Chile. *Oikos* 42: 155–160.
- Moreno, C. 2001. Community patterns generated by human harvesting on Chilean shores: a review. *Aquatic Conservation* 11: 19–30.
- Muck, P., Pauly, D. 1987. Monthly anchoveta consumption of guano birds, 1953 to 1982. In: Pauly, D., Tsukayama, I. (eds). The Peruvian anchoveta and its upwelling ecosystem: three decades of change, pp. 219-233. ICLARM Studies and Reviews, 15, International Center for Living Aquatic Resources Management, Manila.
- Murphy, M.A., Kendall, K.C., Robinson, A., Waits, L.P. 2007. The impact of time, and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. *Conservation Genetics* 8: 1219-1224.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. Kent, J. 2000. *Nature* 403: 853–858.
- Nei, M., Tajima, F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics* 97:145.

- Nei, M., Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Science* 76(10): 5269-5273.
- O'Grady, J.J., Brook, B.W., Reed, D.H., Ballou, J.D. Tonkyn, D.W., Frankham, R. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation* 133: 42-51.
- Ortiz, R.M., 2001. Osmoregulation in marine mammals. *Journal of Experimental Biology* 204: 1831-1844.
- Osgood, W. 1943. The Mammals of Chile. Zoological Series. Field Museum of Natural History 30 (542): 268p.
- Ostfeld, R., Ebersperger, L., Klosterman, L., Castilla, J.C. 1989. Foraging, activity budget and social behavior of the South American Marine Otter *Lutra felina* (Molina, 1782). *National Geographic Research* 5(4): 422-438.
- Packer, C., Altizer, S., Appel, M., Brown, E., Martenson, J., O'Brien, S.J., Roelke-Parker, M., Hofmann-Lehmann, R., Lutz, H. 1999: Viruses of the Serengeti: Patterns of infection and mortality in African lions. *Journal of Animal Ecology* 68: 1161-1178.
- Parera, A. 1996. Las nutrias verdaderas de la Argentina. *Boletín Técnico de la Fundación Vida Silvestre Argentina*. Buenos Aires. 38 p.
- Pellis, S. M. 1988. Agonistic versus amicable targets of attack and defense: Consequences for the origin, function, and descriptive classification of play-fighting. *Aggressive Behavior* 14: 85-104.
- Peltonen, A., Hanski, I. 1991. Patterns of island occupancy explained by colonization and extinction rates in shrews. *Ecology* 72: 1698–1708.

Pimm, S.L., Raven, P. 2000. Biodiversity: Extinction by numbers. *Nature* 403: 843-845.

Pizarro, J. 2008. Mortality of the Marine Otter (*Lontra felina*) in Southern Peru. *IUCN Otter Specialist Group Bulletin*. 25 (2): 94-99.

Posada, D., Crandall, K.A. 2001. Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution* 16: 37-45.

Ralls, K., Ballou, J. 1983. *Genetics and Conservation: A Reference for Managing Wild Animal and Plant Populations*. Benjamin/Cummings, Menlo Park, CA, Chapter Extinction: Lessons from Zoos: 164-184.

Red List of Threatened Species. Version 2011.1. www.iucnredlist.org. Accessed 16th of February 2012.

Redford, K. H., Eisenberg, J. F. 1992. *Mammals of the Neotropics, The Southern Cone: Chile, Argentina, Uruguay, Paraguay*. University of Chicago Press, Chicago.

Rogers, A.R., Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* 9: 552-569.

Rozas, J., Rozas, R. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* 15: 174-175.

Rozzi, R., Torres-Mura, J.C. 1990. Observaciones del Chungungo (*Lutra felina*) al sur de la Isla Grande de Chiloé: antecedentes para su conservación. *Medio Ambiente*. 11(1): 24-28.

Ruiz, E. 2009. Estudio etológico de la nutria marina *Lontra felina* (Molina, 1782) en una 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.

Sánchez, R., 1992. Distribución y abundancia del gato marino *Lutra felina* en el Departamento de Ica. Informe no publicado. 28p.

Sánchez, R., Ayala, L. 2006. Evaluacion de la distribucion y abundancia del gato marino *Lontra felina* en la costa central y norte del Peru durante el otono del 2006. Reporte APECO. 33p.

Santillán, L., Caro, K. 2007. Mamíferos en Salaverry. Reporte CONCIMAR.

Schweigge, E. 1964. El litoral peruano. Universidad Nacional Federico Villareal. Lima.

Sharma, R., Stuckas, H., Bhaskar, R., Rajput, S., Khan, I., Goyal, S.P., Tiedemann, R. 2009. mtDNA indicates profound population structure in Indian tiger (*Panthera tigris tigris*). Conservation Genetetics 10: 909-914.

Sielfeld, W. H. 1989. Sobreposicion de nicho y patrones de distribucion de *Lutra felina* y *L. provocax* (Mustelidae, Carnivora) en el medio marino de Sudamerica Austral. Anales Del Museo De Historia Natural De Valparaiso 20: 103-108.

Sielfeld, W. K. 1990a. Dieta del Chungungo (*Lutra felina* (Molina, 1782)) Mustelidae, Carnivora) en Chile austral. Investigaciones en Ciencia y Tecnología. Serie Ciencias del Mar 1: 23-29.

- Sielfeld, W. K. 1990b. Características del hábitat de *Lutra felina* (Molina) y *L. provocax* Thomas (Carnivora, Mustelidae) en Fuego-Patagonia. Investigaciones en Ciencia y Tecnología. Serie Ciencias del Mar 1: 30-36.
- Sielfeld, W. K. 1992. Abundancias relativas de *Lutra felina* (Molina, 1782) y *L. provocax* (Thomas, 1908) en el litoral de Chile austral. Investigaciones en Ciencia y Tecnología. Serie Ciencias del Mar. 2: 3-11.
- Sielfeld, W.K., Castilla, J.C. 1999. Estado de conservación y conocimiento de las nutrias en Chile. Estudios Oceanológicos 18: 69-79.
- Simmons, M.J., Crow, J.F. 1977. Mutations affecting fitness in *Drosophila* populations. Annual Review of Genetics 11: 47-78.
- Soulé, M.E. 1980. Threshold for survival. Maintaining fitness and evolutionary potential. In: Soule´ , M.E., Wilcox, B.A. (Eds.); Conservation Biology. An evolutionary- ecological perspective. Sinauer, Sunderland: 151-169.
- Spielman, D., Brook, B.W., Frankham, R. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Science USA 101: 15261–15264.
- Staib, E. 2002. Öko-Ethologie von Riesenottern (*Pteronura brasiliensis*) in Peru. PhD Dissertation. Munich.
- Stanton, D.W.G., Hobbs, G.I., Chadwick, E.A., Slater, F.M., Bruford, M.W. 2008. Mitochondrial genetic diversity and structure of the European otter (*Lutra lutra*) in Britain. Conservation Genetics 10: 733-737.
- Stoneking, M. 2000. Hypervariable Sites in the mtDNA Control Region Are Mutational Hotspots. The American Journal of Human Genetics 67(4): 1029-1032.

