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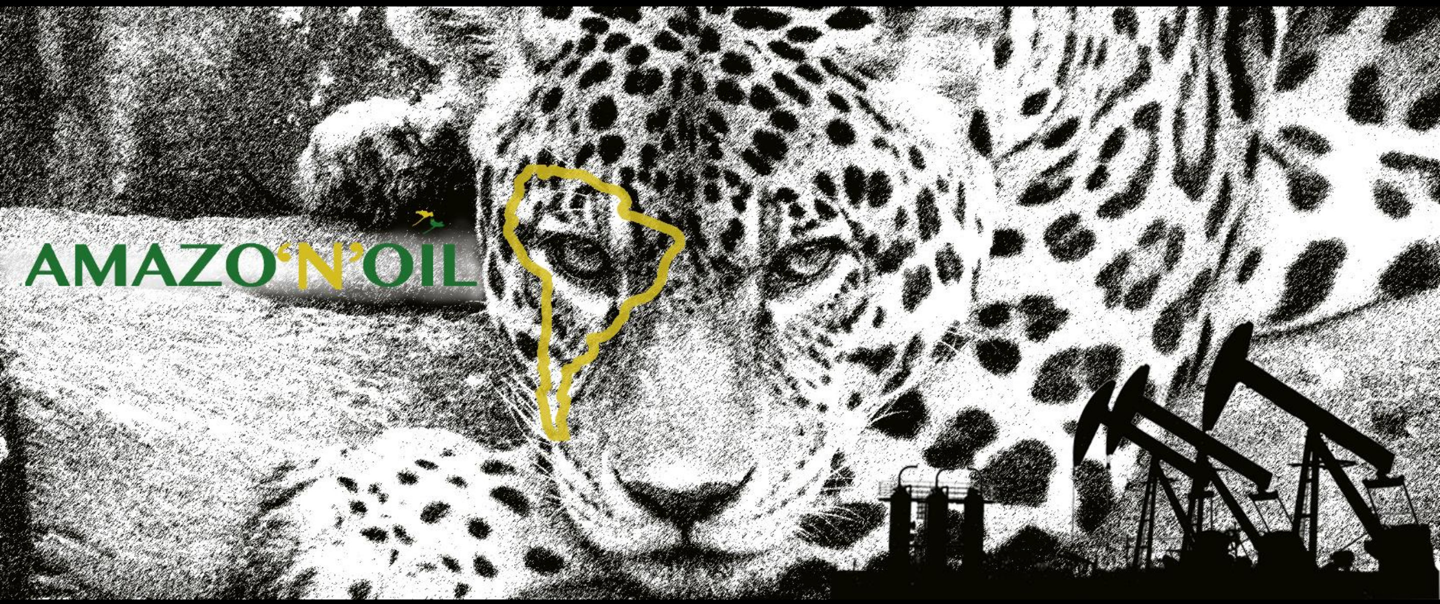
AMAZO'N'OIL: EXPOSURE TO OIL AND LEAD FOR AMAZONIAN WILDLIFE

Mar Cartró-Sabaté



AMAZO'N'OIL: EXPOSURE TO OIL AND LEAD FOR AMAZONIAN WILDLIFE

PhD Thesis | May 2018

Mar Cartró-Sabaté

PhD in Environmental Science and Technology
Institut de Ciència i Tecnologia Ambientals (ICTA)
Universitat Autònoma de Barcelona (UAB)

Directors:
Dr. Martí Orta-Martínez
Dr. Pedro Mayor Aparicio
Dr. Victoria Reyes-García
Mentor:
Dr. Antoni Rosell-Melé

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PhD Dissertation

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Under the direction of:

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Dr. Pedro Mayor Aparicio

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La selva pide tregua, ya no puede resistir.
Navegan en petróleo la guatusa y el tapir.
En un mundo obcecado por comprar y consumir,
me pregunto dónde queda el sentido de vivir.

¿Dónde paseará su canto la serpiente cascabel?
¿Dónde danzará el nenúfar como un barco de papel?
¿Y en qué otra acuarela pintaremos al pincel
los vapores misteriosos de esta tierra de satén?

En la selva cadencias de verdes se pierden en la oscuridad…

Noches de Malva - Nòmades

CONTENT

LIST OF FIGURES

LIST OF TABLES

PREFACE

This manuscript is the result of a PhD Project based at the *Institut de Ciència i Tecnologia Ambientals*, *Universitat Autònoma de Barcelona* (ICTA-UAB). It has been supervised by Dr. Martí Orta-Martínez, Dr. Victoria Reyes-García, Dr. Pedro Mayor-Aparicio and Dr. Antoni Rosell-Melé.

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This dissertation includes four chapters: a general introduction, two research chapters and the final conclusions. Additional information and supplementary materials are presented in the Appendices.

SUMMARY

Tropical rainforests harbour over half of the planet's life forms and are also home to many Indigenous Peoples, despite covering only 7% of the Earth's land surface. Oil activities impacts on tropical rainforest ecosystems and human populations have been scarcely evaluated, although about 30% of the world tropical rainforests overlap with hydrocarbon reservoirs. This PhD thesis is the result of a four-year interdisciplinary study aiming to shed light on one of the unstudied potential impacts of oil activities in tropical rainforests: the ingestion of oil-polluted soils and water by Amazonian wildlife.

Indigenous populations living in a remote oil extraction area in the Peruvian Amazon originally reported the ingestion of oil-polluted soils and water by Amazonian wildlife. In 2013, a two-week camera trap pilot study was conducted to investigate this claim. Videos clearly showed three ungulate and one rodent species ingesting oil-polluted soil and water, illustrating an animal behaviour unknown to the scientific community and raising questions about the environmental and public health implications of this behaviour. In this context, the overarching goals of this thesis were 1) to study this potential new route of exposure to oil-related pollutants for wildlife and 2) to determine whether wild species consumed by local populations living in the vicinity of oil extraction areas was polluted by lead (Pb), a pollutant related to the oil extraction industry.

Over the course of four years, a myriad of methodologies from diverse disciplines (i.e., ethology, conservation biology, environmental chemistry, environmental forensics, but also spatial analysis, community-based monitoring, and citizen science) have been used framed in the field of environmental sciences. To study the ingestion of oil-polluted soil and water by wildlife and the bioaccumulation of lead (Pb), this thesis relied on (1) the analysis of 2,206 videos recorded by trap-cameras during 452 camera days in four oil-polluted sites and three natural salt licks; (2) the analysis of metals and hydrocarbon content in soil samples collected in the seven study sites; (3) a spatial analysis of the oil infrastructure; (4) the analysis of Pb concentration and isotopic fingerprint in 315 liver samples from 18 free-ranging species hunted for food by Indigenous Peoples in an oil concession and in two different control areas of the Peruvian Amazon. The participation of local people has been crucial to develop this research, including developing the research questions, designing the methodology, collecting and analysing data, and interpreting results.

Two main results derive from this dissertation. First, the ingestion of oil-polluted soil and water by wildlife is a frequent widespread behaviour, both in taxonomical and geographical terms. The ingestion of oil-polluted soil has been detected in eight species from the mammalian orders Rodentia, Artiodactyla and Perissodactyla and the avian order Psittaciformes. Seven more species have been recorded visiting these polluted sites. Moreover, this behaviour was frequently reported in all the oil-polluted sites studied. The

extent of the behaviour might be explained by the fact that animals are, indeed, redirecting geophagy from natural salt licks to oil-polluted sites.

Second, liver tissues from wild and free-ranging Amazonian species presented high Pb concentrations, comparable to those found in wildlife in industrial countries and mining areas. About half (49.8%) of the biological samples studied had Pb concentrations above acceptable limits of offal for human consumption according to the UE Commission Regulation. Lead-based ammunition and oil pollution are identified as the main sources of Pb pollution. These findings suggest that mainly lead-based ammunition, but also, oil extraction activities may pose an environmental and health risk to wild species and local human populations that rely on subsistence hunting for their livelihoods. These results suggest that, similarly to oil-related Pb, other toxic and cumulative compounds related with oil extraction might also be entering the food chain and threatening wildlife and human populations, but more research on this topic is needed.

Given these findings, this thesis argue for the need to increase research on oil extraction impacts in tropical rainforests. The results also highlight that the phase-out of lead-based ammunition around the world is urgently needed. Finally, governmental institutions and oil companies are invited to promptly and efficiently review the operational standards and remediation practices of oil companies operating in tropical areas.

RESUMEN

Los bosques tropicales albergan más de la mitad de las formas de vida que se encuentran en el planeta y son también el hogar de muchos pueblos indígenas, a pesar de solo cubrir el 7% de la superficie terrestre. Los impactos de las actividades petroleras en los ecosistemas y las poblaciones humanas de los bosques tropicales apenas se han evaluado, aunque aproximadamente el 30% de los bosques tropicales del mundo se superponen con reservas de hidrocarburos. Esta tesis doctoral es el resultado de un estudio interdisciplinario de cuatro años que tenía por objetivo explorar uno de los impactos potenciales de la actividad petrolera en los bosques tropicales que aún no había sido estudiado: la ingesta de suelos y agua contaminados por petróleo por parte de la fauna amazónica.

Los pobladores indígenas que habitan una concesión petrolera remota en la Amazonía peruana fueron los primeros en reportar la ingesta de suelos y agua contaminados por petróleo por parte de la fauna amazónica. En el año 2013 se llevó a cabo un estudio piloto de trampeo fotográfico de dos semanas para investigar este fenómeno. Los vídeos muestran claramente tres especies de ungulados y una especie de roedor ingiriendo suelos y aguas contaminadas por petróleo, ilustrando un comportamiento animal desconocido por la comunidad científica y planteando interrogantes sobre las implicaciones ambientales y de salud pública de este comportamiento. En ese contexto, los objetivos generales de esta tesis fueron 1) estudiar esta potencial nueva vía de exposición a los contaminantes de la industria hidrocarburífera para la fauna silvestre y 2) determinar si las especies consumidas por las poblaciones locales que viven en las zonas de extracción de petróleo están contaminadas por el plomo (Pb), un contaminante relacionado con la industria de extracción de petróleo.

A lo largo de cuatro años, se han utilizado metodologías de diversas disciplinas (i.e., etología, biología de conservación, química y forénsica ambiental, así como análisis espacial, monitorización participativa y ciencia ciudadana), enmarcadas en el campo de las ciencias ambientales. Para analizar la ingesta de suelos y aguas contaminados por petróleo por parte de la fauna silvestre y la bioacumulación de Pb en los tejidos animales, esta tesis se basa en (1) el análisis de 2.206 vídeos grabados con cámaras trampa durante 452 días de muestreo en cuatro sitios contaminados por petróleo y tres salitrales; (2) el análisis del contenido de metales y de hidrocarburos en las muestras de suelo recogidas en los siete sitios de estudio; (3) un análisis espacial de la infraestructura petrolera; (4) el análisis de la concentración de Pb y su huella isotópica en 315 muestras de hígado de 18 especies de animales silvestres cazados como alimento por parte de los cazadores locales en una concesión petrolera y en dos zonas control de la Amazonía peruana. La participación de la población local ha sido fundamental para desarrollar esta

investigación, incluyendo la formulación de las preguntas de investigación, el diseño del estudio, la recogida y el análisis de datos, y la discusión de los resultados.

De esta tesis se derivan dos resultados principales. El primer resultado es que la ingesta de suelo y agua contaminados por petróleo por parte de la fauna silvestre es un comportamiento frecuente generalizado, tanto en términos taxonómicos como geográficos. La ingesta de suelo contaminado por petróleo se ha detectado en ocho especies de mamíferos de los órdenes Rodentia, Artiodactyla y Perissodactyla y en aves del orden Psittaciformes. Se han registrado siete especies más visitando estos sitios contaminados. Además, este comportamiento se detectó en todos los sitios de estudio contaminados por petróleo. El grado de extensión de este comportamiento podría explicarse por el hecho de que los animales están redirigiendo la geofagia de los salitrales a los sitios contaminados con petróleo.

El segundo resultado es que los tejidos hepáticos de especies amazónicas silvestres presentaban altas concentraciones de Pb, comparables a las que se encuentran en la fauna silvestre de los países industriales y de zonas mineras. El 49,8% de las muestras biológicas estudiadas tenían concentraciones de Pb por encima de los límites aceptables en las vísceras para el consumo humano, según el Reglamento de la Comisión Europea. La munición de Pb y la contaminación por petróleo destacan como principales fuentes de contaminación por Pb. Estos hallazgos sugieren que tanto la munición de Pb como las actividades de extracción de petróleo pueden suponer un riesgo ambiental y sanitario para las especies silvestres y para las poblaciones humanas locales que dependen de la caza para su subsistencia. Estos resultados sugieren que, igual que el Pb, otros compuestos tóxicos y bioacumulabes relacionados con la industria petrolera también podrían entrar en la cadena alimentaria y amenazar la vida silvestre y la salud de la población humana local, aunque son necesarios estudios adicionales en este campo.

Ante estos hallazgos, es necesario que haya un mayor esfuerzo en la investigación sobre los impactos de la extracción de hidrocarburos en los bosques tropicales. Los resultados también señalan la importancia de abandonar urgentemente el uso de munición de Pb a nivel mundial. Finalmente, también se invita a las instituciones gubernamentales y las compañías petroleras a que analicen de forma urgente y eficaz las prácticas operacionales y de remediación de las compañías petroleras que operan en las zonas tropicales.

RESUM

Els boscos tropicals alberguen més de la meitat de les formes de vida que es troben al planeta i també són la llar de molts pobles indígenes, malgrat cobrir només el 7% de la superfície terrestre. Tot i que, aproximadament, el 30% dels boscos tropicals del món se superposen amb reserves d'hidrocarburs, els impactes de les activitats petrolieres en els ecosistemes i les poblacions humanes tropicals s'han avaluat de manera escassa. Aquesta tesi doctoral és el resultat d'un estudi interdisciplinari de quatre anys que tenia per objectiu explorar un dels potencials impactes de les activitats petrolieres en els boscos tropicals que encara no havia estat estudiat: la ingesta de sòls i agua contaminats pel petroli per part de la fauna amazònica.

La població indígena que habita una remota concessió petrolera a l'Amazònia peruana fou la primera en reportar la ingesta de sòls i aigua contaminats per petroli per part de la fauna amazònica. L'any 2013 es va dur a terme un estudi pilot de trampeig fotogràfic de dues setmanes per investigar aquest fenomen. Els vídeos mostren clarament tres espècies d'ungulats i una espècie de rosegador ingerint sòls i aigües contaminades per petroli, il·lustrant un comportament animal desconegut per la comunitat científica i plantejant interrogants sobre les implicacions ambientals i de salut pública d'aquest comportament. Partint d'aquest context, els objectius generals d'aquesta tesi foren 1) estudiar aquesta nova potencial via d'exposició a contaminants de la industria petroliera per a la fauna silvestre i 2) determinar si les espècies consumides per les poblacions humanes que viuen a zones d'extracció de petroli estan contaminades per plom (Pb), un contaminant relacionat amb la indústria d'extracció de petroli.

Al llarg de quatre anys, s'han utilitzat metodologies de diverses disciplines (i.e., etologia, biologia de conservació, química i forènsica ambientals, així com anàlisi espacial, monitoratge participatiu i ciència ciutadana), emmarcades en el camp de les ciències ambientals. Per poder analitzar la ingesta de sòls i aigua contaminats per petroli per part de la fauna silvestre i la bioacumulació de Pb en teixits animals, aquesta tesi es basa en (1) l'anàlisi de 2.206 vídeos gravats per càmeres trampa durant 452 dies de mostratge a quatre zones contaminades per petroli i tres afloraments salins naturals; (2) l'anàlisi del contingut de metalls i d'hidrocarburs en les mostres de sòl recollides a set zones d'estudi; (3) una anàlisi espacial de la infraestructura petroliera; (4) l'anàlisi de la concentració i de l'empremta isotòpica del Pb en 315 mostres de fetge de 18 espècies d'animals silvestres caçats com aliment per part de la població indígena que viu dins d'una concessió petroliera i a dues zones control de l'Amazònia peruana. La participació de la població local ha estat fonamental per desenvolupar aquesta recerca, des de la formulació de les preguntes de recerca, al disseny de l'estudi, la recollida i l'anàlisi de dades fins la discussió dels resultats.

D’aquesta tesi es deriven dos resultats principals. El primer resultat és que la ingesta de sòl i aigua contaminats per petroli per part de la fauna silvestre és un comportament freqüent generalitzat, tant en termes taxonòmics com geogràfics. La ingesta de sòl contaminat per petroli s'ha detectat en vuit espècies de mamífers de les ordres Rodentia, Artiodactyla i Perissodactyla i en aus de l'ordre Psittaciformes. També s'han registrat set espècies més visitant les zones d’estudi contaminades. A més, aquest comportament fou detectat de forma freqüent en totes les zones estudiades. El grau d’extensió d’aquest comportament es podria explicar pel fet que els animals estan redirigint la geofagia dels afloraments salins naturals a zones contaminades per petroli.

El segon resultat és que els teixits hepàtics d'espècies amazòniques silvestres presentaven altes concentracions de Pb, comparables a les que es troben en la fauna silvestre dels països industrials i de zones mineres. El 49,8% de les mostres biològiques estudiades tenien concentracions de Pb per sobre dels límits acceptables en les vísceres pel consum humà segons el Reglament de la Comissió Europea. La munició de Pb i la contaminació per petroli destaquen com a principals fonts de contaminació per Pb. Aquestes troballes suggereixen que tant la munició de Pb com les activitats d'extracció de petroli suposen un risc ambiental i sanitari per a les espècies silvestres així com per les poblacions humanes locals que depenen de la caça de subsistència. Aquests resultats suggereixen que, de manera similar al Pb, altres compostos tòxics i bioacumulables relacionats amb la industria petroliera també podrien entrar a la cadena alimentària i amenaçar la vida silvestre i la salut de la població humana, però caldria recerca addicional en aquest camp per a estudiar-ho.

Davant aquestes troballes, és necessari un major esforç en la investigació sobre els impactes de l’extracció de hidrocarburs en els boscos tropicals. Els resultats també indiquen que és necessari i urgent l’abandonament de l’ús de munició de Pb a nivell mundial. Finalment, també es convida a les institucions governamentals i a les companyies petrolieres a que revisin de manera urgent i eficaç les pràctiques operacionals i de remediació de les companyies petrolieres que operen a les zones tropicals.