Swartzman, G., Bertrand, A., Gutiérrez, M., Bertrand, S., Vasquez, L. 2008. The relationship of anchovy and sardine to water masses in the Peruvian Humboldt Current System from 1983 to 2005. *Progress in Oceanography* 79: 228-237.

Tello, E. 1972. *Anotaciones Sobre el Camarón*. Documentación Ministerio de Pesquería. Lima 18: 59.

Thiel, M., Macaya, E., Acuña, E., Arntz, W. 2007. The Humboldt Current System of northern-central Chile: oceanographic processes, ecological interactions and socioeconomic feedback. *Oceanography and Marine Biology: An Annual Review* 45: 195–344.

Thorne-Miller, B. 1999. *The living ocean: understanding and protecting marine biodiversity*. Washington DC.

Tovar, A., Rivera, G. 2003. *La Nutria Marina del Perú*. Facultad de Ciencias Forestales – Centro de Datos para la Conservación, Universidad Nacional Agraria – La Molina. Informe Interno. Lima.

Trinca, C.S., Waldemarin, H.F., Eizirik, E. 2007. Genetic diversity of the Neotropical otter (*Lontra longicaudis* Olfers, 1818) in southern and southeastern Brazil. *Brazilian Journal of Biology* 67 (Suppl.): 813-818.

Tschudi, J., 1844. *Reiseskizzen aus Peru*. Leipzig.

UICN-OSG 1998. Simposio del Grupo especial de Nutrias de UICN. Resumen: 34-36.

Ulloa, R., Zambrano, M., Chevarria, A., 2010. Expediente Técnico Área de conservación regional Morro Sama-Quebrada de Burros Tacna, Perú. 119 pp.

- Valqui, J. 2004. Comportamiento de la nutria marina *Lontra felina* (Molina, 1782) en una ambiente antrópico, la bahía de Pucusana -Lima, Perú. Tesis para optar el título de Biólogo. Universidad Nacional Agraria La Molina (UNALM). Lima. 58 p.
- Valqui, J., Hartl G.B., Zachos, F.E. 2010. Non-invasive genetic analysis reveals high levels of mtDNA variability in the endangered South-American marine otter (*Lontra felina*). *Conservation Genetics* 11: 2067-2072.
- Valqui, J. 2011. The marine otter *Lontra felina* (Molina, 1782): A review of its present status and implications for future conservation. *Mammalian Biology* 77: 75-83.
- Van Zyll de Jong, C.G. 1987. A phylogenetic study of the Lutrinae (Carnivora; Mustelidae) using morphological data. *Canadian Journal of Zoology* 65: 2536-2544.
- Vaz Ferreira, R. 1979. Situación poblacional y conservación de los mamíferos marinos en Latinoamérica. *Acta Zoologica Lilloana*. 34: 91-101.
- Viacava, C., Aitken, R., Llanos, J. 1978. Estudio del Camarón en el Perú: 1975 - 1976. Instituto del Mar del Perú. Boletín, 3(5): 161-232.
- Vianna. J., Ayerdi, P., Medina-Vogel, G., Mangel, J.C., Zeballos, H., Apaza, M., Faugeron, S. 2010. Phylogeography of the Marine Otter (*Lontra felina*): Historical and Contemporary Factors Determining Its Distribution. *Journal of Heredity* 101(6), 676-689.
- Villegas, L., Huamán, E. 1989. Algunas observaciones de la bioecología de *Lutra felina* "Chungungo" en la Bahía de Catarindo-Arequipa. In: Libro de Resúmenes. I Simposium sobre Ecología y Conservación en el Perú. Lima.

- Villegas, M., Aron, A., Ebensperger, L.A. 2006. The influence of wave exposure on the foraging activity of marine otter, *Lontra felina* (Molina, 1782) (Carnivora: Mustelidae) in northern Chile. *Journal of Ethology* 25 (3): 281-286.
- Villesen, P. 2007. FaBox: an online toolbox for fasta sequences. *Molecular Ecology Notes* 7: 965–968.
- Waits, L. P., Paetkau, D. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. *Journal of Wildlife Management* 69(4): 1419-1433.
- Wang, C., Fiedler, P.C. 2006. ENSO variability and the eastern tropical Pacific: A review. *Progress in Oceanography* 69: 239-266.
- Wilson, D.E., Reeder, D.M. 2005. *Mammal species of the world: a taxonomic and geographic reference*. Baltimore
- Wisdom, M.J., Mills, L.S. 1997. Sensitivity analysis to guide population recovery: prairie chickens as an example. *Journal of Wildlife Management* 61: 302-312.
- Wisely, S.M., Buskirk, S.W., Russell, G.A., Aubry, K.B., Zielinski, W.J. 2004. Genetic diversity and structure of the fisher (*Martes pennanti*) in a peninsular and peripheral metapopulation. *Journal of Mammalogy* 85: 640–648.
- Wu, C.F.J. 1986. Jackknife, bootstrap and other resampling methods in regression analysis (with discussions). *Annals of Statistics* 14: 1261-1350.
- Xu, H., Fu, Y-X. 2004. Estimating effective population size or mutation rate with microsatellites. *Genetics* 166: 555-563.

Yañez, P. 1948. Vertebrados Marinos Chilenos. *Revista de Biología Marina* 1(2): 103.

Young T.P. 1994. Natural die-offs of large mammals: implications for conservation. *Conservation Biology* 8: 410-418.

Zachos, F., Althoff, C., Steynitz, Y., Eckert, I., Hartl, G. 2007. Genetic analysis of an isolated red deer (*Cervus elaphus*) population showing signs of inbreeding depression. *European Journal of Wildlife Research* 53(1): 61-67.

Zachos, F. E., Hajji, G. M., Hmwe, S. S., Hartl, G. B., Lorenzini, R., Mattioli, S. 2009. Population viability analysis and genetic diversity of the endangered red deer population from Mesola, Italy. *Wildlife Biology* 15: 175-186.

Zachos, F.E., Karami, M., Ibenouazi, Z., Hartl, G.B., Eckert, I., Kirschning, J. 2010. First genetic analysis of a free-living population of the threatened goitered gazelle (*Gazella subgutturosa*). *Mammalian Biology* 75: 277-282.