MAIN ABBREVIATIONS AND ACRONYMS

AIDESEP	*Asociación Interétnica de Desarrollo de la Selva Peruana*
Al	Aluminium
As	Arsenic
Ba	Barium
Be	Beryllium
BPD	Barrels Per Day
Ca	Calcium
CCME	Canadian Council of Ministers of the Environment
Cd	Cadmium
CITES	Convention on International Trade in Endangered Species
Cl	Chlorine
CO_2	Carbon dioxide
Cr	Chromium
Cr (VI)	Hexavalent Chromium
Cu	Copper
DDT	Dichlorodiphenyltrichloroethane
DIGESA	*Dirección General de Salud Ambiental*
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
FECONACOR	*Federación de Comunidades Nativas del Corrientes*
FECONAT	*Federación de las Comunidades Nativas del Tigre*
FEDIQUEP	*Federación Indígena Quechua del Pastaza*
FPIC	Free, Prior and Informed Consent
GC-MS	Gas Chromatography-Mass Spectrometry
GIS	Geographic Information System
GPS	Global Positioning System
HCl	Hydrochloric acid
HF	Hydrofluoric acid
Hg	Mercury
HNO_3	Nitric acid
HR	Home Range
I./V.	Individuals per Video
IARC	International Agency for Research on Cancer
ICP-MS	Inductively coupled plasma mass spectrometry
ICTA	*Institut de Ciència i Tecnologia Ambientals*
IIAP	*Instituto de Investigaciones de la Amazonía Peruana*

ILO	International Labour Organization
INEI	*Instituto Nacional de Estadística e Informática*
IPCC	Intergovernmental Panel on Climate Change
IPC-OES	Inductively Coupled Plasma Optical Emission Spectrometer
IQR	Interquartilic Range
IUCN	International Union for Conservation of Nature
K	Potassium
MELPGIS	Monitor Environmental Liabilities through Participatory GIS
MEM	*Ministerio de Energía y Minas*
Mg	Magnesium
MINSA	*Ministerio de Salud del Perú*
Mn	Manganese
MPL	Maximum Permissible Levels
MSL	Mobility to Salt Lick
Na	Sodium
NGO	Non-Governmental Organisation
Ni	Nickel
OECD	Organisation for Economic Co-operation and Development
OEFA	*Organismo de Evaluación y Fiscalización Ambiental*
ONERN	*Oficina Nacional de Evaluación de Recursos Naturales*
ORPIO	*Organización Regional de Pueblos Indígenas del Oriente*
OSINERG	*Organismo Supervisor de la Inversión en Energía y Minería*
OSINERGMIN	*Organismo Supervisor de la Inversión en Energía y Minería*
P	Phosphorus
PAH	Polycyclic Aromatic Hydrocarbon
PAR	Participatory Action Research
Pb	Lead
PCB	Polychlorinated Biphenyl
SD	Standard Deviation
UAB	*Universitat Autònoma de Barcelona*
UE	European Union
UN	United Nations
UNEP	United Nations Environmental Programme
USD	United States Dollar
USGS	United Sates Geological Survey
V	Vanadium
VD	Visit Duration
VF	Visit Frequency
WGS	World Geodetic System
WHO	World Health Organisation
Zn	Zinc

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CHAPTER I

INTRODUCTION

Picture in the previous page: *Oil spill reaches an Indigenous community in the Corrientes River basin, 2018.*
Source: Roberto Sandi Maynas and FENOCANOR.

CHAPTER I • INTRODUCTION

BACKGROUND AND MOTIVATION

Conventional oil represents the main source of the energy consumed in the entire world (International Energy Agency, 2017a). Over 95 million barrels per day (BPD) are consumed daily worldwide (International Energy Agency, 2017b). The main producer countries of crude oil[1] are Saudi Arabia (13.5%), Russian Federation (12.6%), United States of America (12.4%), Canada (5.1%), Islamic Republic of Iran (4.6%), People's Republic of China (4.6%), Iraq (4.4%), United Arab Emirates (4.2%), Kuwait (3.7%) and Brazil (3.1%) (International Energy Agency, 2017a). Although 50% of the top ten crude-oil-producers countries are from Middle East, OECD[2] countries are, by far, the main oil consumers, followed by China, Russia and other Asian, Eurasian and European countries not included in the OECD (International Energy Agency, 2017a).

In addition to being the ultimate supplier of the most relevant world sources of CO_2 emissions (International Energy Agency, 2017a), the oil industry generates many different impacts on human populations and on the ecosystem. Oil industry impacts vary depending on the industry sector (e.g., exploration, extraction, transport, storage, and refinery) and on the type of operation (i.e., offshore or onshore) (Epstein *et al.*, 2002; Van Hinte *et al.*, 2007; Orta-Martínez and Finer, 2010; O'Callaghan-Gordo *et al.*, 2016).

While the impacts of oil spills on the health of clean-up workers and neighbour inhabitants in coastal areas affected by oil spills have been well studied, much less is known about the health repercussions on human populations residentially exposed to oil extraction, which normally occurs in low-middle income countries (O'Callaghan-Gordo *et al.*, 2016). Similarly, much more research has been carried out to assess the impacts of oil spills on marine than on terrestrial ecosystems. Notwithstanding, 71% of oil

[1] "Includes the production of crude oil, natural gas liquids, feedstocks, additives and other hydrocarbons. Excludes liquids from other fuel sources (renewable, coal and natural gas)" (International Energy Agency, 2017a).

[2] The Organisation for Economic Co-operation and Development (OECD) includes 35 countries, which include 21 countries from the European Union, the United States of America, Japan, Korea, Canada, Australia, New Zealand, Chile, Mexico, Israel, Switzerland, Iceland, Norway and Turkey (OECD, 2017).

production is obtained in onshore operations, while only the remaining 29% comes from offshore operations (U.S. Energy Information Administration, 2016).

Despite covering only 7% of the Earth's land surface, tropical forests probably harbour over half of the planet's life forms (Wilson, 1988 in Laurance, 1999) and they are also home to many Indigenous Peoples[3] (Alcorn, 1993). An alarming fact is that 30% of world's tropical forests overlap with hydrocarbon concessions (Orta-Martínez, Rosell-Melé, *et al.*, 2018), turning oil extraction into a major threat to the most diverse and ecologically complex land communities on earth (Myers, 1985; Heywood, 1995; Laurance, 1999; Lewis *et al.*, 2004). However, the impacts of oil activities on tropical rainforests' human populations and ecosystems have been scarcely evaluated (O'Callaghan-Gordo *et al.*, 2016; Orta-Martínez, Rosell-Melé, *et al.*, 2018). Arguably, the discovery of unstudied impacts generated by oil activities in these areas will not be very surprising. This thesis aims to shed light on one of these unstudied potential impacts: the ingestion of oil-polluted soils and water by Amazonian wildlife.

The ingestion of oil-polluted soils and water by Amazonian wildlife was originally reported by Indigenous populations in a remote area in the Peruvian Amazon where oil extraction takes place. Indigenous Peoples in the area repeatedly described wild animals' ingestion of soil and water in places affected by the dumping of oil and other by-products of the oil extraction industry. They even pointed out the presence of oil in the gastrointestinal contents and faeces of wild animals hunted for human consumption (Orta-Martínez and O'Callaghan-Gordo, pers. observ.). In 2013, a pilot study was performed to

[3] Although no universally agreed definition for the term "Indigenous Peoples" exists, one of the most used is that formulated by Jose Martínez Cobo, the UN Secretariat of the Permanent Forum on Indigenous Issues, in 1989: "Indigenous communities, peoples and nations are those which, having a historical continuity with pre-invasion and pre-colonial societies that developed on their territories, consider themselves distinct from other sectors of the societies now prevailing on those territories, or parts of them. They form at present non-dominant sectors of society and are determined to preserve, develop and transmit to future generations their ancestral territories, and their ethnic identity, as the basis of their continued existence as peoples, in accordance with their own cultural patterns, social institutions and legal system. This historical continuity may consist of the continuation, for an extended period reaching into the present of one or more of the following factors: a) Occupation of ancestral lands, or at least of part of them; b) Common ancestry with the original occupants of these lands; c) Culture in general, or in specific manifestations (such as religion, living under a tribal system, membership of an indigenous community, dress, means of livelihood, lifestyle, etc.); d) Language (whether used as the only language, as mother-tongue, as the habitual means of communication at home or in the family, or as the main, preferred, habitual, general or normal language); e) Residence on certain parts of the country, or in certain regions of the world; f) Other relevant factors. On an individual basis, an indigenous person is one who belongs to these indigenous populations through self-identification as indigenous (group consciousness) and is recognized and accepted by these populations as one of its members (acceptance by the group). This preserves for these communities the sovereign right and power to decide who belongs to them, without external interference" (Stephensen et al., 2006).

investigate this claim. A trap camera was installed for two weeks in two different sites within an oil concession in the northern Peruvian Amazon (Oil block 1AB/192) where, according to the local population, game species gathered to ingest polluted soil. These sites were locally considered as good hunting spots although both places were oil-polluted and close to oil infrastructure. Videos clearly showed three ungulates species (*Tapirus terrestris, Mazama Americana* and *Pecari tajacu*) and one rodent species (*Cuniculus paca*) ingesting oil-polluted soil and water (Mayor *et al.*, 2014; Orta-Martínez, Rosell-Melé, *et al.*, 2018). These images recorded illustrated the existence of an animal behaviour unknown to the scientific community and which raised numerous questions about its associated environmental and public health implications. The finding also highlighted the importance of filling the large knowledge gaps in identifying and understanding the myriad of impacts generated by oil activities in tropical rainforests.

Considering that hydrocarbon activities were, and still are, rapidly spreading over the remaining worldwide rainforests, it was necessary and urgent 1) to assess this potential new route of exposure to oil-related pollutants for both wildlife and human populations and 2) to answer whether game species consumed by local populations living in the vicinity of oil extraction areas were polluted. The present doctoral thesis contributes to these relevant and emerging environmental and health issues.

OIL ACTIVITIES IN RAINFORESTS – THE PERUVIAN AMAZON

THE AMAZON: VALUES AND THREATS

The Amazon is the largest remaining extension of tropical rainforest (Foley *et al.*, 2007) containing a high portion of the world's diversity (Myers *et al.*, 2000), and harbouring one out of every five mammal, fish, bird and tree species on earth (IPCC, 2007; Nepstad *et al.*, 2008). Moreover, it is the largest extension of primary forest (FAO, 2010b, 2010a) and one of the three core areas with the highest biocultural diversity on earth (Loh and Harmon, 2005). The Amazon also hosts an incredible diversity of human societies, where at least 300 languages are spoken (Aikhenvald, 2012; Epps and Salanova, 2013) - most of them are endemic languages and 75% of them with 1,000 or fewer speakers (Gorenflo *et al.*, 2012). The region is also the home of most (~50) of the last isolated Indigenous groups on earth, the so-called Indigenous Peoples living in voluntary isolation[4] (Gamboa and Santillán, 2006; Napolitano and Ryan, 2007; Lawler, 2015).

Despite the high biocultural diversity of Amazonian rainforests and the multiple goods and services that they provide, these ecosystems face a number of threats related to human activities (Foley *et al.*, 2007). High rates of deforestation have affected the region for many years and are jeopardizing the ecological integrity of the rainforests (Foley *et al.*, 2007). Furthermore, rapid deforestation is likely to continue since its main drivers keep increasing: there is a growing expansion of cattle ranching, a rising soybean production, and the road network through the core of the rainforests is becoming larger (Foley *et al.*, 2007; Malhi *et al.*, 2008). Many other human activities, such as overhunting, illegal logging, oil palm plantations, mining projects, and fire leakage are also directly threatening the Amazon (Malhi *et al.*, 2008; Bass *et al.*, 2010). Around the 64% of the Amazon is located in Brazil and 80% of Amazonian deforestation has occurred in this country, mostly caused by cattle ranching (Soares-Filho *et al.*, 2006). Amazonian deforestation is concentrated on the southern and eastern margins of the Amazon (in Rondônia, Pará and Mato Grosso, the so-called "arc of deforestation") and also along the Andean piedmont (Malhi *et al.*, 2008). The eastern Amazon in Brazil has obtained much global attention since it is likely to experience continued massive deforestation (Soares-Filho *et al.*, 2006) and drought in the future decades (Malhi *et al.*, 2008). By contrast, the western Amazon, lying on Bolivia, Colombia, Ecuador, Peru, and western Brazil, still preserves large extensions of roadless and relatively intact rainforests (Bass *et al.*, 2010), presenting a high probability of stable climatic conditions in the face of global warming (Killeen *et al.*, 2007; Finer *et al.*, 2008). The region is considered one of the world's Pleistocene refuges and a biological refuge, hosting the highest biodiversity of the Amazon. It is also the least vulnerable region of the Amazon to climatic drying (Malhi *et al.*, 2008). The Western Amazon biodiversity is one of the highest of the planet for many taxa, such as mammals, birds, plants and amphibians (Stotz *et al.*, 1996; Ter Steege *et al.*,

[4] Indigenous Peoples living in voluntary isolation are Indigenous groups that avoid contact with individuals not belonging to their social group. This decision was largely taken based on the dreadful losses by disease and persecution during the rubber boom (late 1800s-1915). They retreated into very remote areas of the Amazon to avoid contact with outsiders and they continue living there (Napolitano & Ryan 2007).
They are described as some of the world's most vulnerable people (Lawler, 2015) as they are extremely vulnerable to illnesses brought by outsiders, due to their lack of resistance and immunity, (Albán, 2005; Napolitano and Ryan, 2007). Between a third and half of the population is estimated to die within the first five years after the first 'face-to-face' contact with outsiders (Hill & Hurtado, 1995; Napolitano & Ryan, 2007).

2003; Young *et al.*, 2004; Ceballos *et al.*, 2005). Recently, a global study on road building categorized the western Amazon as a "priority road-free" area, due to its environmental values (i.e., biodiversity and wilderness) and its moderated agricultural potential (Laurance *et al.*, 2014).

HYDROCARBON ACTIVITIES IN THE WESTERN AMAZON

The Amazon also faces a highly relevant and increasing menace: the oil industry. Indeed, many oil and gas concessions largely overlap with Amazonian rainforest: 39.4% of the Amazonian rainforests overlap with conventional oil and gas reserves, a percentage that resembles the ~30% of the estimated worldwide rainforests overlapping with oil and gas reserves (Orta-Martínez, Rosell-Melé, *et al.*, 2018).

Moreover, this overlap is expected to increase since Amazonian countries are strongly promoting hydrocarbon exploration (Finer *et al.*, 2008, 2015) as a consequence of the globally growing oil demand. Indeed, oil demand increased from 85.3 to 96.56 million BPD between 2006 and 2016 (BP, 2017), pushing the hydrocarbon frontier towards more remote territories and towards unconventional sources whose exploitation is generally associated to higher costs, risks and impacts (Orta-Martínez and Finer, 2010).

Indeed, the territorial extension of hydrocarbon blocks was already alarmingly high in 2008 –over 688,000 Km^2 of the western Amazon, including the blocks in exploration phase (Finer *et al.*, 2008). However, the western Amazonian hydrocarbon frontier expanded to up to 733,414 Km^2 in 2014. Moreover, this frontier is likely to continue pushing into more remote areas, since many untapped hydrocarbon discoveries have been suspected and/or confirmed in the last few years (Finer *et al.*, 2015). Simultaneously, over the last decades governments in the western Amazon have created new laws to allow oil exploration and exploitation in natural protected areas to promote hydrocarbon activities. This is the case of the Bolivian government (Fernández-Llamazares and Rocha, 2015). This is also the case of the Ecuadorian government, which allows the overlapping of oil activities and protected areas if authorized by the President and declared by the Congress (Finer *et al.*, 2010). In Peru only areas designated as National Parks and National and Historic Sanctuaries are strictly off-limits to extractive activities for oil and gas (Finer and Orta-Martínez, 2010), and the overlap of hydrocarbon activities with other less strictly protected areas, Indigenous People's titled lands, and proposed or created reserves for Indigenous People in voluntary isolation is very common (Finer and Orta-Martínez 2010). This leeway is affecting emblematic protected areas of the aforementioned countries, such as the Madidi (Bolivia), the Yasuní (Ecuador) and the Manu (Peru) National Parks (Finer *et al.*, 2008, 2015).

The oil industry has been pointed out as the cause of many cultural, social and environmental injuries (Kimerling, 2006; Van Hinte *et al.*, 2007; Orta-Martínez and Finer, 2010; Campanario and Doyle, 2017; McKenzie *et al.*, 2017). Diverse oil activities have been associated to different types of impacts. The impacts of oil exploration activities (seismic exploration) are usually seen as low-level, short-term, and temporary, despite affecting huge areas (UNEP Technical Publication & E&P Forum, 1997). These impacts consist in "transient" deforestation in seismic lines, basecamps, heliports, and noise from seismic detonations. However, in the Amazon these impacts cannot be considered low-level nor short-term, but rather grave and irreversible (Orta-Martínez and Finer, 2010). The mere presence of outsiders in remote areas may be a lethal threat for Indigenous Peoples living in voluntary isolation, as an accidental contact with the oil workers might suppose the death of up to half their population (Hill and Hurtado, 1995). Even if there is no contact, isolated people may be forced to move to other territories to avoid the risks of contact (Napolitano and Ryan, 2007; Orta-Martínez and Finer, 2010). Noise may also affect animals' behaviour, consequently impacting both the whole ecosystem and local people who depend on wild meat for their livelihood (Van Hinte *et al.*, 2007).

Differently, oil extraction impacts are normally described as long-term and chronic, but affecting small areas (UNEP Technical Publication & E&P Forum, 1997). Deforestation and noise persist during oil extraction and normally increase as more roads, base camps, airports, wells, pipelines and other infrastructure are built to accommodate the staff and conduct extractive activities. In the western Amazon, oil extraction impacts are particularly alarming since hydrocarbon projects are normally located in remote areas and oil and gas roads are potentially "the first cut" into areas that would otherwise be relatively well conserved and road-free (Laurance *et al.*, 2014; Finer *et al.*, 2015). Oil access roads are reported to trigger new deforestation fronts and to facilitate many other social-environmental impacts, such as colonization, illegal logging, and over-hunting (Sierra, 2000; Suárez *et al.*, 2009; Laurance *et al.*, 2014).

With the opening of roads linked to the oil industry, access to the area is normally eased and therefore increased. This, in turn, raises the exposure risk to other impacts associated with the arrival of foreigners, such as merchants or illegal loggers. Examples of these indirect impacts are the cultural erosion and the integration of Indigenous Peoples and Local Communities to the market economy and the loss of their traditional ecological knowledge, the introduction and transmission of diseases, overhunting, or illegal logging, to name a few (Van Hinte *et al.*, 2007). These impacts might be further exacerbated by the racist and disrespectful attitudes of outsiders, in addition to the long story that carry the Amazonian people, through many types of abuses, mockery, and the unrecognition of their rights (Martín-Beristain *et al.*, 2009; Orta-Martínez and Finer, 2010).

Pollution is another major threat related to oil extraction. Although there is a global tendency on improving environmental standards and regulations for oil activities, operations in these remote areas do not stand at the technological forefront (López and Napolitano, 2007). Moreover, in some cases oil companies operations do not even meet minimum internationally accepted standards. This situation is aggravated by the fact that Indigenous communities are often unaware of the risks of oil pollution, like the toxicity of the different compounds present in crude oil. Thus, they do not start complaining against contamination caused by low operational standards until damages become very visible (i.e., normally with the appearance of intoxication cases and new illnesses, the increase of miscarriage cases or other health impacts) (Martín-Beristain *et al.*, 2009; Orta-Martínez, 2010; Orta-Martínez and Finer, 2010). Sometimes Indigenous Peoples do not even have the opportunity to lodge their complaints against the oil company to other external stakeholders such as state agencies, NGOs or courts and the conflict is muted (Orta-Martínez and Finer, 2010). Since the State is nearly absent in these regions, Indigenous communities, normally isolated and misinformed, are in a very vulnerable situation when facing oil activities threats. Moreover, oil companies often cover some immediate needs of Indigenous communities, such as zinc roofs, electric generators, or health assistance. In some cases, oil companies are the most important source of cash since employing most young men in the area. This dependence relation might weaken the negotiation capacity of local people (Orta-Martínez and Finer, 2010).

Since Indigenous Peoples depend on their territories for their livelihood, environmental damages to them carry health, social and cultural impacts (Maffi, 2005; Orta-Martínez and Finer, 2010; Gorenflo *et al.*, 2012; Ens *et al.*, 2016). Therefore, it is not surprising that oil activities are posit to entail direct and indirect health problems. Simultaneously, it is documented that cultural degradation is often linked with biodiversity loss (Raymond, 2007). Therefore, the integration of Indigenous Peoples and Local Communities to the market economy, the cultural erosion and the loss of practices and knowledge from Indigenous Peoples might also foster further environmental degradation.

The growing oil industry in Amazonian rainforests, and their associated health and environmental risks could have further exacerbated the underlaying causes of the poor health indicators of the Indigenous Peoples. Indigenous Peoples present, today, one of the poorest indices of wellbeing and health in their countries (Stephens *et al.*, 2006).

HYDROCARBON ACTIVITIES IN THE PERUVIAN AMAZON

The Peruvian and the Ecuadorian Amazon present the highest overlap with hydrocarbon blocks in the western Amazon (Finer *et al.*, 2008). The Peruvian Amazon comprises the second largest area of the Amazon Basin and the conservation of its 661,000 Km^2 of tropical forests is considered a priority in most of the global biodiversity inventories (Brooks *et al.*, 2006; Oliveira *et al.*, 2007). The area is also home to 60 Indigenous ethnic groups from 13 linguistic families (Benavides, 2005; INEI, 2009; Huamán, 2017). Moreover, there are at least 14-15 Indigenous groups living in voluntary isolation in the Peruvian Amazon (Albán, 2005; Finer and Orta-Martínez, 2010). The Peruvian Amazon is a complex puzzle where protected areas, Indigenous titled lands, and territorial reserves for voluntary isolated Indigenous groups largely overlap with active hydrocarbon concessions that are already in the exploration or exploitation phase (Finer and Orta-Martínez, 2010). Moreover, seismic lines spread over a wide part of the Peruvian Amazon (Figure 1.1). Around 84% of the Peruvian Amazon has been covered by active and proposed oil and gas blocks at some point in time during the last 40 years (Finer and Orta-Martínez, 2010) and ~14 untapped hydrocarbon discoveries have been suspected and/or confirmed in the last years (Finer *et al.*, 2015). Of concern is the fact that only 10% of the Peruvian Amazon (national parks and sanctuaries) is completely protected from oil and gas activities (Orta-Martínez and Finer, 2010). More than one-third (~40%) of the active blocks overlapped with less strictly protected areas, covering the 17.1% of the Peruvian Amazon protected area system. Around 90% of the active blocks extended along Indigenous Peoples titled lands, affecting ~55% of the Indigenous lands. Even more worrisome, ~33% of the blocks overlaid with proposed or official reserves for Indigenous

Peoples in voluntary isolation, affecting ~61% and ~17% of the proposed and created reserves, respectively (Finer and Orta-Martínez, 2010). Not surprisingly, several deathful contact cases between Indigenous Peoples in voluntary isolation and oil companies have been reported in Peru in the last half century (see review in Orta-Martínez & Finer, 2010). The increasing recent contact situations have been described as genocide by many anthropologists and missionaries (Shepard *et al.*, 1999; Napolitano and Ryan, 2007).

The oil history of South America began in the northern pacific coast of Peru, where the first oil well of the region was drilled. Oil extraction did not reach the Peruvian Amazon until 1939, with the beginning of oil activities in Aguas Calientes (Orta-Martínez and Finer, 2010). The most productive period of the Peruvian oil history occurred between the 1970s-1980s, tattooing the Peruvian Amazon with at least 670 wells and 100,000 Km of seismic lines. The national peak oil was in 1979. However, hydrocarbon activities are likely to be far from finishing in the country (see Figure 1.2 and 1.3 for hydrocarbon concessions in operation and those available for contract in 2007 and 2017, respectively). Natural gas and liquid natural gas reservoirs have not reached their peak yet, and the impacts of this industry are reportedly similar to those of the oil industry. Moreover, in 2008 the firsts concessions for unconventional hydrocarbon were leased (Orta-Martínez and Finer, 2010).

Currently, the most important hydrocarbon projects in the country are located in the northern pacific coast and in the Amazonian rainforest (MEM, 2016b). The most important petroleum reserves are located in the north-western part of the Peruvian Amazon -Oil blocks 39, 67, 64, 192 and 8- (MEM, 2016b). The controversial Camisea project, Peruvian major gas reserve, overlaps the Nahua-Kupakagori reserve for Indigenous Peoples living in voluntary isolation. In 2013, the Environmental Evaluation Report of the 88 oil block, part of the Camisea project, reported the contact between oil workers and Indigenous Peoples living in voluntary isolation, and the Peruvian Ministry of Health suggested hydrocarbon activities as a probable cause of many deaths occurring among the local Indigenous groups (Campanario and Doyle, 2017).

At a global level, in 2015 Peru was the 40th major petroleum producer worldwide (U.S. Energy Information Administration, 2016). According to Orta-Martínez & Finer (2010) and using the consume ratio of 2009, the total amount of oil extracted in the Peruvian Amazon (996 million barrels) would supply the world oil demand for only 12 days.

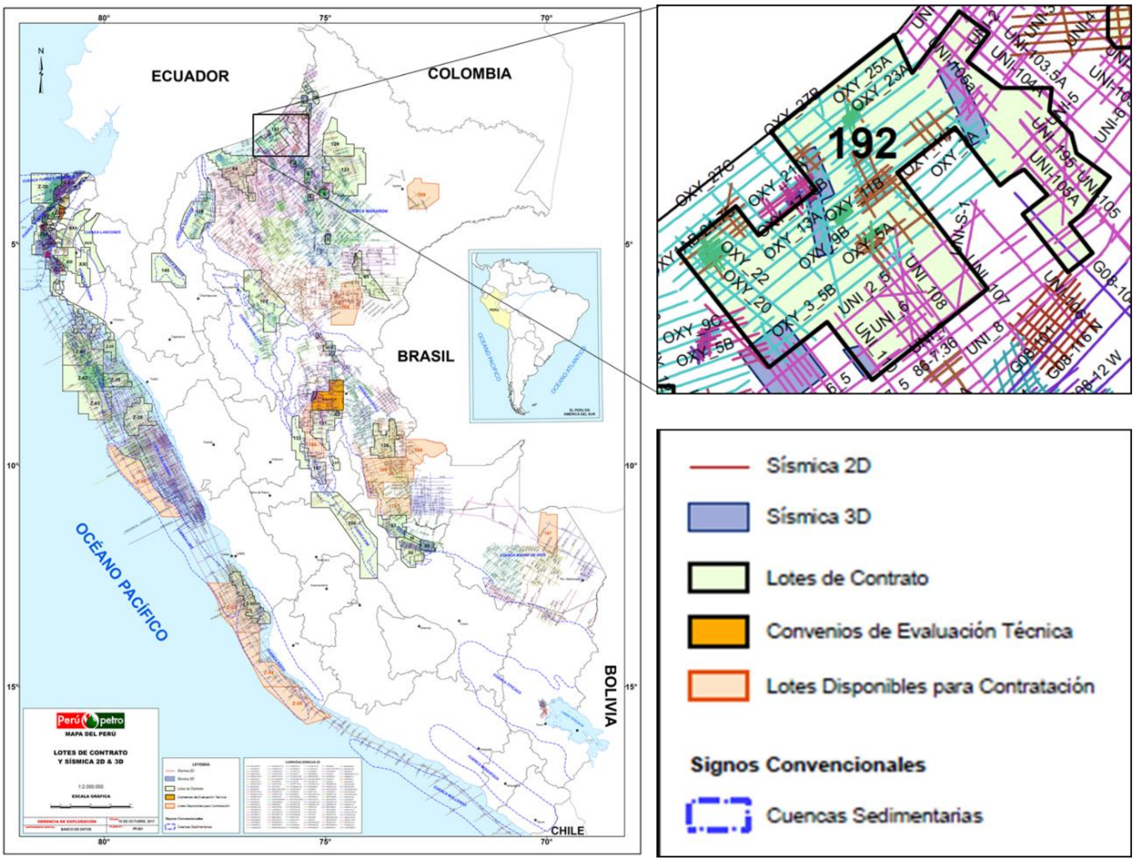


Figure 1.1 | Seismic lines in the Peruvian Amazon.
Note: Map showing the seismic lines opened for hydrocarbon exploration in the Peruvian Amazon until 2017. On-going oil blocks are represented in green polygons; blocks being evaluated are displayed as black-lined orange polygons; blocks available for contract are shown as orange-lined pastel-orange polygons. Source: PeruPetro, S.A. (www.perupetro.com.pe) Consulted in October 2017.

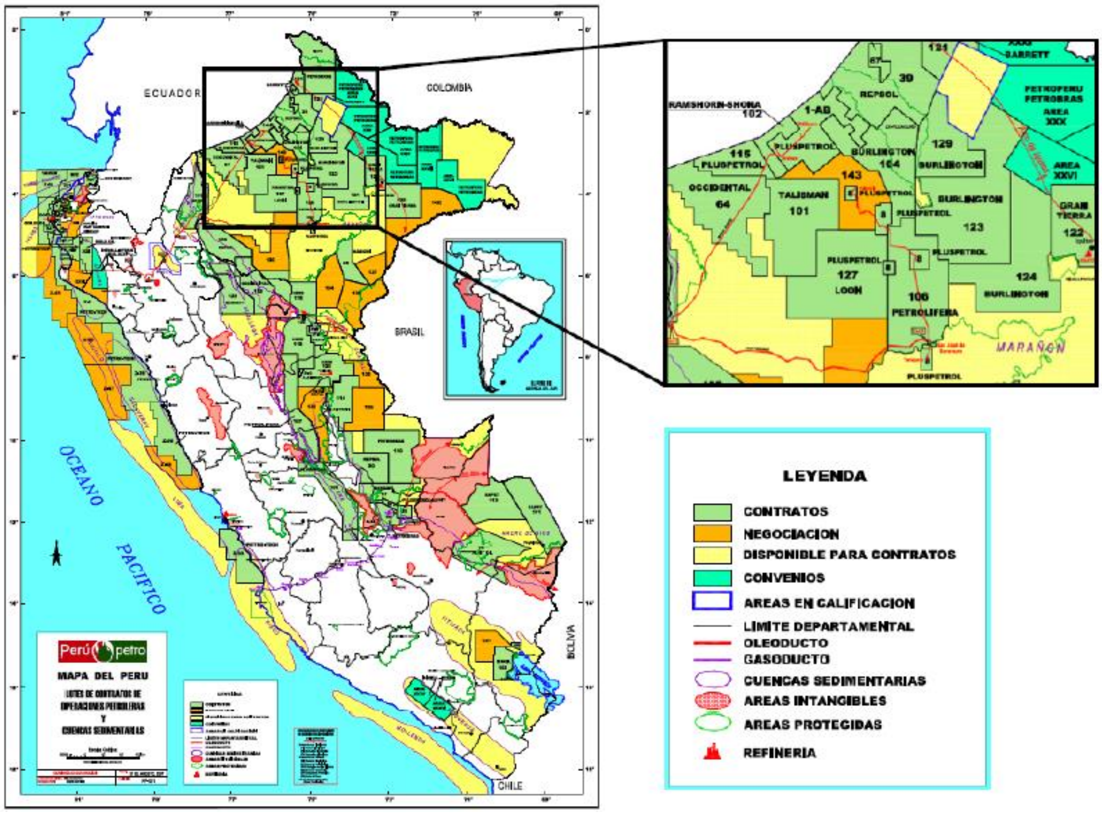


Figure 1.2 | Hydrocarbon concessions in the Peruvian Amazon in 2007.
Note: Map showing the hydrocarbon blocks in the Peruvian Amazon in 2007. On-going oil blocks are represented in green polygons; blocks being evaluated are displayed as orange polygons; blocks available for contract in 2007 are shown as green polygons, respectively. Source: PeruPetro, S.A. (www.perupetro.com.pe) Consulted in October 2017.

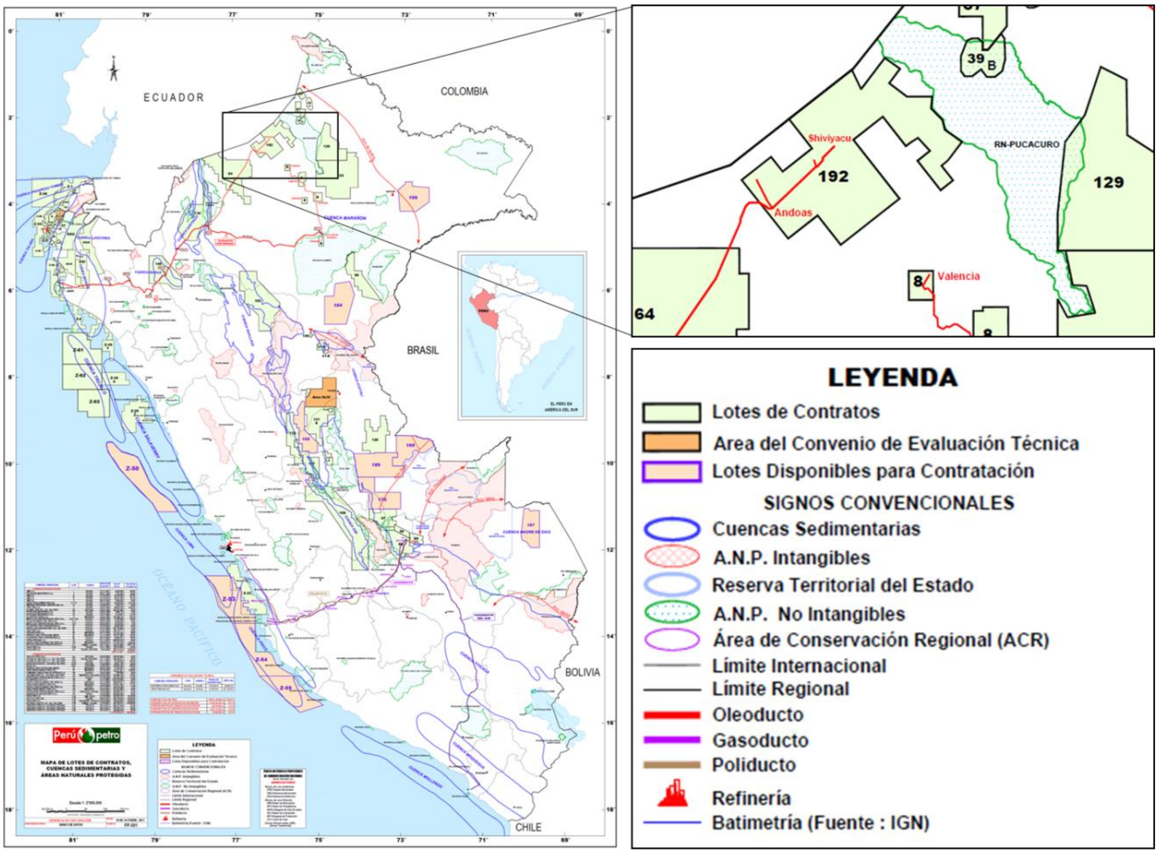


Figure 1.3 | Hydrocarbon concessions in the Peruvian Amazon in 2017.
Note: Map showing the hydrocarbon blocks in the Peruvian Amazon in 2017. On-going oil blocks are represented in green polygons; blocks being evaluated are displayed as orange polygons; blocks available for contract in 2007 are shown as purple-lined pale-orange polygons. National Protected Areas are displayed as green and red-doted polygons. Source: PeruPetro, S.A. (www.perupetro.com.pe) Consulted in October 2017.

OIL POLLUTANTS

The oil extraction industry can be very hazardous for the environment (Epstein *et al.*, 2002; Van Hinte *et al.*, 2007). Accidental or deliberate spillages and regulated discharging and dumping of oil-related materials may contaminate the surrounding environment (Yavari *et al.*, 2015). Because of the hazardous and toxic nature of its components, oil extraction industry impacts can reach the whole ecosystem, including human populations (San Sebastian, 2001; Sebastián *et al.*, 2002; Murakami *et al.*, 2008; Yavari *et al.*, 2015).

The main by-product of oil extraction industry is produced water (also called formation or production water). According to the E&P Forum/UNEP (1997), this is 'water originating from the natural oil reservoir that is separated from the oil and gas in the production facility' (UNEP Technical Publication & E&P Forum, 1997). In average produced water represents 70% of the liquid extracted from a well (Fakhru'l-Razi *et al.*, 2009). The physical and chemical characteristics of produced water varies among oil fields and over the years, but in general it presents a very high concentration of salts – it can be several times saltier than ocean water- and potentially toxic agents, such as hydrocarbons (e.g., benzene, xylene, toluene and polyaromatic hydrocarbons (PAHs), among others), heavy metals (e.g., barium (Ba), arsenic (As), cadmium (Cd), chrome (Cr) and mercury (Hg), among others, and it can also contain radioactive isotopes (Doyle, 1994; Fakhru'l-Razi *et al.*, 2009; Konkel, 2016). Among them, there are carcinogenic and mutagenic compounds, such as many PAHs and the metals Cd, As or hexavalent Cr, among others (IARC, 1988, 2012). Although good practices and many laws stipulate the reinjection of produced waters into wells, this practice is not always followed, especially in low and middle income countries, where sub-standard technologies are frequently used (Jernelöv, 2010).

Other by-products commonly dumped into the environment are drilling muds and cuttings. The first are a mixture of clays, water (sometimes oil), and chemicals which are used in the drilling process to balance underground hydrostatic pressure, cool the bit, and flush out rock cuttings (UNEP Technical Publication & E&P Forum, 1997). Heavy metals such as Ba, Cd, zinc (Zn) or lead (Pb) are commonly found in these muds. Their hazardous effects depend on their bioavailability, which varies depending on the oil content of the muds (UNEP Technical Publication & E&P Forum, 1997). However, some authors argue that common components of these muds make metals bioavailable, turning muds into a very dangerous waste (Doyle, 1994). Dumping of drilling muds is usually accompanied by drill cuttings, which are broken pieces of rocks removed from the borehole.