Zhang, Q., Guan, Y., Yang, H. 2008. ENSO Amplitude Change in Observation and Coupled Models. *Advances in Atmospheric Sciences* 25 (3): 331–6.

ANNEX I

LIST OF FIGURES

GENERAL INTRODUCTION

Figure 1. The marine otter *Lontra felina* (Molina 1782).

Figure 2. Marine otter habitat with difficult access to samples.

Figure 3. Field work at the rocky shore coast of Peru.

Figure 4. Fishing port at the Peruvian coast.

CHAPTER I

Figure 5. The marine otter *Lontra felina* in a cave.

Figure 6. Historical, potential and current distribution of *Lontra felina* at the Pacific coast.

Figure 7. Rocky shore habitat of the marine otter.

CHAPTER II

Figure 8. Geographic location of the sample sites of marine otter faeces along the Peruvian coast within four sampling groups.

Figure 9. 4x4 truck to access collection sites.

Figure 10. Sampling in a fishing boat.

Figure 11. Sampling on a dock.

Figure 12. Median-joining network of the 14 marine otter mtDNA CR haplotypes found in this study. Circles are proportional to haplotype frequencies and show haplotype origin. Black dashes refer to mutational events.

Figure 13. Median-joining network of the 6 marine otter mtDNA cyt b haplotypes found in this study. Circles are proportional to haplotype frequencies and show haplotype origin. Black dashes refer to mutational events.

Figure 14. Median-joining network of the concatenated (CR and cyt b) marine otter mtDNA haplotypes from Vianna et al. (2010) and Valqui et al. (2010) (*) completed for this thesis. Haplotype frequencies are not shown. Yellow diamonds denote a median vector (hypothetical haplotype); mutational events are only shown if greater than one.

CHAPTER III

Figure 15. Population numbers in a deterministic scenario with growth rate $det-GR = 0.0048$.

Figure 16. Mean N , GR and H_E for different inbreeding scenarios (data from Table 6)

Figure 17. Mean N , GR and H_E for different catastrophe scenarios (data from Table 7)

Figure 18. Mean N for different decrease levels in K , in a scenario with no inbreeding and no catastrophes.

Figure 19. Development of subpopulations in a scenario without decrease in K , two catastrophes and different levels of inbreeding, expressed as number of lethal equivalents (LE). The upper line (green) represents the metapopulation, all lower lines are the subpopulations. Extinct populations may reappear as the simulations keep running, but do not count in the final surviving numbers.

LIST OF TABLES

CHAPTER I

Table 1. Occurrence and abundance of the marine otter *L. felina* along its distribution. Due to the one-dimensional distribution, locations are indicated with southern latitude (S. lat.) only. Type of record (Record) is specified as: REF (references by fishermen or locals), DS (direct sightings), or SBT (scat, blood or tissue samples collected). References⁽¹⁻⁸⁾ for type of records are listed. The least number of individuals is given for each location (Individuals). Locations in or close to protected areas are marked with *.

CHAPTER II

Table 2. Field work effort.

Table 3. Success rate in sampling and laboratory methods.

Table 4. Distribution and frequencies of the 14 CR haplotypes found in the present study within four sampling groups.

Table 5. Distribution and frequencies of the 6 cyt b haplotypes found in the present study within four sampling groups.

Table 6. Distribution and frequencies of CR, cyt b and concatenated haplotypes found in the present study within four sampling groups. Haplotypes occurring in at least two location are represented by grey squares, private haplotypes by diagonally striped squares.

Table 7. Pairwise F_{ST} values among all groups within Peru and Chile.

CHAPTER III

Table 8. Input summary for the PVA simulation.

Table 9. Mean number of surviving individuals (N), growth rate (GR) and expected heterozygosity (H_E) in a metapopulation at different inbreeding scenarios.

Table 10. Mean number of surviving individuals (N), growth rate (GR) and expected heterozygosity (H_E) in a metapopulation at different catastrophe scenarios.

Table 11. Effect of different levels in decrease of K (columns, 0%, -1%, -2% and -5%) and type of catastrophes (rows) on number of surviving individuals (N),

growth rate (GR) and expected heterozygosity (H_E) for the marine otter metapopulation, after 40 years and an initial population size of 4473 individuals. Each run was performed with 1000 iterations.

Table 12. Survival after 100 years under different levels of decrease in K.

ANNEX II**Non-invasive genetic analysis reveals high levels of mtDNA variability in the Endangered South-American marine otter (*Lontra felina*)**

Juan Valqui, Günther B. Hartl, Frank E. Zachos

Zoological Institute, Christian-Albrechts-University, Olshausenstrasse 40, 24118 Kiel, Germany

Conservation Genetics 11: 2067-2072 (2010).

ABSTRACT

Scats from marine otters were collected from the entire Peruvian distribution range along the Pacific coast. Partial mtDNA control region sequences (265 bp) were successfully amplified and analysed in 37 out of 87 samples. Based on spatial distribution and home range information of marine otters we assumed our final data set to represent at least 24 different individuals, yielding surprisingly high genetic variability (11 haplotypes, $h = 0.86$, $\pi = 0.0117$). No unequivocal evidence of genetic substructuring, a bottleneck or isolation by distance could be detected. This study presents the first genetic data in this endangered species and highlights the significance of the Peruvian gene pool for the establishment of reserves, potential future expansion, recolonisation or translocations.

Keywords: *Lontra felina*, mitochondrial control region, non-invasive genetic sampling, Peru

INTRODUCTION

The marine otter (*Lontra felina*) is the smallest marine mammal of the world and the only *Lontra* species confined solely to marine habitats. This monophyletic genus of otters comprises four species and is the sister to all remaining otters (*Hydriectis*, *Enhydra*, *Lutra*, *Lutrogale* and *Aonyx*) except for the most basal species, the giant otter (*Pteronura brasiliensis*) (Koepli et al. 2008). The historical distribution of the marine otter has been reduced in the last decades due to anthropogenic factors such as habitat destruction, pollution and poaching, and in the southern part of their distribution range (Cape Horn and Southern Tierra del Fuego) marine otters are on the brink of extinction (Red List of Threatened Species Version 2009.1; Parera 1996; Apaza et al. 2004). It is likely that the population will decrease by at least 50% in the next 30 years (Red List of Threatened Species Version 2009.1). Therefore, the species is listed as Endangered by the IUCN, in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), in Appendix I of the Convention on Migratory Species (CMS), and it is protected by Peruvian law.

Marine otters occur along the Pacific coast of South America from 6° S (today more probably 8°S, personal observation) to 56°S in Peru, Chile and Argentina, but because they depend upon habitats with rocky outcroppings and caves above the water at high tide, the distribution is disjunct (Larivière 1998; Red List of Threatened Species Version 2009.1). Chile holds the largest number of animals; studies on spatial distribution (Medina-Vogel et al. 2008) showed that strong predictors for marine otter presence are rocky seashore patches, and studies on spacing behaviour (Medina-Vogel et al. 2007) showed home ranges of <5 km along coastlines and suggested availability of land refuges and food as decisive factors in the species' distribution.

Research on the status of *Lontra felina* in Peru has so far been limited to some basic census surveys with divergent results: While earlier studies described numbers of about 200-300 (Castilla and Bahamondes 1979), a more recent survey yielded an estimate of c. 700 animals for the Peruvian coast (Apaza et al. 2004). In light of the bleak prospect for the coming decades, the species is in urgent need of help but as yet, no genetic analyses on marine otters have been

carried out, although knowledge on genetic variability and structure is of great relevance to conservation measures. The present study from the Peruvian part of the species' distribution range, therefore, aims at closing this gap by providing the first molecular data for this endangered mustelid. We particularly want to quantify the amount of genetic diversity left in marine otters and also analyse its spatial distribution to find out if there are already clear signs of disrupted gene flow among the remnant populations along the coast.

MATERIALS AND METHODS

Scat samples were collected along the Peruvian Pacific coast from Vesique (9°12'S, 78°29'W) to the port of Vila Vila (18°07'S, 70°43'W), covering more than 1400 km of coastline and the entire distribution range of marine otters in Peru (UICN-OSG 1998), in August and September 2008. Samples came from four main regions: Norte, Lima, Ica and Sur (Figure 1)

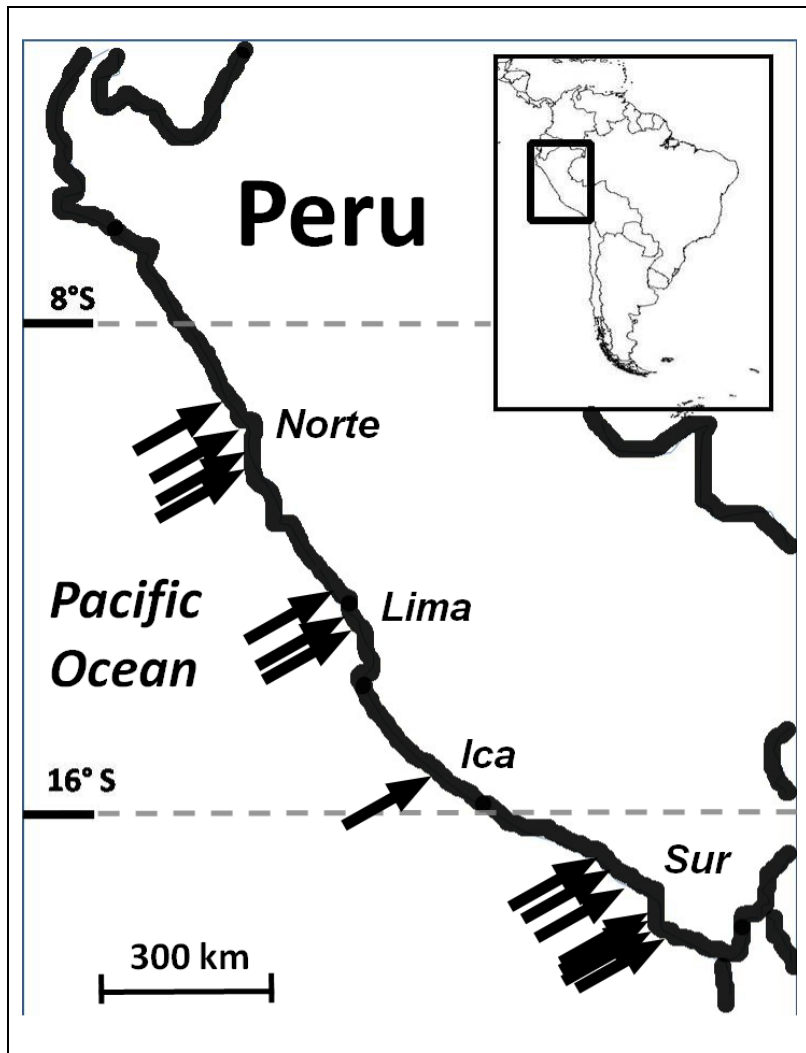


Figure 1. Geographic location of the sample sites of marine otter faeces along the Peruvian coast within four sampling groups.

which were separated by 340 km, 347 km and 370 km of unsampled coastline. Altogether 25 localities were visited, and a total of 87 samples were taken at 20 of them. Faeces were primarily collected at latrines used for scent-marking. Fresh scats were preferred as it has been shown that success rates of DNA amplification drop substantially after a few days (Murphy et al. 2007). Samples were taken using surgical gloves, stored in 96% ethanol, sealed with Teflon tape and transferred to a refrigerator as quickly as possible. DNA was extracted from the samples after they had been transferred to Germany (about two months after collection) using the QIAMP DNA stool mini kit. A blank extraction control was included to monitor potential contamination. A portion of the

mitochondrial control region was amplified with the primers DLH (5'-CCTGAAGTAAGAACCAGATG-3') and ProL (5'-CACCACCAACACCCAAAGCT-3') at an annealing temperature of 57°C and 35 amplification cycles. Amplicons were then sequenced with an automated MegaBACE sequencer.

Sequences were aligned using BioEdit (Hall 1999) and collapsed into haplotypes using the collapse function implemented in the FaBox package available at <http://www.birc.au.dk/fabox/> (Villesen 2007). Because we used faecal material, we used the following approach to validate our results: each sample was sequenced in both directions, and all haplotypes were confirmed by at least two independent PCR and sequencing runs, i. e. singletons were re-analysed, and in case of haplotypes occurring more than once, at least one sample was re-analysed. No differences were detected within any pair of runs for the same individual which we interpreted as evidence of reliable sequences. Marine otters are mostly solitary, and radio-tracking of animals in Chile revealed that home ranges comprised no more than 4,134 m of coastline (Larivière 1998; Larivière and Jennings 2009; Medina-Vogel et al. 2007). Therefore, identical haplotypes were only treated as different individuals if the sample locations were at least 5 km apart.

Haplotype and nucleotide diversity were calculated with DnaSP (Rozas and Rozas 1999). Differentiation among the four sample regions was tested for with Arlequin (Excoffier et al. 2005) by means of an analysis of molecular variance (AMOVA) and pairwise Φ_{ST} -values under the Tamura and Nei model of nucleotide substitution (this model was closest to the GTR model inferred for our data set with Findmodel (<http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html>) which is not implemented in Arlequin). To test for a pattern of isolation by distance we applied a Mantel test on matrices of the number of pairwise nucleotide differences and geographical distances between individuals.