Finally, oil spills occurring in oil infrastructure (e.g., pipelines, tanks and wells) are also common in many oil concessions (Van Hinte *et al.*, 2007; Martín-Beristain *et al.*, 2009; Orta-Martínez, 2010; Yavari *et al.*, 2015). This spillage may involve the release into the environment of many toxic oil-related compounds, including a wide range of hydrocarbons, sulphur compounds, nitrogen-oxygen compounds, and heavy metals (Murakami *et al.*, 2008).

On land ecosystems, a slow recovery of oil-polluted soils is expected because an important fraction of oil spilled (i.e., components with alkanes with carbon numbers higher than 20 and PAHs) will remain in the soil micropores for years due to their resistance towards biodegradation (Yavari *et al.*, 2015). These compounds present mutagenic and carcinogenic potential and are able to bioaccumulate[5]. Therefore the effects of the oil and produced water spillage on land ecosystems are expected to be acute and to affect the whole food web. Oil spills and the dumping of produced water on aquatic systems also lead to important threat to aquatic life affecting the whole trophic network (see review in Perhar and Arhonditsis, 2014) for long periods of time. For instance, almost 20 years after the Exxon Valdez spill in Alaska, many marine species were still suffering its negative effects (Peterson *et al.*, 2003). In freshwater environments, like tropical rivers, the solubility of some oil components, like PAHs, is increased because of the decreasing salt concentration (Whitehouse, 1984). Thus, the bioavailability and hazardous potential of these components is also increased.

GEOPHAGY AS POTENTIAL OIL EXPOSURE ROUTE

The risk of wildlife exposure to contaminants depends on a mixture of biotic and abiotic factors. These include the characteristics of the contaminants and the physicochemical properties of the environment –which determine their transport and fate in the environment- and those of the animals staying in the polluted site (Smith *et al.*, 2007). In general, contaminants exposure dynamics are better known for aquatic organisms than for terrestrial and semi-terrestrial animals, since distributions of contaminants in aquatic systems can be considered more homogeneous (Smith *et al.*, 2007). "Ingestion of polluted matter –biotic or abiotic-, pollution absorption through skin, or via inhalation of volatile, aerosolized or particle bound contaminants" are the exposure routes to contaminants among terrestrial vertebrates, ingestion being the most predominant (Smith *et al.*, 2007). Besides the consumption of polluted vegetable and animal matter and water for feeding purposes, for many species the ingestion of soil and sediment –deliberated or accidental- can be an important route of contaminant exposure (Beyer *et al.*, 1994).

The deliberate ingestion of soils (geophagy) in nutrient-poor ecosystems, such as the Amazon, is a widespread behaviour frequently observed in herbivores, frugivorous, and omnivorous wildlife species (Kreulen, 1985). Many mammals such as ungulates

[5] If bioaccumulation occurs, compounds' concentrations in the tissues are higher than those in the environment.

(Montenegro, 2004; Ayotte *et al.*, 2006; Tobler *et al.*, 2009), rodents (Blake *et al.*, 2011; Molina *et al.*, 2014; Varanashi, 2014), primates (Izawa, 1993; Krishnamani and Mahaney, 2000; Blake *et al.*, 2011; Link *et al.*, 2011), and bats (Bravo *et al.*, 2008; Voigt *et al.*, 2008), and many families of birds like the Cracidae, the Psittacidae, or the Columbidae (Emmons and Stark, 1979; Diamond *et al.*, 1999; Brightsmith, 2004; Blake *et al.*, 2011) ingest soil in sites known as salt licks (also called mineral or clay licks).

There are several non-mutually exclusive hypotheses for explaining geophagy and the reasons for ingesting soils might indeed differ depending on the species and the season (Kreulen, 1985; Klaus and Schmidg, 1998; Blake *et al.*, 2011). A common explanation for geophagy is mineral supplementation. Due to abundant precipitation in the Amazon basin, most soils in the area face an important leaching of soluble nutrients, which results in a reduced essential elemental content in the vegetation (Stark, 1970; Emmons and Stark, 1979). Animal species seem to complement their diet with the ingestion of soils in salt licks to achieve an adequate level of nutrients such as sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), or iron (Fe), among others (Emmons and Stark, 1979; Klein and Thing, 1989; Klaus and Schmidg, 1998; Klaus *et al.*, 1998; Montenegro, 1998; Molina *et al.*, 2014). In particular, many studies around the world, including in the Amazon, point out at Na supplementation as the principal cause of geophagy (Weeks, 1978; Holdø *et al.*, 2002; Brightsmith and Muñoz-Najar, 2004; Dudley *et al.*, 2012), since Na is one of the most limiting nutrients to vertebrates in many regions of the Neotropics (Freeland *et al.*, 1985). Moreover, the western Amazon is a region deprived geographically of salt, as aerosol deposition of salts declines with distance from oceanic sources (Dudley *et al.*, 2012).

However, since in some salt licks the minerals analysed present concentrations equal or lower than the concentrations found in untouched surrounding soil (Hladik and Gueguen, 1974; Arthur and Alldredge, 1979), geophagy cannot be uniquely explained by mineral supplementation. Thus, other researchers have suggested other drivers of geophagy such as the ingestion of clay (e.g., bentonite, zeolite) to adsorb toxins from secondary plant compounds or to alleviate digestive disorders (e.g., diarrhoea or acidosis) through the increase of the buffering capacity (Oates, 1978; Kreulen, 1985; Diamond *et al.*, 1999; Krishnamani and Mahaney, 2000; Voigt *et al.*, 2008).

Although geophagy seems to offer diverse benefits for the animals, it also entails some costs. For instance, animals are more exposed to predation, poaching, and hunting in salt licks since these sites are frequently visited by predators seeking easy preys (Montenegro, 1998; Varanashi, 2014) and are also important hunting sites for local people (Montenegro, 1998; Tobler *et al.*, 2009; Blake *et al.*, 2011) and poachers (Seidensticker and McNeely, 1975; Klaus and Schmidg, 1998; Klaus *et al.*, 1998). Moreover, since some animals may walk long distances and even exceed their home ranges to visit a salt lick (Tobler, 2008), the energy invested on seeking out and visiting licks is expected to be high (Wiles and Weeks, 1986; Klein and Thing, 1989; Tobler, 2008). Exposure to diseases is also high, since at salt licks there is a large contact between animals (Hebert and Cowan, 1971; Henshaw and Ayeni, 1971). The ingestion of clay may provoke tooth wear (Mayland *et al.*, 1975) and soil can also contain excessive concentrations of otherwise essential minerals that would lead to mineral imbalances (Kreulen, 1985), or even contain toxic elements such as Pb, Cd, Hg, As or radionuclides (Mayland *et al.*, 1975; Arthur and Alldredge, 1979; Kreulen, 1985).

Since industrial activities expands into remote areas and the anthropogenic contaminants (from pesticides to heavy metals) on the ground are becoming more frequent and widespread, geophagy may emerge as an increasingly important route for contaminant exposure (Beyer *et al.*, 1994; Hui, 2004), negatively impacting animal's health (Beyer and Fries, 2002). Studies on livestock have found that geophagy has been an exposure route to many environmental pollutants like the pesticide Dichlorodiphenyltrichloroethane (DDT) or the Polychlorinated biphenyl (PCBs), among others (Fries, 1982; Fries *et al.*, 1982). The ingestion of polluted soil by free-ranging wildlife has also been documented. For instance, Arthur & Alldredge (1979) documented the ingestion of radioactively polluted soil by mule deer (*Odocoileus hemionus*) and Weeks (1978) reported the ingestion of soil by white-tailed deer (*Odocoileus virginianus*) in sites where unknown chemicals were dumped as well as in natural salt licks. The average content of inorganic matter in the faeces was 29.4% and the highest value was 87.5% (Weeks and Kirkpatrick, 1976). So, although soil ingestion varies among species and among seasons and age classes, normally large amounts of soil (up to ~30% of digesta) are ingested by some vertebrate species visiting salt licks (Beyer *et al.*, 1994; Hui, 2004). Furthermore, concentrations of some elements and pollutants in ingested soil may be much higher than those in animals' diet (Arthur and Alldredge, 1979).

Despite its relatively small spatial dimensions, salt licks are important in many species ecology (Blake *et al.*, 2011), including predators and humans (Montenegro, 1998; Tobler *et al.*, 2009; Blake *et al.*, 2011; Varanashi, 2014), and may influence their health (Montenegro 2004). Therefore, it has been argued that salt licks study and appropriate management should be taken in consideration in strategic conservation planning (Tobler *et al.*, 2009; Molina *et al.*, 2014) but also in public health studies, especially in human communities that consume wild meat, since some pollutants ingested by wildlife through geophagy may bioaccumulate and enter the food chain.

STUDY AREA

The study area is an oil concession located in the northern part of the Loreto Department, an emblematic Peruvian oil extraction area and the ancestral land of the Achuar, Quechua and Kichwa Indigenous Peoples. The study area can be considered quite remote, since it is only accessible by boat (3–5 days upstream travel time from the city of Iquitos, which in turn is only reachable by boat or plane), or by plane using the private (oil-company owned) airfield.

The oil concession known as Block 1AB/192 (formerly called 1AB, first held by Occidental Petroleum Corporation -Oxy-, then transferred to Pluspetrol Corporation S.A. in 2001, and to Frontera Energy in 2015) was leased at the early 1970's, overlapping the Corrientes, Pastaza and Tigre River basins. This oil block, together with the adjacent oil block 8 (initially operated by PetroPeru, then transferred to Pluspetrol Corporation S.A. in 1996), have come to be the longest running oil project in the Peruvian rainforest and the most productive one in Peru (Orta-Martínez & Finer 2010).

Oil Block 1AB/192 is located in the large sedimentary Marañón River basin and it covers an area of 5,123.47 Km^2, overlapping the Pastaza, Corrientes and Tigre Rivers watersheds (Figure 1.4). Although its productivity has decreased in the last years, it has an accumulated production of 709,778.6 million barrels, representing the 26.72% of the whole Peruvian oil accumulated production (own calculations based on (MEM), 2016). In this oil concession, there are 11 central production facilities, 250 wells[6], and 360.3 Km

[6] Including abandoned wells as well as active wells for oil extraction and for the reinjection of produced waters.

of main pipeline routes, which join with the North-Peruvian Pipeline, an 802 Km long pipeline that connects the oil concession with the Bayóvar harbour terminal, in the Peruvian pacific coast.

Around 10,000 Indigenous Achuar, Quechua and Kichwa people inhabit this oil block. Eleven Indigenous settlements are located inside the block 1AB/192. Nearby communities not settled in the block also depend on this territory for their daily subsistence (Figure 1.4). The subsistence activities of these Indigenous communities include hunting, fishing and small-scale agriculture. However, during the last decades, most adult men in some of these Indigenous communities temporarily work for the oil company – normally during two or three months per year-. Thurs, oil company has become the most relevant source of money income in the area (Orta-Martínez and Finer, 2010). Local people are employed as unskilled workers doing vegetation cleaning tasks along pipelines, in seismic lines and drilling sites, and working on remediation and road maintenance. Moreover, since the Peruvian State has always been absent in these very remote areas, oil companies supply some needs (e.g., health assistance, air transport for health emergencies, drinking water, among others) (Orta-Martínez and Finer, 2010). Consequently, the degree of dependency of the communities on oil companies is very high.

The Indigenous population living in the study area has a low access to schooling and public health and is highly vulnerable to illnesses such as malaria, leishmaniosis, hepatitis and tuberculosis, among others (MINSA, 2006). They display high rates of illiteracy (INEI, 2009), infant mortality, and chronic malnutrition (INEI, 1993 consulted in Orta-Martínez *et al.* 2007).

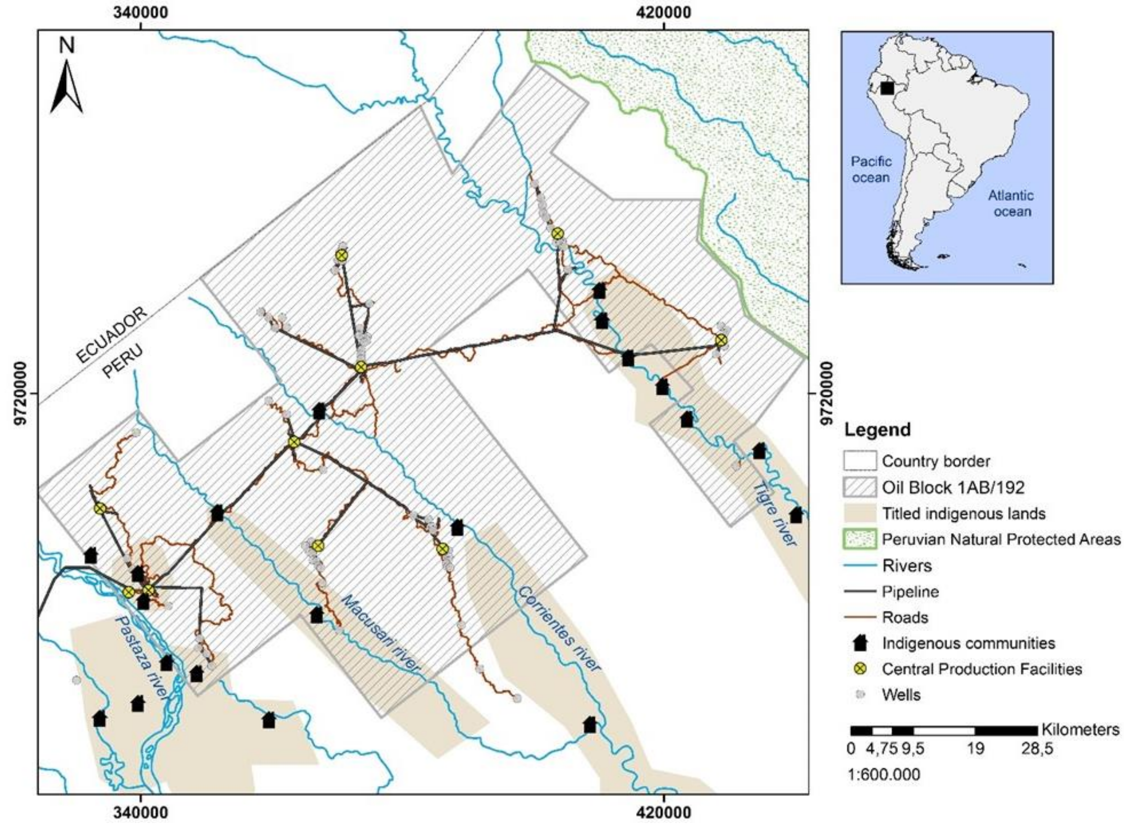


Figure 1.4: Study area.
Note: Map showing the location of oil concession 1AB/192 and oil infrastructure, as well as the Indigenous communities and titled territories. Source: Own elaboration.

The eastern part of the oil concession borders a proposed territorial reserve for people living in voluntary isolation: the *Reserva Territorial Napo-Tigre* (Figure 1.5). Many local people, loggers, hunters, soldiers and, lately, oil workers confirmed the presence of people living in isolation in this area (Álvarez and Trigoso, 2002; Albán, 2005; ORPIO, 2008; Huertas, 2010). Adjacent to the northern frontier of the proposed reserve there is an Ecuadorian reserve for Indigenous Peoples in voluntary isolation "*Zona Intangible Tagaeri Taromenani-ZITT*". Despite the multiple evidences and the fact that the territorial reserve was already proposed in 2005, the reserve has not been approved yet. This postponement has been linked to the vast interest of the Peruvian Government and oil companies to operate in the area -oil blocks 39 and 67- (Huertas, 2010; Tipula, 2016).

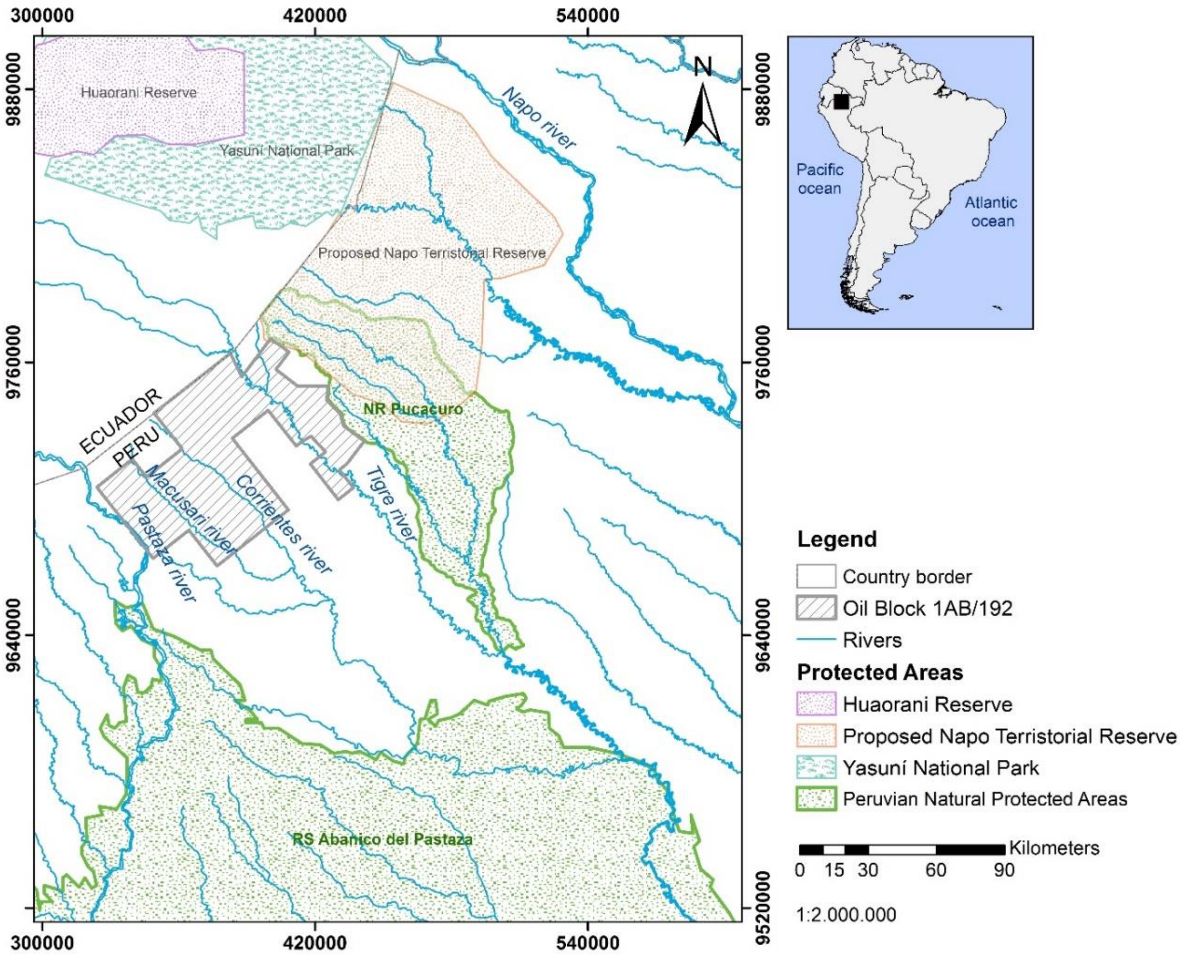


Figure 1.5 | Oil Block 1AB/192 and protected areas.
Note: Map showing the location of the oil concession and the nearby protected areas, including Ecuadorian reserves and a proposed territorial reserve in Peru. Source: Own elaboration based in Huertas Castillo (2010).