Haplotype relationships were depicted by a median-joining network with the Network software (Bandelt et al. 1999). Networks are more appropriate for intraspecific phylogenies than tree algorithms because they explicitly allow for the co-existence of ancestral and descendant alleles in a sample, whereas

trees treat all sequences as terminal taxa (see Posada and Crandall 2001 for a review). Demographic history was inferred by a mismatch analysis and calculation of Fu's F_s (Fu, 1997) with the Arlequin software. Mismatch analyses depict the frequency distribution of pairwise differences between the sequences found in a sample. This distribution is dependent upon demographic processes. Unimodal distributions are typical of populations that experienced a recent expansion, while ragged and multimodal distributions are found in populations at demographic equilibrium (Rogers and Harpending 1992) and bimodal distributions often result from admixture of two previously separate lineages. Bottlenecked populations typically produce L-shaped distributions with a single peak at low pairwise differences. Arlequin tests for a mismatch distribution fitting the sudden expansion expectations. Fu's F_s statistic is a neutrality test but can also be used to infer demographic histories with large and statistically significant positive values of F_s being indicative of a deficit of rare haplotypes compared to expectations for a stable population and thus being a sign of a bottleneck, while large and significant negative values suggest an excess of recent mutations (many rare haplotypes), which is typical of recent expansions.

RESULTS AND DISCUSSION

Forty-one out of the 87 collected scat samples were amplified for a fragment of the mtDNA control region. Four samples yielded shorter sequences and were not included in subsequent analyses, leaving 37 successfully sequenced samples. The final alignment comprised 265 bp. The success rate of amplification was 43%, a value similar to those from other scat-based analyses (e. g. Centrón et al. 2008; Sharma et al. 2009). GenBank comparisons ruled out the possibility of contamination from other species by showing highest sequence similarity of our data with the North-American *Lontra canadensis* (no marine otter sequences are available). Thirteen polymorphic sites with 16 mutations (nine transitions, two transversions and five indels) defined 11 haplotypes (accession numbers GU982296-GU982306) one of which (LF08) was present in all four sampling groups (Table 1).

Table 1. Distribution and frequencies of the 11 haplotypes found in the present study with geographic coordinates within four sampling groups.

Haplotype	Sampling groups				Total
	Norte	Lima	Ica	Sur	
LF01	-	-	-	3 17°00'S 72°06'W 17°01'S 71°02'W 17°17'S 71°28'W	3
LF02	-	1 12°28'S 76°47'W	-	-	1
LF03	-	1 12°28'S 76°47'W	-	-	1
LF04	1 9°43'S 78°17'W	-	-	-	1
LF05	-	1 13°01'W 76°29'W	-	-	1
LF06	-	-	-	1 17°17'S 71°28'W	1
LF07	-	1 13°01'S 76°29'W	1 15°26'S 75°04'W	2 18°00'S 70°53'W 18°01'S 70°50'W	4
LF08	3 9°13'S 78°29'W 10°05'S 78°10'W 9°56'S 78°13'W	1 12°57'S 76°30'W	1 15°26'S 75°04'W	3 18°00'S 70°63'W 18°01'S 70°50'W 18°07'S 70°43'W	8
LF09	-	-	-	1 17°59'S 70°53'W	1
LF10	-	-	-	1 18°07'S 70°43'W	1
LF11	-	1 12°28'S 76°47'W	-	1 18°00'S 70°53'W	2
Total	4	6	2	12	24

Thirteen samples of the 37 were excluded from further analyses because they showed haplotypes identical to others within a distance of 5 km (possible

pseudoreplication). Therefore, calculations were based on a final data set comprising 24 sequences assumed to be from different individuals (see Table 1). This sample size is identical or similar to previous genetic analyses of other *Lontra* species in South America (Centrón et al. 2008; Trinca et al. 2007).

Overall haplotype and nucleotide diversity was 0.86 (± 0.053 SD) and 0.0117 (± 0.002 SD), respectively. None of the calculated overall or pairwise Φ_{ST} -values was significantly different from zero (all $p > 0.18$). A significant substructuring could, therefore, not be detected with our data. However, in spite of the ubiquitous distribution of LF08 this may be due largely to the small sample sizes within the four groups. In the absence of further data these groups should be considered as sample regions rather than genetically defined units. Further sampling and high-resolution markers such as microsatellites may elucidate differentiation along the Peruvian coast. We found no significant correlation of pairwise genetic and geographical distances between individuals (Mantel test, $r = -0.024$, $p = 0.795$).

Network analysis (Figure 2) revealed two frequent central haplotypes, but also a number of relatively distant satellite haplotypes of low frequency.

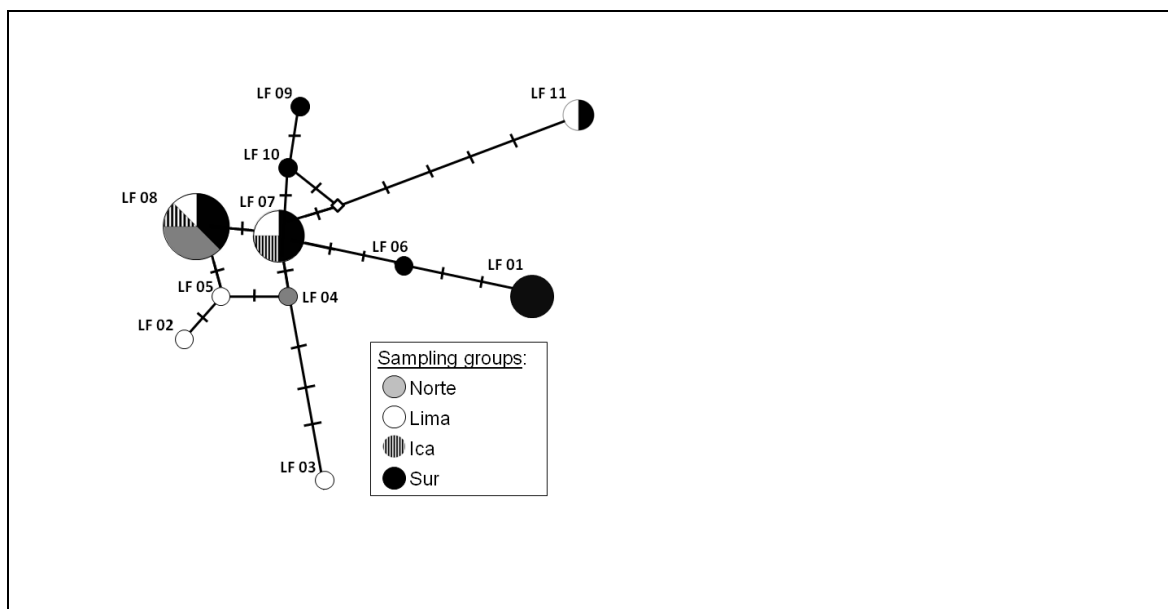


Figure 2. Median-joining network of the eleven marine otter mtDNA control region haplotypes found in this study. Circles are proportional to haplotype frequencies and show haplotype origin. The white diamond denotes a median vector; black dashes refer to mutational event.