The area is located at the end of the Amazonian Great-Plain, where first inclines towards the Andean Mountain Range, with an elevation oscillating between 267 and 182 meters above sea level. It shows a tropical rainy weather, with a humid season from March to November and a drier season from November to February and with maximum and minimum temperatures of 31ºC and 22ºC, respectively (Orta-Martínez, 2007). As mentioned, three main rivers cross the oil concession: the Pastaza, the Corrientes and the Tigre Rivers. The Pastaza River comes from the mostly volcanic *Cordillera Real* in the Ecuadorian Andes. The Corrientes and Tigre Rivers are originated in the lowland forest. The three rivers, after traversing the oil concession, feed a big extension of flooded forest included in the National Reserve Pacaya-Samiria and the *Abanico del Pastaza* Ramsar Site. Finally, they discharge their waters into the Marañon River. Soils in the area are typical tropical rainforests soils, with low concentrations of nutrients and strongly lixiviated thanks to the high temperatures and the abundant rains (Orta-Martínez, 2007).

PRE-OIL HISTORY

The Achuar and the Quechua and Kichwa Indigenous inhabitants of the study area belong to the Jivaroan and Kichwan peoples, respectively, and their contact with the European began in the 16th century. Jivaroan people, famous for their head shrinking rituals, live in the frontier territories between Ecuador and Peru and have always been one of the most resistant Indigenous groups to domination attempts (Mayor and Bodmer, 2009). In the study area, they mostly inhabit the Corrientes River basin. First Spanish expeditions to their territories date back to the mid-16th century, when Indigenous Peoples destroyed any attempt to build towns in the Jivaroan people territory. In the late 17th century, missionaries reached their territories in the Santiago River, but it was not until the late 18th century when they reached the region of study and funded the missions *Nuestra Señora de los Dolores de Muratos* and *Sagrado Corazón de Jesús de los Jíbaros* in the Pastaza River, where they reduced 130 Jivaroan people. Shortly-after, with the expulsion of the Jesuits, the Jivaroan people were again relatively isolated until the rubber boom in the late-19th century.

The Kichwan people from the region mostly inhabit now in the Pastaza and Tigre River basins and are composed by many families from the Canelo, Coronado, Urarina, Romayna, Shimiagés, Arabela, Murato and Achuar groups that lost their ethnic identity during the rubber boom. Escaping from the slave raids, these groups sought refuge to the Jesuit missions, where they learned the Quechua language used by the Jesuits, although it is also believed that Inka Peoples already began to disseminate the Quechua language in the pre-Colombian period. (Mayor and Bodmer, 2009). In the 18th century, Dominican missionaries funded a mission in Andoas in the Pastaza River. In the late 19th century, during the rubber boom, many Kichwa people were moved to the Tigre and Ucayali River basins.

The rubber boom marked the start of a ferocious exploitation boom of Amazonian products and people, which continues until nowadays. The rubber boom was the driver of an important population loss among Amazonian Indigenous Peoples because of the bad labour conditions (usually slavery) and the exposure to new illnesses brought by outsiders to whom they had no resistance. In the Jivaroan territory, because of the warlike character of the Jivaro, rubber extraction was less aggressive than in other places. However, it was common the exchange of rubber collected by the Jivaroan people for steel tools, arms and fabrics offered by the rubber barons, an exchange that was always done through the debt peonage system[7] disadvantaging the Jivaroan people. After the rubber boom, some commercial exchanges with outsiders continued with balata and lechecaspi –also rubber products-, and later with timber, furs, mullein, wild meat and fish, with the same patron-peon system (Mayor and Bodmer, 2009). Around the 1960s, with the arrival of the evangelizers of the Summer Institute of Linguistics, local populations began to settle down in communities next to churches (Orta-Martínez, 2010). At about the same time, oil reserves were discovered under their territories, marking the beginning of a new exploitation boom.

OIL CONFLICT IN THE STUDY AREA

When the first well was drilled in the block 1AB/192 in 1971 by the company Occidental Petroleum Corporation, Indigenous communities of the area were not informed nor consulted. Peru ratified the International Labour Organization (ILO) Convention 169 in 1993 (Legislative Resolution 26253-1993) which gave all Indigenous communities the right to Free, Prior and Informed Consent (FPIC) of the activities to be conducted in their territories, even for those without land title. However, when a new contract began with the oil company Pluspetrol in 2001, the FPIC was also not considered, breaking the law and violating the Indigenous Peoples' rights (Campanario and Doyle, 2017).

The adoption of low and inappropriate environmental standards by the oil companies operating in the Block 1AB/192 caused several and diverse impacts on the ecosystem and on human populations (see Campanario & Doyle, 2017; Orta-Martínez & Finer, 2010; Orta Martínez et al., 2007 for reviews). Orta-Martínez *et al.* (2007) summarized the findings of many official reports on oil pollution impacts produced by Peruvian state agencies. Among them, it is worth highlighting a report published by the Research Institute of the Peruvian Amazon (IIAP, 1985) were high levels of Pb were described in two biological samples of fish from rivers in the region. The same research institute

[7] This system is similar to bonded labour and implies the provision of commercial goods (or money) by a patron to a peon in advance of the peon's future labour. The patron has the use of the peon labour until he/she pays the debt, plus interest rates (Fernández-Llamazares Onrubia, 2015).

reported the pollution by hexavalent chromium (Cr(VI)) in tributaries of the Corrientes and Marañón Rivers (Gomez, 1995). Similarly, both the Ministry of Energy and Mines (ONERN, 1984; MEM, 1998) and the Peruvian Regulatory Body for Energy Investment (OSINERG, 2004) reported high concentrations of hydrocarbons, Ba, Pb, Hg and chlorides in local rivers and river sediments, and the governmental agency for natural resources classified the region as one of the most environmentally damaged areas of Peru (ONERN, 1984).

In 2005, the Ministry of Health, found that 98.6% and 66.2% of Achuar children (2-17 years of age) and 99.2% and 79.2% of adults had blood levels of Cd and Pb, respectively, above the acceptable limits for people not occupationally exposed (DIGESA, 2006). Some authors did not associated Pb levels with oil activities, since Pb values in environmental samples presented relatively low values (Anticona *et al.*, 2011; Anticona, Ingvar A Bergdahl, *et al.*, 2012). More recently, Yusta-García *et al.* (2017) reported the dumping of 0.37, 2.54 and 4.72 metric tons/year of Pb as well as 0.02, 0.16 and 0.30 metric tons/year of Cd along with the release of produced waters in the Pastaza, Corrientes and Tigre Rivers.

Considering that since the beginning of oil activities until 2010, one million barrels of produced water have been daily dumped in the waters and soils of the study region (Orta-Martínez, 2010), the potential impact of the industry in the area might be not negligible. Indeed, Moquet *et al.* (2014) have linked oil activities in the study region with 12% and 20% increase of dissolved Na and chlorine (Cl) fluxes in the Amazon River, thousands of kilometres downstream in Brazil. Similarly, Reátegui-Zirena *et al.* (2014) linked the presence of PAHs in river sediments and water with oil-related activities in the oil blocks 8 and 1AB/192 and Webb *et al.* (2017) found PAHs metabolites' levels in human urine higher than expected for an Indigenous community, suggesting hydrocarbon pollution exposure as a very plausible cause.

Additionally, systematic dumping of crude oil regularly occurred in oil extraction wells: probably until 2001, between 2 and 6 barrels of crude oil were dumped weekly in each well of Block 1AB/192 due to maintenance and pressure control activities (Orta-Martínez, 2010). In addition, probably until the same year, the 300-600 tonnes of drilling mud and 1000-1500 tonnes of cuttings that a drilling of a typical well produces (UNEP-

IE/E&P-Forum 1997) were abandoned on open waste pits or discharged straight into the nearest water bodies (Orta Martínez *et al.*, 2007).

As oil activities were developing in the area and their environmental and health impacts started to be evident, inhabitants from the study area initiated protests, mobilizations and claimed for the respect of rights and health (see review in Orta-Martínez & Finer 2010). As early as in 1983, Achuar leaders met the Peruvian President, asking for land titles and for the shutdown of oil activities (Orta-Martínez & and Finer 2010). Several decades ago, the local Indigenous population also reported the occurrence of oil spills into water bodies and linked them with acute cases of poisoning and cancer (Álvarez, 2008). They also denounced other impacts, from sexual abuses to the reduction of their livelihood stocks due to illegal hunting or logging by oil companies (La Torre, 1998). However, social unrest in relation to oil activity impacts first emerged when alarming blood levels of Pb and Cd were reported (DIGESA, 2006). Since then, in several occasions the Indigenous population has occupied oil facilities (e.g., wells, airports) and blocked oil roads in protests to oil impacts. They have also attended a myriad of meetings with governmental authorities. As a result of their steady actions, they have achieved some improvements in the oil operational standards–such as the reinjection of produced waters -, in the health care systems and, in the environmental remediation management. The fact that escalating conflict has been effective in boosting changes of the operational procedures and standards and improving socio-environmental performance of the oil companies operating in the area has been defined as “the conflict imperative” (Orta-Martínez, Pellegrini, *et al.*, 2018).

More specifically, in 2007 five Achuar communities brought suit against Occidental Petroleum Corporation for the impacts of its activity on their health and the environment. The court case, that took place in US courts, finished with an out-of-court settlement. The settlement included the payment of development programmes for the Indigenous communities by the oil company (AIDESEP, 2015). Governmental offices also imposed economic administrative sanctions to the oil companies. For instance, in 2012, Pluspetrol was fined to pay a USD 6 million sanction by the Agency for Environmental Assessment and Enforcement (OEFA) for the irreparable (and unreported) damage to a lagoon. From 2007 to 2014, the company was also sanctioned with a 16 million and 24 million USD penalty fees by the Regulatory Body for Energy and Mines Investment (OSINERGMIN) and OEFA, respectively (Campanario and Doyle, 2017).

An important keystone of this history has been and continues to be the Independent Community-Based Monitoring Programme, a locally run monitoring programme which has played a crucial role in the identification, documentation, and mapping of oil activities impacts since their creation in 2005 (Orta-Martínez, 2010). The programme was designed to Monitor Environmental Liabilities through participatory GIS (MEPLGIS) and was implemented first in the Achuar territory by their Indigenous organization FECONACNO (now FECONACOR) jointly with two Peruvian NGOs (Racimos de Ungurahui and Shinai) and a research team of ICTA-UAB. Shortly after, with the support of a numberless NGOs (*alterNativa Intercanvi amb Pobles Indígenes*, Rainforest Foundation, *Mouvement pour la Coopération Internationale*, etc.), the programme expanded to the whole Indigenous territory under the influence of the oil concession by the Indigenous organisations FEDIQUEP, FECONACO (now FECONACOR) and FECONAT (now OPIKAFPE) from Pastaza, Corrientes and Tigre River basins, respectively. From 2006 onwards, and as a product of this monitoring programme, a detailed database of oil spills with GPS-coordinates and images has been constructed (See more information in Orta-Martínez, 2010).

In 2013, as a result of the official analyses conducted as response to the Indigenous demands, the Peruvian government declared the environmental state of Emergency in the whole Pastaza (Ministerial Resolution 094-2013-MINAM), Corrientes (Ministerial Resolution 263-2013-MINAM) and Tigre (Ministerial Resolution 370-2013-MINAM) River basins. In May 2014, the Peruvian government also declared the Health Emergency in the Pastaza, Corrientes, Tigre and Marañón River basins (Supreme Decree 006-2014-SA). At that time, the OEFA identified 92 polluted sites that were not previously declared by the oil company and that must be remediated by the same (Report Nº 411-214-OEFA / DH-HID, 2014). The Environmental and Health Emergency Declarations did not really change the paradigm for the Indigenous communities, who keep on complaining (with strikes, road and river blocks, and occupation of oil infrastructures) to pressure the Government for the fulfilment of the agreements arranged after the aforementioned declarations. These agreements included the remediation of polluted sites, a proper land titling of Indigenous territories, the building of water treatment plants in the communities, a detailed and specific health plan for the communities, and the elaboration of a toxicological and epidemiological study on oil pollution impacts.

In August 2015, the oil concession expired and Pluspetrol left the area without an approved decommissioning plan. Indeed the company presented a plan that did not include the remediation of all the environmental impacts identified by the OEFA, and that was rejected twice by the Peruvian Government (Report Nº 411-214-OEFA / DH-HID, 2014). Furthermore, the company judicially appealed this report. This illustrates the weakness of the Peruvian State to defend its citizens and the environment in front of powerful multinational corporations (Campanario and Doyle, 2017). Afterward, the oil company Frontera Energy (formerly called Pacific Stratus Energy) began new extraction activities in the oil block. Since the short oil contract with Frontera Energy will conclude in February 2019 and the oil concession will be leased under a 30-years contract, Indigenous communities claim the need that their right to have FPIC to have oil extraction activities in their areas is recognized (Campanario and Doyle, 2017). The UN Rapporteurs on Hazardous waste and on Indigenous Peoples Rights, many NGOs, and even environmental governmental agencies urged the Peruvian Government to undertake immediate action to guarantee the Indigenous rights in the oil Block 1AB/192 (Campanario and Doyle, 2017). Nowadays, there is still a big uncertainty on the future of the oil block.

Despite of the numerous evidences of environmental impacts, the associations between petroleum contamination detected in surface water, sediments and soils, the presence of these substances in human, and the exposure routes and possible long term health effects have not yet been completely established.

AIMS AND METHODS OF THE DISSERTATION

This PhD dissertation is structured as follows: Chapter I, the introduction chapter, is followed by two empirical chapters (Chapter II and III), which cover the main results of the thesis. Chapter IV concludes the thesis highlighting the most relevant scientific contributions, the policy implications of the results presented in the previous chapters, and the take-away messages derived from this research. The main caveats of this work are also highlighted in the last chapter, in which potential further research is also suggested. Finally, a list of publications complementary to this PhD project is presented (Appendix I) together with supplementary information to the chapters of this thesis (Appendices II-IV).

This thesis aims to further our understanding of the impacts associated to oil activities in tropical rainforests. Concretely, the goal of this thesis is to shed light on the recently suggested potential new route of exposure to petrogenic pollutants for wildlife: the ingestion of oil-polluted soil and water by wildlife.

The main research objectives of the empirical chapters of this thesis are the following:

CHAPTER II

Main objective

To study the ingestion of oil-polluted soil and water by wildlife

Specific objectives

1. To describe the ingestion of oil-polluted soil and water by wildlife and discuss its potential causes.
   1.1. To determine the occurrence of petroleum biomarkers (sterans and hopanes) and oil-related heavy metals in the soil of the studied salt licks;
   1.2. To identify the whole range of animal species that ingest oil-polluted soil and the frequency of this behaviour;
   1.3. To explore the potential causes behind this behaviour;
   1.4. To quantify the local Indigenous hunting grounds at risk by the presence of oil-polluted salt licks;
2. To study other potential species behavioural changes due to noise disturbance related to the hydrocarbon activity;
3. To better understand the relationship between elemental composition of salt licks and the composition of visitor species.

CHAPTER III

Main objective

To study the bioaccumulation of oil-related pollutants in wildlife.

Specific objectives

1- To identify the main sources of Pb found in wild game species through the analysis of Pb isotopic ratios and Pb concentrations (Subchapter III-A);

2- To study the variability of Pb concentrations in Amazonian wildlife according to a set of explanatory variables selected based on previous studies, including oil-activity, basin, species (interspecies differences in metabolism and kinetics), body mass, habitat and trophic level (Subchapter III-B).

METHODOLOGICAL APPROACH

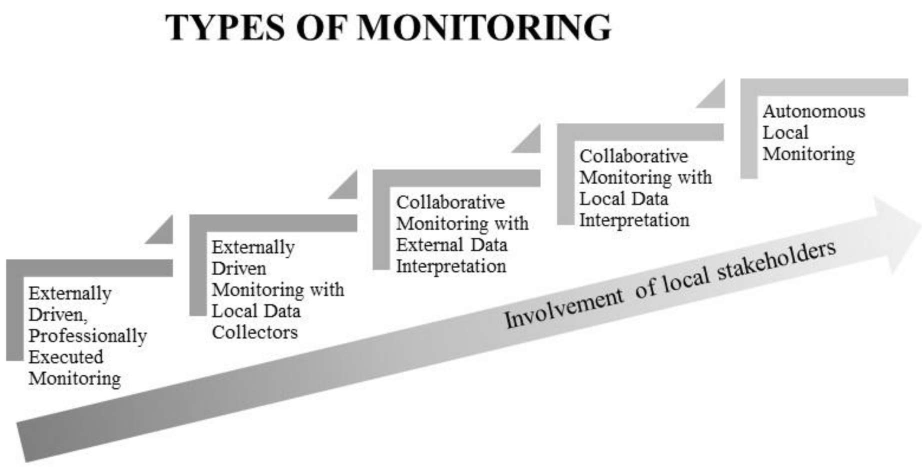
This thesis is built on the basis of Participatory Action Research (PAR) (Whyte, 1991; Baum *et al.*, 2006), an approach that aims to conduct research by and for local people. In this research, local people has been engaged not only as volunteers collecting or analysing data, but also as co-researchers. A Radical Citizen Science approach was applied, taking the concept of Citizen Science a step further: local Indigenous Peoples set the agenda and choose the research questions, addressing issues of great concern to them. Local people, collectively with scientists, designed the research, collected and analysed the data, and discussed the results. The methodological approach used is grounded in the idea that the collective generation of knowledge empowers people and spurs action to solve the problems affecting them. In this vein, this thesis draws on the inputs of very varied actors –ranging from community members and local Indigenous monitors of the community-based monitoring programme to scientists from diverse fields. It also uses a myriad of interdisciplinary methodologies and approaches to answer its main research questions.

Originally, local Indigenous Peoples were the first ones to suggest the research objectives of this thesis and over the years that this project unfolded, they have largely and actively contributed to its accomplishment. The Indigenous environmental monitors have been active researchers of this thesis, collaborating in many of their phases, including the raising of the research questions, data collection, and data interpretation. Following Danielsen et al., (2009), this thesis would be a hybrid between "Collaborative Monitoring with Local Data Interpretation" and "Collaborative Monitoring with External Data Interpretation". On the one hand, it involves local people participation in the design, collection and interpretation of data, although data analysis (such as video, laboratory and statistical analysis) has been mostly conducted by the scientific team. The results have been periodically shared with the Indigenous leaders, the environmental monitors and, the whole Indigenous population of the study area. Furthermore, the knowledge production process aimed at creating relevant, actionable knowledge with transformative potential.