On average, two sequences differed by 3.094 mutations from one another, and the mismatch analysis did not reject the null hypothesis of a sudden demographic expansion (sum of squared deviations between observed and expected mismatch distribution: 0.01, $p = 0.68$; Harpending's raggedness index: 0.03, $p = 0.84$). Fu's F_s statistic was negative (-2.94) and just above the significance threshold ($p = 0.063$) when transitions, transversions and indels were all weighted equally, but a significant ($p = 0.007$) and slightly negative (-0.785) value was yielded when mutations were weighted according to their intrinsic ratio (1:1.8:4.5 for transitions, indels and transversions, respectively). These results may tentatively be interpreted as hinting at an expansion event (in line with the mismatch analysis).

Although widespread and endangered, relatively little is known about marine otters in spite of a recent conservation focus on this species (e. g. Medina-Vogel et al. 2007; Medina-Vogel et al. 2008). Perhaps the most important of our results is the unexpectedly high genetic variability. The 24 marine otters of the present study yielded more haplotypes at a short fragment of the control region than did the entire European population of *Lutra lutra* at a longer one. Overall haplotype diversity and mean nucleotide diversity in Eurasian otters were reported as 0.16 and 0.0006, respectively (Ferrando et al. 2004 and references therein), but recent studies have revealed higher diversity for Eurasian otters in the UK (0.73 and 0.003, Stanton et al 2008) and Ireland (haplotype diversity of 0.75, Finnegan and Néill in press). Still, marine otter haplotype diversity and nucleotide diversity were considerably higher, the latter at least by an order of magnitude, although population sizes of *Lutra lutra* are doubtless much larger. This genetic depletion is even more pronounced than that of the sea otter (*Enhydra lutris*), which suffered a loss of an estimated 99% of its population due to persecution but still yielded mean haplotype diversities of 0.41 (Larson et al. 2002). Comparable data for other *Lontra* species are rare, but a recent study of Southern river otters (*Lontra provocax*), also classified as Endangered by the IUCN, from two disjunct regions in Argentinean Patagonia yielded only a single mtDNA control region haplotype in 13 individuals, although four different cytochrome b haplotypes were found in 34 animals analysed (Centrón et al. 2008). In an analysis of 491 bp of the mtDNA control region the third South-

American *Lontra* species, the Neotropical otter (*Lontra longicaudis*), was found to exhibit a haplotype diversity similar to ours (0.82), but a more than twofold lower nucleotide diversity of 0.0049 (Trinca et al. 2007). Neotropical otters are also considered to be threatened, but, due to lack of information, their present IUCN status is Data Deficient. As it seems, the marine otter is genetically the most diverse South-American *Lontra* species. Both the network with its various rare and divergent haplotypes and the demographic tests (mismatch analysis and Fu's F_s) did not show obvious signs of a bottleneck but rather some weak indication of a population expansion.

It is also noteworthy that we did not find significant substructuring in the mitochondrial genome, suggesting that either there is gene flow among the sample localities or that the recent disruption of the meta-population has not yet resulted in significant differentiation through genetic drift. The fact that we did not find an isolation-by-distance pattern has to be viewed with caution given our approach: because we excluded identical sequences within the same 5 km, we actually might have considered whole maternal lineages as a single individual, which may bias correlations between geographical proximity and genetic similarity. In any case, disjunct occurrence is not only the result of human-caused habitat fragmentation; the specific habitat requirements of marine otters result in a naturally disjunct distribution pattern, and adaptations to dispersal may facilitate gene flow despite human pressure and mitigate the negative effects of fragmentation (long-distance swimming has been observed by one of us, JV, in the field). Future analyses making use of high-resolution markers (microsatellites) are needed to further evaluate if there is differentiation along the Peruvian coast or not.

The bleak prospect of future population reductions of 50% or more within the next 30 years will certainly lead to a substantial loss of gene pool diversity. Our results are a first step towards a genetic underpinning for future conservation which should include information on the partitioning and distribution of genetic diversity. They are also interesting with respect to possible reserve areas. Some twenty islands and ten mainland sites along the Peruvian coast are managed by the government as guano harvesting areas. Based on the law *Decreto Supremo N°024-2009-MINAM*, these areas will be transformed into a system of reserves

(*Reserva Nacional Islas y Puntas Guaneras*) comprising a total area of more than 100,000 ha to protect threatened populations of seabirds (Humboldt penguin, Peruvian diving petrel, inka tern, Peruvian booby), marine mammals (South American fur seal, South American sea lion, marine otter) and reptiles (sea turtles). Although this measure will help maintain meta-populations of marine otters and facilitate gene flow, areas with high levels of genetic diversity along the southern Peruvian coast are not included. The genetic data presented here suggest that protection in the south should be implemented in the conservation program and also provide information to select appropriate individuals should translocations and re-stocking of abandoned areas become necessary. Further genetic studies based on larger sample sizes, nuclear loci and, above all, covering also the Chilean and Argentinean part of the distribution range are what is now most urgently needed.

ACKNOWLEDGEMENTS

Execution of field work was made possible by Authorization N° 98-2008-INRENA-IFFS-DCB given by the National Institute of Natural Resources (INRENA) from the Ministry of Agriculture of the Republic of Peru. Natalia Ortiz, Daniella Biffi and Jerico Solis assisted in the field. Yennifer Hernández assisted in the laboratory work. IDEAWILD provided logistic material through Elisa Ruiz. Financial support from The Society for Marine Mammology (SMM) is gratefully acknowledged.

ANNEX III**New record of the marine otter *Lontra felina* (Molina, 1782)
north to its current distribution**

Joanna Alfaro-Shigueto^{1,2}, Juan Valqui^{3,4}, Jeffrey C. Mangel^{1,2}

¹ ProDelphinus. Enrique Palacios 630-204, Lima 18, Perú

² University of Exeter, Penryn, Cornwall, TR10 9EZ, United Kingdom.

³ Centro de Ornitología y Biodiversidad (CORBIDI). Calle Santa Rita 105 Of. 202, Lima 33, Perú.

⁴ Instituto de Zoología, Universidad de Kiel. Am Botanischen Garten 1-9, 24118 Kiel, Alemania.

***Ecologia Aplicada* 10(2): 87-91 (2011)**

ABSTRACT

The marine otter *Lontra felina* is an endangered species and uncertainty exists regarding the limits of its current range, which is considered to be Chimbote (9°10'S). Here we present the first documented evidence of the species' presence 115 km to the north of Chimbote, in the artisanal port of Huanchaco (8°04'S). The note seeks to promote the generation of research to help clarify the factors that determine the presence of marine otters in a given location and which determine the northern limit to their range. Local (fishing ports and towns) and regional (provinces and countries) conservation measures safeguarding the species and the coastal marine habitat are recommended and should take advantage of the flagship species character of *L. felina*.

Key words: marine otter, *Lontra felina*, distribution, northern Peru, habitat

ANNEX IV

The order Carnivora (Mammalia) in Peru: research priorities for its conservation

E. Daniel Cossíos^{1,2}, Paloma Alcázar García^{3,4}, Ursula Fajardo Quispe⁵, Kelly Chávez⁶, Joanna Alfaro^{7,8}, Susana Cárdenas-Alayza⁹, **Juan Valqui**^{4,10}, Francesca G. Montero¹¹, Jesús Lescano Gómez¹², Miryam Quevedo Urday¹², Elena Vivar⁵, Renata Leite¹³, Karim Ledesma⁴, César Medina⁶, Leonardo Maffei¹⁴, Jessica Amanzo¹⁵, César Chávez Villavicencio¹⁶, M. Enciso¹⁷, Álvaro García Olaechea¹⁶, Jeffrey Mangel^{7,8}, Joel A. Mendoza Oblitas¹⁸, Gianmarco Rojas¹⁹, Larissa Silva Romero¹⁸, J. Villegas²⁰, Robert S.R. Williams¹⁸, Alfonso Zúñiga²¹, Alex Cruz²², Elisa Ruiz²³

1: University of Geneva. 30 Quai Ernest Ansermet 1211, Genève 4, Switzerland.

2: Kawsay Pacha-Asociación Biodiversidad. Av Caminos del Inca 2436, Surco, Lima, Perú.

3: Universidad Ricardo Palma, Escuela Académico Profesional de Ciencias Veterinarias, Facultad de Ciencias Biológicas. Av. Benavides 5440, Lima 33 – Perú.

4: Centro de Ornitología y Biodiversidad (CORBIDI). Calle Santa Rita 105 Of. 202, Lima 33, Perú.

5: Museo de Historia Natural de la Universidad de San Marcos, Av. Arenales 1256, Jesús María, Lima 14, Perú.

6: Museo de Historia Natural de la Universidad Nacional San Agustín. Av. Alcides Carrión s/n. Arequipa, Perú.

7: Pro Delphinus. Octavio Bernal 572-5, Lima 11, Peru

8: Centre for Ecology and Conservation, School of Biosciences, University of Exeter, Cornwall Campus, Penryn, Cornwall, UK

9: Centro para la Sostenibilidad Ambiental, Universidad Peruana Cayetano Heredia. Av. Armendariz # 445, Miraflores. Lima, Peru.

10: Instituto de Zoología, Universidad de Kiel. Am Botanischen Garten 1-9, 24118 Kiel, Alemania.

11: Universidad Nacional Agraria La Molina. Av. La Molina s/n, La Molina, Lima.

12: Laboratorio de Anatomía Animal y Fauna Silvestre, Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos. Av. Circunvalación Cdra. 28 s/n - San Borja. Lima.

14: Wildlife Conservation Society. Arias Araguez 152, Miraflores, Lima.

15: Laboratorio de Estudios en Biodiversidad. Departamento de Ciencias Biológicas y Fisiológicas, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia. Honorio Delgado 430, Urb. Ingeniería, SMP, Lima.

16: Centro Neotropical de Entrenamiento en Humedales – Internacional. Calle David Lewellyn 870, Coquimbo, Región de Coquimbo, Chile.

17: Departamento de Reprodução Animal. Faculdade de Medicina Veterinária e Zootecnia. Universidade de São Paulo. Av. Prof. Dr. Orlando Marques de Paiva nº87. Cidade Universitária. São Paulo/SP. Brasil.

18: Frankfurt Zoological Society. Clorinda Matto de Turner 305, Urb. Magisterio, Cusco.

29: Parque Zoológico Huachipa. Av Las torres s/n Ate Vitarte, Lima.

21: Word Wildlife Fund – Perú.

23: Dirección General Forestal y de Fauna Silvestre del Ministerio de Agricultura. Calle Diecisiete 355, urb El Palomar, San Isidro, Lima.

ABSTRACT

The high diversity of Peruvian carnivore species may pose problems when deciding which taxa and topics should receive new research efforts. In this publication, we evaluated the research effort made on each taxa -by assessing the number of publications about each one-, note the knowledge gaps that are important to the conservation of each species and present the first evaluation of research priorities for this group of animals to the country. We registered 145 publications about Peruvian carnivores made since 1943. The number of publications is significantly different between taxa, between subjects and between ecoregions where the research was conducted. According to the proposed priority scale, the species to be studied with greater priority is *Nasua olivacea* and the lowest priority is for *Leopardus pardalis*. The results of our study highlight the urgent need to conduct researchs on certain species of carnivores about which there are few published data, nationally and globally, and which occupies a low number of ecoregions in the country. Both the scale of research priorities and the list of knowledge gaps presented here, should be useful to guide logistical and financial efforts, as well for individual researchers as for private or governmental institutions.

LIST OF PUBLICATIONS

Relevant and related publications appearing in peer-reviewed scientific journals:

- ***Mammalian Biology* 77: 75-83 (2012)**

Valqui, J. The marine otter *Lontra felina* (Molina, 1782): A review of its present status and implications for future conservation.

- ***Conservation Genetics* 11: 2067-2072 (2010)**

Valqui, J., Hartl, Zachos, F.E., G.B. Non-invasive genetic analysis reveals high levels of mtDNA variability in the endangered South-American marine otter (*Lontra felina*).

- ***Revista de Ecología Aplicada* 10(2): 87-91 (2011) –Spanish**

Alfaro-Shigueto J., Valqui, J., Mangel, J.C. New record of the marine otter *Lontra felina* (Molina, 1782) north to its current distribution.

- ***Revista Peruana de Biología* 19(1): in press (2012) –Spanish**

Cossíos D., Alcázar P., Fajardo U., Chávez K., Alfaro J., Cárdenas S., Valqui J., Montero F., Lescano J., Quevedo M., Vivar E., Leite R., Ledesma K., Medina C., Maffei L., Amanzo J., Chávez C., Enciso M., García A., Mangel J., Mendoza J., Rojas G., Silva L., Villegas J., Williams R., Zúñiga A., Cruz A., Ruiz E. The order Carnivora (Mammalia) in Peru: research priorities for its conservation.