The original idea of this research was born out of a crisis: the local Indigenous population was afraid of the impacts of oil activities in their territories. Their concerns were based on the many impacts of the oil activities that they had detected over the five decades of oil extraction in the area; impacts that were rarely reported to the State agencies by the companies operating in the area. So, the research idea explored in these pages was born from Indigenous Peoples' aim to better protect their health by gaining knowledge on the mentioned new potential exposure route to petrogenic pollutants, as they expected that evidence on this impact would prompt governmental actions.

The relationship between the scientists involved in this thesis and the local population has been of mutual training and sharing of knowledge. Workshops on trap camera and samples' collection have been carried out by the scientists and, simultaneously, the monitors have shared their knowledge on wildlife ecology, history and geography with me and other scientists in the group. From a scientific point of view, this close collaboration with the local population also offered many practical advantages, such as the possibility of collecting a bigger amount of data and samples for a longer period of time, and the possibility to take advantage of the local knowledge of the territory, the location of oil impacts and wildlife behaviour to facilitate the research design. Local people were, in fact, key in order to shed light on issues that remained unknown for the scientific community.

Although this research was requested by the leaders of the Indigenous organizations of the study area, permission of community leaders and inhabitants was obtained in each community where work was conducted. The project was explained to the communal assemblies before any data collection started in a particular community.



Participation of local stakeholders					
Data collection	☒	☑	☑	☑	☑
Design	☒	☒	☒	☑	☑
Interpretation	☒	☒	☒	☑	☑
Decision making	☒	☒	☑	☑	☑

Figure 1.6: Different types of monitoring according to the involvement of local stakeholders.
Note: Own elaboration based on Danielsen *et al.* (2009).

The methodology used in this thesis embraces very diverse methods, which include trap-cameras, geographic information system (GIS) analysis, environmental forensics, and Pb isotopic fingerprint analysis.

CHAPTER II

WILDLIFE INGESTION OF OIL-POLLUTED SOIL AND WATER

Picture in the previous page: *Two Tapirus terrestris licking an abandoned oil well*
Source: Camera trap programme. FENOCANOR, FEDIQUEP, ICTA-UAB.

CHAPTER II • WILDLIFE INGESTION OF OIL-POLLUTED SOIL AND WATER

ABSTRACT

In this chapter, novel data on wildlife ingestion of oil-polluted soil and water is reported. Eight vertebrate species ingesting oil-polluted soil and water in an oil concession located in the northern Peruvian Amazon have been recorded: the ungulates *Tapirus terrestris, Mazama americana* and *Pecari tajacu*, the rodents *Cuniculus paca* and *Coendou prehensilis* and three parrot species (*Ara chloropterus, Ara macao* and *Brotogeris sp*.). Proofs of how these species have redirected geophagy from natural salt licks to oil-polluted sites are presented. Data show that oil-polluted sites used as artificial salt licks have similar concentrations of most minerals allegedly sought for wildlife in natural salt licks. Oil-polluted sites used as artificial salt licks exert a similar pull effect on wildlife than natural salt licks. Similarly as natural salt licks, these sites are also key hunting sites for both predator species and human populations. Considering species' mobility range, the species conducting redirected geophagy can access to potential oil-polluted salt licks from almost any spot of the oil concession. In other words, more than 80% of the oil concession area might be considered hunting grounds frequented by game species probably exposed to oil pollutants through geophagy in oil-polluted salt licks. While the health effect that the ingestion of oil-polluted soils by Amazonian animals may have primarily on wildlife and secondary on human populations has not yet been studied, this behaviour could allegedly result in bioaccumulation and biomagnification of petrogenic hydrocarbons and heavy metals. This behaviour could, therefore, be an important threat to the conservation of top predators in the tropical rainforests and to the health of local Indigenous Peoples.

Keywords: Amazon; Geophagy; Indigenous health; Oil extraction; Trap cameras; Wildlife.

INTRODUCTION

The western Amazon is one of the most biocultural diverse, road-less and relatively-well conserved regions on earth (Foley *et al.*, 2007; Bass *et al.*, 2010). Hydrocarbon activities are one of the main threats for the conservation of this area (Finer *et al.*, 2008, 2015) due to their innumerable social, cultural and environmental impacts (Orta-Martínez and Finer, 2010; Laurance *et al.*, 2014). Nevertheless, scientific research on the impacts of hydrocarbon activities in the Amazon is scarce (O'Callaghan-Gordo *et al.*, 2016), especially regarding hydrocarbon activities' impacts on wildlife. One of the potential severe impacts of oil extraction activities in Amazonian wildlife comes from the ingestion of oil-polluted soil and water. This potential behaviour reported in only one previous pilot study (Mayor *et al.*, 2014; Orta-Martínez, Rosell-Melé, *et al.*, 2018) has been suggested to be explained by the high salinity content of oil spills which might exert an effect similar to that of natural salt licks (Emmons and Stark, 1979). In other words, it was hypothesized that animals might visit oil-polluted sites attracted by salts, redirecting their behaviour from natural to oil-polluted salt licks. On the one hand, geophagy is commonly explained by Na supplementation: in regions deprived of salt, animal species seem to ingest soils in salt licks to achieve an adequate level of nutrients in their diets (Emmons and Stark, 1979; Klein and Thing, 1989; Klaus and Schmidg, 1998; Klaus *et al.*, 1998; Montenegro, 1998; Molina *et al.*, 2014). On the other hand, the main by-product of oil activities -produced waters- presents a very high salinity (Fakhru'l-Razi *et al.*, 2009). (see *Introduction* for a detailed explanation to oil extraction activities in the Amazon, oil pollution and geophagy). Therefore, oil-polluted salt licks might exert a similar or even higher effect, posing at risk vast hunting grounds. In fact, natural salt licks exert a pull effect on wildlife and some animals walk long distances and even exceed their home ranges to visit a salt lick (Tobler, 2008). Indigenous communities inhabiting the study area rely on game species for their livelihoods and they visit both natural salt licks and oil-polluted sites for hunting purposes (Mayor *et al.*, 2014; Orta-Martínez, Rosell-Melé, *et al.*, 2018). There is also a broad concern about the impacts of anthropogenic noise on wildlife behaviour, largely due to the recent global increase of noise fostered by the expansion of resource extraction activities, human populations, and transportation networks (Shannon *et al.*, 2016). Despite the wide range of noise types and exposure levels and the intrinsic different responses among taxonomic groups, many authors agree that noise pollution is a relevant environmental stressor that might cause severe impacts on wildlife (Francis and Barber, 2013). The biological responses to noise pollution observed range from changes in individual behaviours to changes in ecological communities, including negative effects on animals' abilities to detect important sounds, but also changes in reproductive success, changes in vigilance and foraging behaviour, or changes on the density and structure of ecological communities, among others (Barber *et al.*, 2010; Francis and Barber, 2013; Shannon *et al.*, 2016). However, most studies on noise impact have been performed in Europe and North America, and mostly on songbirds and marine mammals (Shannon *et al.*, 2016) but research is also needed in other regions, like the tropical rainforests, where the expansion of resource extraction activities is especially acute and the biodiversity is very high (Orta-Martínez, Rosell-Melé, *et al.*, 2018).

In the same vein, another anthropogenic impact on wildlife behaviour is hunting pressure. For instance, hunting pressure might modify the species composition and the activity pattern of salt lick visitors (Parish, 2001; Bitetti *et al.*, 2008; Blake *et al.*, 2012; Hon and Shibata, 2013), and even have an effect on the size (body mass) of the animals (Peres, 1990). The road network of the oil companies have modified the spatial patterns of hunting pressure and here the subsequent changes on wildlife behaviour are studied.

Given the importance of the potential impacts of the ingestion of oil-polluted soil and water by wildlife in terms of both conservation and public health, the main aim of this chapter is to confirm and describe geophagy of oil-polluted soil and water. To do so, the following aspects are investigated in this chapter: (a) the occurrence of petroleum biomarkers (sterans and hopanes) and oil-related heavy metals in the soil of apparently oil-polluted and non-polluted salt licks; (b) the range of species visiting oil-polluted salt licks, their visitation frequency, and the percentage of visitors showing ingestion evidences; (c) the potential causes behind geophagy of oil-polluted soil and water, in particular, the salt composition of the oil-polluted and non-polluted salt licks; and, (d) the total land area of local Indigenous hunting grounds at risk of being affected by the presence of oil-polluted salt licks. Furthermore, to explore other potential impacts of oil extraction on wildlife, this chapter also examines (e) changes in species behaviour due to noise disturbance -related to the hydrocarbon activity- and to hunting pressure. Given the scarce data that exist on salt licks, this chapter also aims (f) to shed some light into the general understanding of the relationship between elemental composition of salt licks and the type of visitors (i.e., species) of each salt lick.

METHODOLOGY

To achieve the research aims of this chapter, a comparative study among natural and apparently oil-polluted salt licks in the oil Block 1AB/192 was conducted. Together with local Indigenous hunters and environmental monitors from FEDIQUEP and FECONACOR, soil samples were collected and trap cameras were installed in both, natural and apparently oil-polluted salt licks. The last were selected based on an *in situ* organoleptic assessment. Specifically, Bushnell 8MP Trophy Cam HD trap cameras were installed in three natural salt licks and 19 apparently oil-polluted ones located in the oil Block 192/1AB, between July 2013 and October 2015. Camera traps were not active for all this period in all the salt licks (Figure S2.1). Soil samples were collected from all the study sites.

Data were analysed in several steps. First, analyses of soil samples of the salt licks were carried out to determine whether they were indeed polluted by petrogenic compounds (petroleum hydrocarbons and/or oil-related heavy metals). Second, the videos recorded by the trap cameras were analysed not only to document whether wildlife was actually ingesting soil and water in oil-polluted salt licks, but also to describe this behaviour. A massive amount of data has been produced by this project (approximately 50,000 survey hours and 8,200 videos). Here, only the analysis of 2,206 videos recorded in seven sites - three natural salt licks and four apparently oil-polluted sites- is presented. The videos allowed to identify species diversity, species' visit frequency and visit duration, the percentage of species' visits in which ingestion of soil and water occurs, and the pull effect of oil-polluted sites by comparing natural and oil-polluted salt licks. Third, the elemental composition of the soil samples of the salt licks was also analysed to explore the reasons behind this behaviour. Fourth, to determine the spatial extent of the impact of oil-polluted soil and water ingestion, a spatial analysis with geographic information system (GIS) tools, combining the location of oil infrastructure prone to produce spills with animals' mobility data, was conducted. Finally, the analysis of the recorded videos by the camera trapping survey allowed the identification of some other possible species behavioural changes potentially related to noise disturbance and hunting pressure.

STUDIED SALT LICKS

All studied salt licks are located in the Corrientes and Pastaza River basins in the Northern Peruvian Amazon (Fig 2.1). They are inside the actual limits of the oil Block 192/1AB or few kilometres away from it. Both natural and apparently oil-polluted salt licks were active hunting spots and were selected for this study by the Indigenous monitors. Three apparently oil-polluted salt licks [sites 3, 4 and 5] and one natural salt lick [site 1] are found in the Pastaza River basins while one apparently oil-polluted salt lick [site 6] and two natural salt licks [site 2 and 7] are located in the Corrientes River basin. Field visits to the area revealed that animals' fingerprints were very evident in all these sites. The geographic location of all sites was recorded on a Global Positioning System (TwoNav, CompeGPS) using the WGS 84 geographic coordinate system.

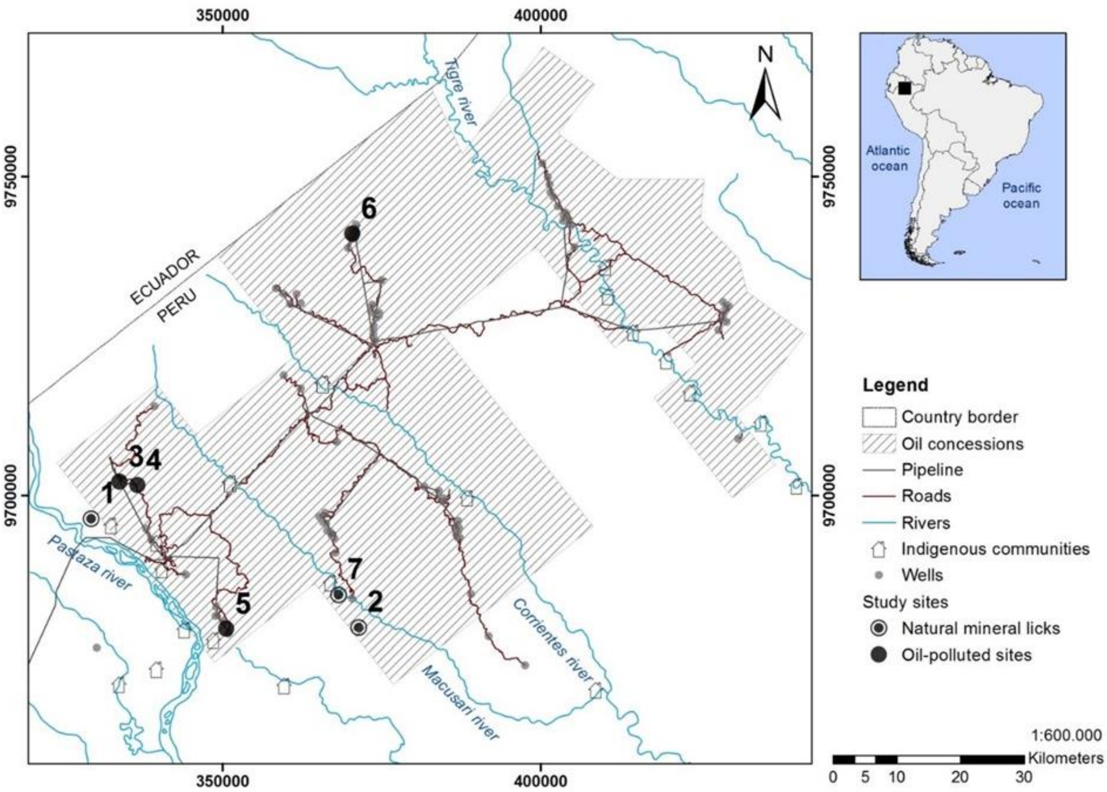


Figure 2.1 I Study area and studied salt licks.

All the studied salt licks were characterized according to their accessibility, distance to Indigenous communities, and distance to oil infrastructure (Table 2.1). Sites 1, 2 and 5 are much more inaccessible to human than the other sites, which are easy reachable by car or boat. Sites 3, 4 and 6 are more prone to be noisy, since they are located close to active oil infrastructures. All natural salt licks are located more than 3 Km away from the oil company road-system. Most sites are separated one from another by more than 10 Km. The Euclidean distance between sites 1-3, 1-4, 3-4 and 2-7 are 7.6 Km, 9.0 Km, 2.9 Km and 6.5 Km, respectively. Site 7 is the smallest salt lick (ca. 10 m^2) and site 2 is the biggest one (ca. 150 m^2).

Table 2.1 | Means of access and proximity of the studied salt licks to Indigenous communities (proxy of hunting pressure) and their distance from ongoing oil activities (proxy of noise disturbance).

Study sites	Hunting pressure				Noise disturbance	
	Distance away from the closest community				Distance away from the closest ongoing oil-activity	
	Kilometres [1]	Hours	Means of transport	0) > 1 hour away 1) ≤ 1 hour away	Kilometres [2]	0) > 3 Kms away 1) ≤ 3 Kms away
Natural salt lick 1	12	3	B + W	0	9	0
Natural salt lick 2	19	2	B + W	0	12	0
Natural salt lick 7	4	0.75	B + W	1	7	0
Oil-polluted salt lick 3	19	0.75	C	1	0	1
Oil-polluted salt lick 4	15	0.5	C	1	0	1
Oil-polluted salt lick 5	7	1.75	C + W	0	4	0
Oil-polluted salt lick 6	40	2	C	0	0.5	1

Note: The set of initials B, C and W stands for boat[8], car/motorcycle and walking, respectively. "Kilometers [1]" have been calculated considering the rivers' and paths' courses. "Kilometers [2]" indicates the Euclidean distance.

Oil-polluted salt licks

Oil-polluted salt licks 3 and 4 are close to the drainage pipe of sump tanks and close to the oil road, 19 Km and 15 Km away from the closest Indigenous community. Sump tanks are designed to contain oil overflowing from a well due to an unexpected increase in pressure. However, in the study area oil is rarely recovered from these tanks, and since most of the tanks are uncovered, oil overflows from tanks with the regular heavy tropical rainfalls (Orta Martínez *et al.*, 2007). Oil-polluted salt lick 6 is a 30-years-old oil spill located close to an oil central production facility and the oil road, but far (i.e., 40 Km) from the closest Indigenous community. Oil-polluted salt lick 5 is an abandoned well drilled in 1970 and sealed in 1982 that was licking when the study was conducted. It is accessible only by foot -one hour away from the oil road- and it is 7 Km away from the closest Indigenous community.

[8] It refers to a canoe with a 5.5 hp motor (locally known as *peke-peke*).

Natural salt licks

Natural salt lick 1 is located close to the Michicuyacu River and access is very limited since the river is not often navigable and it requires an additional 2 hours walk from the river. For this reason, it is used by very few hunters. Natural salt lick 2 is located close to the Macusari River, a tributary of the Corrientes River. It is reachable only by foot, at about one hour walk from the river. It was not used by hunters during many years as it was locally thought that the site was protected by a dangerous spirit (*madre mala*), but in the last decade it has been used. Natural salt lick 7 is also found close to the Macusari River but much closer to the nearest Indigenous community than site 2 (20 minutes' walk from the river) and it is usually used by local hunters. According to the environmental monitors, this site became a salt lick only after a detonation for seismic oil-exploration purposes.

SOIL SAMPLES COLLECTION AND ANALYSIS

Soil samples resulted from the combination and homogenization of three samples taken from the superficial soil (between 0 and 20 cm-depth), separated by at least 1.5 meters in each salt lick and collected using a methacrylate tube (7 cm ø). Soil samples were stored in plastic bags, transported at ambient temperature, and placed in a freezer once arrived at the laboratory. In the laboratory, mostly glass and porcelain materials were used to avoid sample contamination with metals or hydrocarbons. The three samples per site were mixed, homogenised, lyophilised, sieved (0.6 mm), and pulverised with the help of a porcelain mortar. Weights were annotated in each step.

Petroleum biomarkers analysis

To determine the presence of oil-related pollution in the collected soils, the occurrence of petroleum biomarkers (sterans and hopanes) was analysed in the Environmental Forensics Laboratory from the ICTA-UAB. Steranes and pentacyclic triterpanes (hopanes) are compounds typically found in high concentrations in crude oils and petroleum products (e.g., asphalt, heavy residuals oils or lubricating oils). These compounds are particularly useful to define the identity of the spilled product in environmental forensics, when the petrogenic substance is severely weathered (Wang and Stout, 2010; Wang *et al.*, 2013; Aeppli *et al.*, 2014). They are formed during diagenesis and catagenesis of organic matter from the precursors steroids and hopanoids and are more resistant to biotic and abiotic degradation than other compounds typically found in oils, such as n-alkanes and PAHs, which have also significant modern sources in tropical environments (Volkman *et al.*, 1997; Wilcke *et al.*, 2000; Wolfgang Wilcke *et al.*, 2002; Krauss *et al.*, 2005; Wilcke, 2007). The source specificity and low susceptibility to weathering offered by hopanes and steranes make them useful and reliable biomarkers for oil characterisation (Albaigés *et al.*, 2015). Indeed, it is largely agreed that the presence of hopanes and steranes compounds in environmental samples undoubtedly indicates the existence of oil derived products (Volkman *et al.*, 1997; Wang *et al.*, 2006).

The determination of hopanes and steranes compounds was carried out using the analytical method of gas chromatography–mass spectrometry (GC-MS). First, the oil residues from soil samples were extracted. To remove high-boiling compounds that are not eluted from the GC column and may influence the performance of the instrument (Albaigés *et al.*, 2015), the hydrocarbon fractions were separated by column chromatography. Extracts were injected in a GC-MS instrument, where selected ion monitoring was used to retrieve the distribution of hopanes and steranes from the whole GC-MS chromatograph. In this case, the monitoring of ions with a mass-to-charge ratio (m/z) of 191 was selected to obtain the distribution of hopanes, and 217 and 218 for steranes. The whole analysis was performed for the seven soil samples, two blancs and one reference sample. Squalene (25 ng/µg) was added as internal standard.

The extraction of the samples with organic solvents in a microwave (MarsX-CELL) was done using 10 Teflon digestion vessels that were previously cleaned using the same solvent. 5 g of soil for all the samples were extracted, except for soil samples from sites 6, where only 2 g were used, because of the appreciable higher oil pollution load. 25mL of trace analysis grade n-hexane–acetone (1:1, v/v) (Merck, Darmstadt, Germany), a magnetic stirrer and 150 mL of internal standard were added to each vessel. The extraction method started with a 12 minutes temperature ramp from room temperature to 115 ºC and then it kept this temperature for 2 minutes. After the extraction, the vessels' content was transferred to 50 mL test tubes for its centrifugation. Samples were centrifuged during 10 minutes at 2200 rpm in a centrifuge (Rotofix32-Hettich) and the supernatant liquid was transferred to 50 mL pear bottom flasks. Approximately 2mL of hexane were added to each test tube, which were agitated in a pulse-vortexing and then centrifuged again. This process was repeated three times in order to accurate as much as possible the collection of extract. Then the extract was evaporated to 0.5 mL using a rotary evaporator (Büchi Heating bath B-490 and Büchi Rotavapor R-200). The extracts were fractionated by adsorption chromatography with glass columns containing 2.5 g of silica (Scharlau, Barcelona, Spain), 2.5 g of aluminium oxide (Sigma–Aldrich, St. Louis, USA) previously activated at 110 ºC and deactivated 5% with ultrapure water (Mili-Q/Millipore, Cork, Ireland), and 1 g of sodium sulphate (Merck, Darmstadt, Germany). The first fraction of eluate was collected corresponding to aliphatic hydrocarbons eluting in 6 mL of *n*-hexane. The extracts were concentrated first by rotary evaporation and finally with a gentle stream of N_2 to near dryness.

The biomarkers identification was carried out in an Agilent 7890A gas chromatograph (GC) coupled to an Agilent 5975C mass spectrometer (MS) operated in electron impact ionization mode (70 eV) and equipped with a 30 m x 0.25 mm x 0.25 µm DB-5ms capillary column (J&W Scientific, CA, USA) and a 5 m guard column. The instrument was operated in splitless mode. The oven temperature program started at 60 ºC (held for 1 min) then increased to 320 ºC at a rate of 4 ºC min^{-1} and held for 10 min. Injector, transfer line and ion source temperatures were 310 ºC, 320 ºC and 250 ºC respectively. Helium was used as the carrier gas at constant flow of 2 mL min^{-1}. The MS was used in both scan mode and single ion mass mode at a time, monitoring m/z= 191 for the hopanes and m/z= 217-218 for the steranes. The hopanes and steranes more commonly used in oil spill identification, which are highlighted in the European Committee for Standardization (CEN) methodology *Oil spill identification –Waterborne petroleum and petroleum products - Analytical methodology and interpretation of results based on GC-FID and GC-MS low resolution analyses* (European Commitee for Standarization (CEN), 2012), were identified.

<u>Petrogenic heavy metals and elemental soil composition</u>

In order to determine the presence of oil-related pollution in the collected soils, concentrations of heavy metals were also analysed. Specifically, Cd, Ba, Hg, Pb, vanadium (V), Cr, nickel (Ni), beryllium (Be), manganese (Mn), As, Ca, Mg, K, Na, aluminium (Al), Fe, copper (Cu) and Zn were analysed since they are associated to crude oils (Khalaf *et al.*, 1982; Guzmán and Jarvis, 1996) and to produced waters (Fakhru'l-Razi *et al.*, 2009), the major by-product of oil extraction activities (see *Introduction: Oil pollutants*) .

The concentrations of the elements allegedly most sought by animals in Amazonian salt licks were also quantified: Na, Ca, Mg, K, Fe, Mn, and phosphorus (P) (Ayres & Ayres, 1979; Emmons & Stark, 1979; Lips & Duivenvooden, 1991; Molina et al., 2014; Montenegro, 1998; Narváez & Olmos, 1990, see *Introduction: Geophagy as potential exposure route*). Except for P, all of them are also associated to oil activity (Fakhru'l-Razi *et al.*, 2009).

The metals were analysed at the *Servei d'Anàlisi Química*, from the UAB. An aliquot (0.25 g) of each soil sample was digested with HNO_3, HCl and HF in an Ultrawave-Milestone microwave and diluted with HCl 1% (v/v). ICP-MS spectrometry was used to determine the concentration of Be, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Cd, Ba, Hg, and Pb using an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer. Ca, Mg, Na, K, and P content was determined by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) spectrometry using a Perkin-Elmer Optima 4300DV.

The detected concentration of metals was compared with the Maximum Permissible Levels (MPL) according to the Peruvian legislation. These are defined, since 2013, by the Peruvian Supreme Decree 002-2013-MINAM (Table S2.1), which describes the MPL for Pb, Ba, Hg, Cd and Cr VI depending on the soil typology: (a) agricultural, (b) residential/natural protected areas and (c) commercial/industrial/extractive soils. Concentrations were also compared with the levels described by the Canadian Environmental Quality Guidelines for the Protection of Environmental and Human Health (CCME, 1999) (Table S2.2).

CAMERA TRAPPING

Trap cameras triggered by an infrared motion-and-heat detector were used to study the ingestion of oil-polluted soil and water by wildlife. The use of these cameras is becoming very popular since costs have decreased and the equipment technology has improved in recent years (Tobler, 2008). They are used for diverse purposes such as creating species inventories, describing activity patterns and habitat use, and even estimating animal density for terrestrial mammal species (some examples: (Bowkett *et al.*, 2007; Matsubayashi *et al.*, 2007; Rowcliffe *et al.*, 2008; Rovero and Marshall, 2009; Tobler *et al.*, 2009; Brien *et al.*, 2010; Blake *et al.*, 2011; Molina *et al.*, 2014). In comparison with more traditional methods (e.g., line transects, direct sighting…), the use of camera traps presents a very high potential for studying elusive species in tropical rainforests causing minimal disturbance (Bowkett *et al.*, 2007; Rowcliffe *et al.*, 2008; Tobler, 2008; Dobson and Nowak, 2010). Moreover, the efficiency of data collected with camera traps is equal at night and during the daytime and it allows an easy standardization of survey methods across sites (Dobson and Nowak, 2010). Furthermore, images can be sent to experts anywhere in the world for verification (Tobler, 2008). Finally, images make much easier the communication of results for political advocacy and educational purposes. Downsides of trap cameras are the inexistence of a baseline data with which to compare the obtained data, since the use of this technology is very recent (Dobson and Nowak, 2010), and their lower efficiency regarding the collection of data for small mammals and arboreal species (Srbek-araujo and Chiarello, 2005). For all the aforementioned advantages of this methodology and, especially because of the remoteness of the study sites for both scientists and environmental Indigenous monitors, trap cameras equipped with lithium batteries (for a longer-lasting duration) were used in this research.

One trap camera was placed (Bushnell 8MP Trophy Cam HD I model 2) at each site, ca. 1 m above ground surface. Cameras were set in video mode and they recorded 1 minute videos when triggered by the infrared motion-and-heat detector. A period of 60 s of inaction between videos was established and cameras were continuously activated until batteries were discharged. Batteries were replaced when possible and different surveys were performed in the same salt lick, reaching a total of 450 camera days (Fig. 2.2). The camera trap survey was carried out together with the Indigenous environmental monitors.

Survey time was fragmented due to several reasons. On the one hand, the location of some cameras was changed during the study period when (1) the salt licks were abandoned by animals (no new visible footprints) and/or (2) there was risk of theft by oil company staff. Moreover, one camera was broken and one was lost/stolen during the camera trapping survey. Although trap cameras were equipped with lithium batteries for a longer run-time, they run out of battery for several periods. Remoteness of the study areas and inaccessibility of the salt licks make the replacement of the batteries very difficult. Access to the salt licks from the Indigenous communities was constrained by multiple and diverse factors (e.g., poor road conditions due to the heavy rainfall, roadblocks over long periods of time due to conflicts between Indigenous communities, or shortage of fuel, among others).

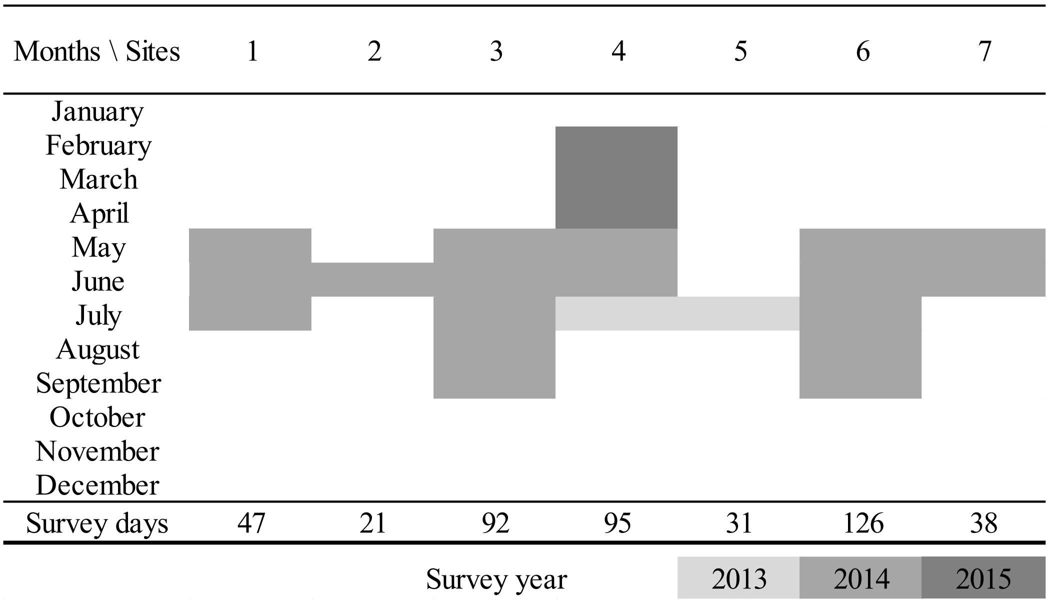


Figure 2.2 | Camera days of the camera trapping programme, per salt lick.

All videos were labelled with site id, camera id, survey id, date and time. For each video, several variables were noted down: species, number of biological units and ingestion proofs. These variables were converted into new variables per each site: species, visit frequency and duration, and percentage of visits with recorded ingestion proofs (from now on called percentage of ingestion evidence) and species' activity patterns (diurnal, nocturnal or cathemeral).

For the purpose of the analysis, a biological unity was equated with one individual for those species that are mostly solitary, like *Tapirus terrestris* or *Panthera onca*. Differently, a biological unit was equated with a group of individuals for those species which usually form herds or flocks, like *Ara macao* or *Pecari tajacu*.

Following Blake *et al.* (2011), to measure visit frequency, consecutive videos recording the same species were considered to belong to a unique visit when time between videos was less than 30 minutes (and no different biological unities were identifiable by unique markings or gender. Following Bowkett *et al.* (2007), 60 minutes were used as a cut-off for visits' length. Visit frequency is expressed as the number of visits per 100 camera days. Visit duration was calculated by subtracting the hour-time of the first video that forms a visit from the hour-time of the final video that belongs to the same visit. As a cut-off of 60 minutes was set for visits' length, visit duration cannot overcome this value. When a visit was represented by a single video, it was assumed that the visit duration was 1 minute even if the animal appeared less than that time in the video. The recorded acts of chewing, eating, drooling, swallowing or licking soil and water, or licking the oil infrastructure were classified as “ingestion proofs”. The percentage of ingestion evidence was calculated as the percentage of visits were ingestion proofs were recorded.

To study the potential pull effect of oil-polluted salt licks, visit frequency and duration for both oil-polluted and natural salt licks were compared. When possible, individual identification was also carried out for *Mazama americana* and *Tapirus terrestris*. These data were also used to study the pull-effect and to give us a sense of the total population affected. Individual identification allowed determining whether oil-polluted soils and water were ingested by a reduced number of individuals that had acquired the taste for them or rather it was a more common behaviour. Although individuals are difficult to identify because they differ little in appearance, permanent or long-lasting marks such as important body deformations and injuries (e.g., one-eyed animals, broken horns and ears, deep skin marks, deviated snout, among others) or exceptional body size (e.g., litters) were used to identify different individuals. These marks are easier to identify for the mentioned two ungulate species than for other species. To consider the possible appearance of the same individuals in different salt licks, identified individuals from different study sites were compared when the distance among sites was smaller than the species mobility.

Species’ activity patterns were also registered. Patterns were defined considering the percentage of visits happening at night (18:30h-5:30h), during the day (6:30h-17:30h), or at twilight (5:30h-6:30h and 17:30h-18:30h). Following Tobler (2008), species with more than 80% of visits recorded during the day were classified as diurnal, species with more than 80% of videos recorded at night as nocturnal, and the remaining species were classified as cathemeral.

Species identification was conducted with the Indigenous environmental monitors and the help of Peruvian scientists. The videos were analysed by five different researchers at the ICTA-UAB, using a guide created for their homogeneous analysis. Most videos were analysed only by one of these researchers, but the videos that were prone to generate confusion or misinterpretation were reanalysed by at least two researchers.

STATISTICAL AND SPATIAL ANALYSES

A descriptive statistical analysis of the variables obtained from the camera traps and from soil analysis was performed. Wilcoxon tests were used 1) to confirm that the apparently oil-polluted salt licks were actually oil-polluted salt licks (H#1: There will be higher concentrations of oil-related metals in soils from apparently oil-polluted salt licks than in soils from natural salt licks); 2) to understand the causes of oil-polluted soil and water ingestion (H#2: There will be similar or lower levels of metals allegedly sought by animals in salt licks found in natural than in oil-polluted salt licks); 3) to investigate a possible higher pull effect of oil-polluted salt licks (H#3: The number of identified individuals of *Mazama americana* and *Tapirus terrestris*, the total number of species visiting each site, the visit duration, visit frequency, and the percentage of ingestion evidence for all the species recorded will be higher in oil-polluted than in natural salt licks); 4) to study the relationship between species composition and the elemental composition of the salt licks (H#4: Differences on species composition of salt licks' visitors will be associated with differences on elemental composition of salt licks). Pearson correlation tests were used to correlate the visit frequency of specific species groups with specific minerals. A significance level of 10% (p-value>0.1) for all Wilcoxon and Pearson tests was established and it was considered that there was a strong correlation when correlation (r) was higher than 0.7 or lower than -0.7 in the correlation test.

To estimate the area indirectly affected by the oil-polluted salt licks in the oil concession 192/1AB, i.e., the hunting grounds where Amazonian game species may have access to oil-polluted salt licks, a spatial analysis was conducted. Oil infrastructure (i.e., wells, pipelines and central production facilities) was used as a proxy for the potential location of oil-polluted salt licks. Secondary data on species mobility to visit salt licks was used to calculate the distance from where wildlife could reach a potential oil-polluted salt lick. When there was no secondary data on the species' mobility to a salt lick, the species' home range was used. Maximum and minimum values from the literature were used to calculate these areas. A buffer surrounding the oil infrastructure was drawn using the mobility of species to salt licks (buffer distance = mobility to salt licks) and their home range data (buffer distance = $2*\sqrt{(\text{home range}/\pi)}$. Oil-polluted salt licks were assumed to be homogeneously distributed along the infrastructure. A potentially higher pull effect of oil-polluted than of natural salt licks was assumed to not affect species' mobility. The same analysis was carried out for all the species identified visiting oil-polluted salt licks, except for those whose mobility data were unavailable. Oil infrastructure information was obtained from Perupetro (the Peruvian state energy company) and own digitalization. Data homogenization, transformation and analysis were performed using ArcGIS x10.

To get a sense of the impact of noise -from oil activity- and subsistence hunting disturbances in animal behaviour, the studied salt licks were classified according to two variables. First, salt licks were classified depending on their distance to ongoing oil activities (including roads, oil wells and central production facilities), as a proxy for noise disturbance. A cut-off distance of 3 Km was established. Buij *et al.* (2007) assumes that further from 1 Km there is no noise impact associated to roads. In this chapter it is assumed that noises related to oil activities would not impact areas located three times further. Second, salt licks were also classified depending on the travel distance to the salt licks from the Indigenous communities, as a proxy for hunting pressure, since it has been repeatedly reported that both variables are strongly correlated (Vickers, 1991; Parish, 2001) (Table 2.1). Wilcoxon tests were conducted to compare the values of the animal behaviour variables (e.g., percentage of diurnal visits or, percentage of ingestion evidence and, visit's duration and frequency) for the most common visitor species in salt licks with different levels of expected noise from ongoing oil activities and hunting pressure. For the analysis, the binary variables shown in Table 2.1 were used. When comparing the visit frequency of the most common visitor species in sites differently affected by noise and hunting pressure, the analysis was restricted to those salt licks where these species accounted for, at least, the 50% of the visits. All statistic tests were performed with R-Studio version 0.98.1062 2009–2013 (RStudio, Inc. with lme4 Package and Deducer JRG version 1,7–9, 2003–2011 RoSuDa, Univ. Augsburg).