CONTRIBUTION OF AUTHORS

All parts from this thesis, including: study design, field work execution, laboratory work, data analysis, graphic design and writing were performed by Juan Valqui, if not otherwise acknowledged. Juan Valqui's contribution in the publication figured in Annex II 'Non-invasive genetic analysis reveals high levels of mtDNA variability in the Endangered South-American marine otter (*Lontra felina*)' comprehended the complete field work logistics, sample collection and laboratory work. Data and statistic analysis were performed in cooperation with Frank Zachos. The manuscript was written by Juan Valqui, Frank Zachos and Günther Hartl.

CURRICULUM VITAE

Personal information

Name	Juan Valqui
Date of birth	19.09.1976
Place of birth	Lima, Peru
Nationality	German
Address	Papenkamp 41 – 24114 Kiel, Gemany

Education

02/2006-07/2012	Zoological Institute Christian-Albrechts-Universität zu Kiel. PhD-Student under the thesis title: Population Genetics and Conservation of the Marine Otter (<i>Lontra felina</i>) at the Peruvian Coast
11/2003-10/2004	La Molina National Agrarian University, Lima – Peru Diploma Degree under the thesis title: Behavior of the marine otter <i>Lontra felina</i> (Molina 1782) within an anthropogenic habitat, the Pucusana Bay – Lima, Peru.
08/1996-12/2001	La Molina National Agrarian University, Lima – Peru Bachelor of Science Degree
03/1986-12/1995	Alexander von Humboldt Schule, Lima – Peru High-School Diploma (Deutsches Abitur)

Research and Scientific Work Experience

07/2008 - present	Proyecto Lontra felina Project Director: Field work of the research project on the population genetics of the marine otter and environmental education in Peru.
02/2012-04/2012	Canadian Journal of Zoology Reviewer: ‘Early Holocene glacial retreat isolated river otter (<i>Lontra canadensis</i>) populations along the Alaskan coast’ by M. Seymour et al.
06/2011-07/2011	Mammalian Biology Reviewer: ‘Site and shelter selection by giant river otters (<i>Pteronura brasiliensis</i>) in the Western Brazilian Amazonia’ by D. Santos Lima et al.
02/2011	Molecular Biology Laboratory - Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru Lecturer: Practical Course in Conservation Biology.

11/2009-01/2010	National Geographic Society Scientific assistant: Photographic Project of Kevin Schafer on the marine otter in Peru and Chile.
08/2009-03/2010	IUCN - INRENA (Natural Resources Agency), Peru Assessor: Elaboration of the categorization file of <i>Lontra felina</i> as an Endangered species.
04/2002-08/2003	INRENA (Natural Resources Agency), Peru Researcher and Logistic Director: Population evaluation of the Humboldt Penguin and the Marine Otter at the Peruvian coast.
01/1999	Wildlife Conservation Society, Lima – Peru Field Assistant: Survey of the population of the Humboldt Penguin in southern Peru.

Peer-Reviewed articles

2012 (in press)	Revista Peruana de Biología 19(1). Cossíos, D., Alcázar García, P., Fajardo Quispe, U., Chávez, K., Alfaro-Shigueto, J., Cárdenas-Alayza, S., <u>Valqui, J.</u> , et al. El orden Carnivora (Mammalia) en el Perú: Estado del conocimiento y prioridades de investigación para su conservación.
2011	Mammalian Biology 77: 75-83. <u>Valqui, J.</u> The marine otter <i>Lontra felina</i> (Molina, 1782): A review of its present status and implications for future conservation.
2011	Revista de Ecología Aplicada 10(2): 87-91. Alfaro-Shigueto, J., <u>Valqui, J.</u> , Mangel, J.C. Nuevo registro de la nutria marina <i>Lontra felina</i> (Molina, 1782) al norte de su distribución actual.
2010	Revista Peruana de Ciencias 7 (2): 69-76. <u>Valqui, J.</u> Primer estudio genético de la nutria marina (<i>Lontra felina</i>) en la costa peruana.
2010	Conservation Genetics 11: 2067-2072. <u>Valqui J.</u> , Hartl G.B., Zachos F.E. Non-invasive genetic analysis reveals high levels of mtDNA variability in the endangered South-American marine otter (<i>Lontra felina</i>).
2005	Cotinga 24: 42-44 Barrio, J. and <u>Valqui, J.</u> Fishing by two Furnariidae: Pacific Hornero <i>Furnarius (leucopus) cinnamomeus</i> and Surf Cinclodes <i>Cinclodes taczanowskii</i> .

Oral presentations, posters and other media (selection)

07/2011-08/2011	Participation in the documentary on the Population genetics of endangered species “Die Arche Noah” – Kiel, Germany
10/2010	14th Workshop of the Sociedad Latinoamericana de Especialistas de Mamíferos Acuáticos (SOLAMAC) – Florianópolis, Brazil. Poster and Oral presentation
10/2008	1st Workshop of the Peruvian Zoological Society – Cusco, Peru Oral presentation
11/2004	11th Workshop of the Sociedad Latinoamericana de Especialistas de Mamíferos Acuáticos (SOLAMAC) – Quito, Ecuador. Poster presentation

Scientific Memberships

07/2008-present	Proyecto Lontra felina Director
05/2011-present	IUCN Otter Specialist Group Member
10/2008-present	Deutsche Gesellschaft für Säugetierkunde Member
01/2008-present	CORBIDI (Centro de Ornitología y Biodiversidad), Lima, Peru Associated Researcher
07/2004-12/2007	CEPEC (Peruvian Centre for Cetacean Research) Associated Researcher

DECLARATION

I hereby declare that this thesis is my own work and effort. Where other sources of information have been used, they have been acknowledged, contributions of other persons or co-authors of publications are clearly highlighted as such. This work was not submitted in whole or in part elsewhere in the context of a review process. The work was created in compliance with the rules of good scientific practice of the "Deutsche Forschungsgemeinschaft". This is my first and only doctoral program. The thesis subject is: Population Genetics.

Kiel, _____.06.2012

 Juan Valqui

ERKLÄRUNG

Hiermit erkläre ich, dass die vorliegende Dissertation selbständig von mir angefertigt wurde. Die Dissertation ist nach Form und Inhalt meine eigene Arbeit und es wurden keine anderen als die angegebenen Hilfsmittel verwendet. Beiträge von anderen als meiner Person wurden deutlich als solche hervorgehoben. Diese Arbeit wurde weder ganz noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach Populationsgenetik erfolgen.

Kiel, den _____.06.2012

 Juan Valqui