RESULTS

WILDLIFE INGESTION OF OIL-POLLUTED SOIL AND WATER: DESCRIPTION AND CAUSES

<u>Soil analysis</u>

Petrogenic hydrocarbons

All soil samples collected in apparently oil-polluted salt licks presented traces of steranes and hopanes (Fig. 2.3 and Table 2.2). In the case of hopanes, the 17α(H),21β(H)-

stereochemistry predominate, indicating a substantial contribution from petroleum (Volkman *et al.*, 1997; Wang *et al.*, 2006; Sakari *et al.*, 2010). The steranes in petroleum, as they are derived from sterols, occur as the C27, C28 and C29 homologous series in different proportions, which reflect the carbon number distribution of the sterols in the precursor organic matter in the source rocks for these oils (Wang and Stout, 2010). Steranes in petroleum have a number of isomeric forms with different stereochemistries at positions 5, 14, 17, 20 and 24. These are the type of distributions found in the samples from apparently oil-polluted salt licks, which further confirms the occurrence of petrogenic inputs to the soils collected in these sites. Contrastingly, traces of steranes or hopanes were not found in the soil samples collected in natural salt licks (Fig. 2.4).

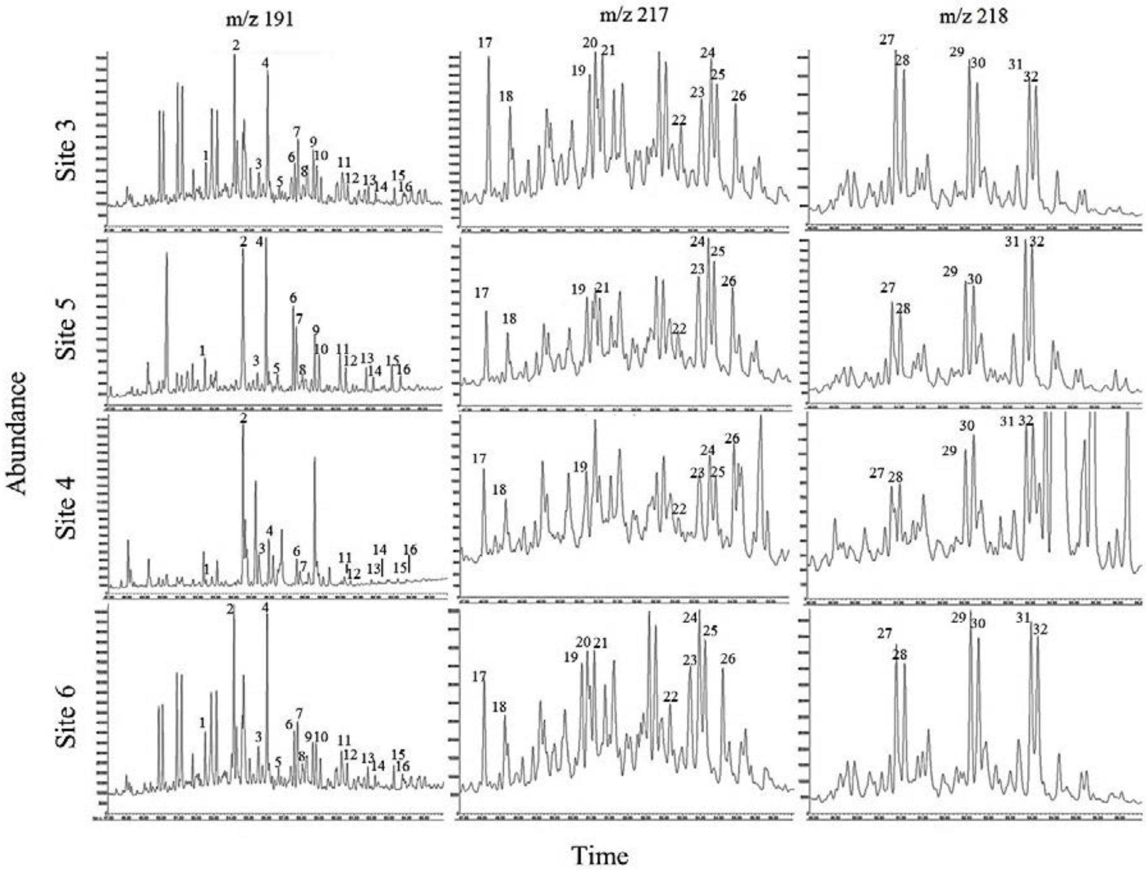


Figure 2.3 | Ion chromatograms and identification of the A) hopanes (m/z 191), B) steranes (m/z 218), and C) steranes (m/z 217) found in the soil samples from the apparently oil-polluted salt licks.
Note: Names are shown in Table 2 and are based on CEN (2012).

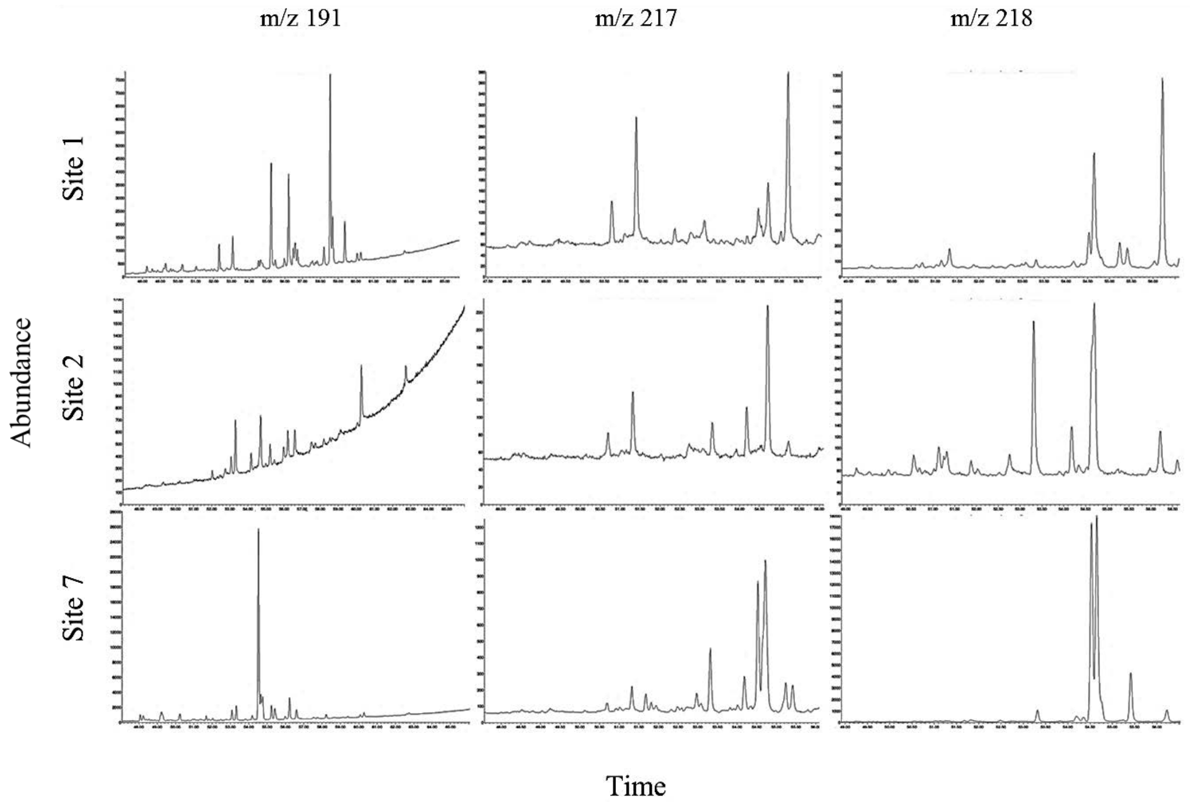


Figure 2.4 | Ion chromatograms for m/z 191, 218 and 217 for soil sample from the natural salt licks.

Table 2.2: Abbreviations and chemical notation of steranes and hopanes found in the soil samples from the apparently oil-polluted salt licks and the m/z fragment in the spectrometric analysis used to identify them.

#		Abbreviation	Name	m/z	
1		27Tm	17α(H)-22,29,30-trisnorhopane	191	HOPANES
2		29ab	17α(H),21β(H)-30-norhopane	191	
3		29ba	17β(H),21α(H)-30-norhopane (normoretane)	191	
4		30ab	17α(H),21β(H)- hopane	191	
5		30ba	17β(H),21α-(H)-hopane (moretane)	191	
6		31abS	17α(H),21β(H), 22S-homohopane	191	
7		31abR	17α(H),21β(H), 22R-homohopane	191	
8		30G	Gammacerane	191	
9		32abS	17α(H),21β(H), 22S- bishomohopane	191	
10		32abR	17α(H),21β(H), 22R-bishomohopane	191	
11		33abS	17α(H),21β(H), 22S- trishomohopane	191	
12		33abR	17α(H),21β(H), 22R-trishomohopane	191	
13		34abS	17α(H),21β(H), 22S-tetrakishomohopane	191	
14		34abR	17α(H),21β(H), 22R-tetrakishomohopane	191	
15		35abS	17α(H),21β(H), 22S-pentakishomohopane	191	
16		35abR	17α(H),21β(H), 22R-pentakishomohopane	191	
17	C27	27dbS	13β (H),17α(H), 20S - cholestane (diasterane)	217	STERANES
18	C27	27dbR	13β (H),17α(H), 20R - cholestane (diasterane)	217	
19	C27	27aaS	5α (H),14α(H),17α(H)-cholestane (20S)	217	
20	C27	27bbR	5α (H),14β(H),17β(H), 20R-cholestane	217	
21	C27	27bbS	5α (H),14β(H),17β(H), 20S-cholestane	217	
22	C28	28aaR	24-methyl-5α(H),14α(H),17α(H), 20R- cholestane	217	
23	C29	29aaS	24-ethyl-5α(H),14α(H),17α(H), 20S- cholestane	217	
24	C29	29bbR	24-ethyl-5α(H),14β (H),17β(H), 20R- cholestane	217	
25	C29	29bbS	24-ethyl-5α(H),14β (H),17β(H), 20S- cholestane	217	
26	C29	29aaR	24-ethyl-5α(H),14α(H),17α(H), 20R- cholestane	217	
27	C27	27bbR	5α (H),14β(H),17β(H), 20R-cholestane	218	
28	C27	27bbS	5α (H),14β(H),17β(H), 20S-cholestane	218	
29	C28	28bbR	24-methyl-5α(H),14β (H),17β(H), 20R- cholestane	218	
30	C28	28bbS	24-methyl-5α(H),14β (H),17β(H), 20S- cholestane	218	
31	C29	29bbR	24-ethyl-5α(H),14β (H),17β(H), 20R- cholestane	218	
32	C29	29bbS	24-ethyl-5α(H),14β (H),17β(H), 20S- cholestane	218	

Petrogenic heavy metals

From all heavy metals related to oil and oil extraction by-products (i.e., Cd, Ba, Hg, Pb, V, Cr, Ni, Be, Mn, As, Ca, Mg, K, Na, Al, Fe, Cu, and Zn), only Al, Ca, and Mn presented higher median concentrations in natural salt licks than in oil-polluted ones. The remaining metals related to oil extraction industry showed higher median concentrations in oil-polluted salt licks. Nevertheless, only Pb presented a statistically significant higher median concentration in oil-polluted salt licks than in natural salt licks (see p-values in Table 2.3). Contrastingly, Ca showed significant higher concentrations in natural than in oil-polluted salt licks, while the remaining metals did not reveal any statistically significant difference.

When comparing these concentrations with the Peruvian MPLs (Table S2.1), only the soil sample collected in the oil-polluted salt lick 5 exceeded the established level of Ba for both agricultural soil and soils of residential/natural protected areas. Metal concentrations in other salt licks do no exceed the MPLs. Some metal levels from five salt licks exceeded MPLs of the Canadian soil quality guidelines (Table S2.2). Ba concentration in the oil-polluted salt lick 5 exceeded the MPL for both residential/parkland and agricultural soils. Ni, Cr (total), and V concentrations in natural salt lick 1 exceeded the established levels (for both residential/parkland and agricultural soils in the case of Ni and, for all soil types in the case of Cr (total) and V). MPLs of V are also exceeded in the oil-polluted salt licks 3, 4, and 6 for all soil categories.

Elemental composition

The concentrations of the minerals allegedly sought for wildlife in salt licks greatly differ among sites (Table 2.3). Although not significantly, median concentrations of Mg, K, Na, Fe, Cu and Zn were higher in the oil-polluted salt licks than in natural ones, while median concentrations of Ca, P and Mn were lower in the oil-polluted than in natural salt licks. Notwithstanding, only Ca showed a significantly higher median concentration in natural salt licks while the other minerals (Mg, K, Na, Fe, Cu, Zn, P and Mn) did not show any statistically significant difference in the median concentrations between natural and oil-polluted salt licks (Table 2.3).

Table 2.3 | Concentrations (dry weight) of heavy metals found in the study sites and the median (IQR) values for both types of salt licks: natural and oil-polluted salt licks.

Sites	Cd (µg/g)~	Ba (µg/g)~	Hg (µg/g)~	Pb (µg/g)~	V (µg/g)~	Cr (µg/g)~	Ni (µg/g)~	Be (µg/g)~	Al (mg/g)~	As (µg/g)~
Natural salt lick 1	0.18	352.53	0.05	12.56	173.29	94.32	76.11	1.03	33.09	1.75
Natural salt lick 2	0.14	60.21	0.05	10.53	97.62	17.60	4.70	0.43	7.15	1.61
Natural salt lick 7	0.13	81.23	0.11	15.04	81.60	24.35	6.68	0.66	7.16	2.88
Oil-polluted site 3	0.13	139.78	0.15	27.28	140.00	29.79	16.77	1.07	6.41	2.32
Oil-polluted site 4	0.05	77.44	0.05	15.81	125.91	31.52	11.32	0.75	7.30	1.81
Oil-polluted site 5	0.26	982.21	0.05	27.77	92.89	23.43	7.42	0.52	3.71	2.81
Oil-polluted site 6	0.34	343.95	0.14	41.91	164.63	53.79	32.59	1.61	6.62	6.90
Natural salt licks	0.14 (0.03)	81.23 (146.16)	0.05 (0.032)	12.56 (2.26)	97.62 (45.85)	24.35 (38.36)	6.68 (35.71)	0.66 (0.30)	7.61 (12.97)	1.75 (0.63)
Oil-polluted sites	0.20 (0.17)	241.86 (379.82)	0.10 (0.09)	27,52 (6,89)	132.96 (28.50)	30.65 (8.89)	14.05 (10.38)	0.91 (0.51)	6.51 (1.06)	2.57 (1.64)
Wilcoxon test - p-value	0.857	0.629	0.559	0.057*	0.857	0.857	0.629	0.400	0.229	0.400

Sites	Ca (mg/g)~^	Mg (mg/g)~^	K (mg/g)~^	Na (mg/g)~^	P (mg/g)^	Mn (µg/g)~^	Fe (mg/g)~^	Cu (µg/g)~	Zn (µg/g)~
Natural salt lick 1	30.32	18.80	4.06	18.63	0.85	792.06	46.06	45.43	95.77
Natural salt lick 2	1.55	0.53	1.36	< 1	< 0,1	295.81	23.41	8.41	30.40
Natural salt lick 7	2.65	0.39	2.00	< 1	0.23	872.42	19.01	9.62	36.74
Oil-polluted site 3	< 1	1.12	4.25	1.17	0.28	511.58	33.78	32.37	94.41
Oil-polluted site 4	< 1	0.93	4.63	1.18	0.12	387.69	30.99	27.80	56.78
Oil-polluted site 5	< 1	0.16	2.79	< 1	0.10	111.67	17.32	12.73	59.78
Oil-polluted site 6	2.19	0.75	5.65	2.59	0.85	414.60	38.22	37.89	128.73
Natural salt licks	2.65 (14.38)	0.53 (9.21)	2.00 (1.35)	0.50 (9.06)	0.23 (0.40)	792.06 (288.31)	23.41 (13.52)	9.62 (18.51)	36.74 (32.69)
Oil-polluted sites	0.50 (0.42)	0.84 (0.38)	4.44 (1.00)	1.17 (0.53)	0.20 (0.31)	401.14 (120.16)	32.39 (7.32)	30.09 (9.72)	77.10 (43.97)
Wilcoxon test - p-value	0.099*	1.000	0.114	0.854	0.857	0.400	1.000	0.629	0.400

Note: The symbol (~) indicates the association of the metal to oil activities and (^) indicates a metal allegedly sought by wildlife in salt licks. Values "<1" and "<0.1" are considered equivalent to 0.5 and 0.05 when performing statistical tests. Symbol (*) indicates a significant difference between both medians (p-value < 0.10).

Camera trapping

A total of 2,206 videos were recorded during 450 camera days in four oil-polluted salt licks and three natural salt licks. 79.24% of the videos contained animals and the remaining were activated mostly by the rain and by abrupt changes in light (maybe transformed into sudden changes of temperature of the recorded landscape). A total of 1,072 visits from 21 different species have been documented. Visit frequency was 237.41 visits/100 camera days, with similar values among natural and oil-polluted salt licks (285.59 and 222.54 visits/100 camera days, respectively). The highest visit frequency was reported in natural salt lick 2, followed by the oil-polluted salt licks 5 and 3, with 574.48, 510.76 and 459.04 visits/100 camera days, respectively (Table 2.4).

The ungulate *Tapirus terrestris* accounted for the 62.04% of the visits, followed by the ungulate *Mazama americana* (24.44%), the birds *Ortalis guttata* (4.94%), *Ara chloroperus* (1.87%) and *Ara macao* (1.12%), and the rodents *Cuniculus paca* (1.40%) and *Dasyprocta fuliginosa* (1.12%). The remaining 3.07% visits were done by the rodents *Coendou prehensilis* and *Myoprocta pratti*, the birds *Psophia leucoptera*, *Brotogeris sp.*, *Momotus momota*, *Cacicus cela* and *Pitangus sulphuratus*, the ungulate *Pecari tajacu*, the felines *Panthera onca* and *Leopardus sp.*, the mustelid *Eira Barbara*, the monkey *Saguinus sp.*, the squirrel *Sciurus sp.* and the lizard *Kentropyx sp.* Ten species have been recorded chewing, eating, drooling, swallowing or licking soil and/or water or licking the oil infrastructure either in the natural or the oil-polluted salt licks studied: *Tapirus terrestris*, *Mazama americana*, *Pecari tajacu*, *Cuniculus paca*, *Coendou prehensilis*, *Ara chloroperus*, *Ara macao*, *Brotogeris sp.*, *Ortalis guttata* and *Momotus momota,*. The first eight species have been recorded ingesting in the oil-polluted salt licks. Although bats do also ingest soil in salt licks (Bravo *et al.*, 2008; Voigt *et al.*, 2008) and were also recorded in the oil-polluted salt licks 3, 5 and 6 and in natural salt licks 2 and 7, they have not been included in the analysis that follow because in the study areas they are not hunted.

Two types of salt licks have been identified: those visited mainly by birds (sites 1 and 6) and those visited primarily by ungulates (sites 2, 3, 4, 5 and 7) (Fig. 2.5).

Considering the animals' mobility and the possible appearance of the same individual in different salt licks by comparing identified individuals from nearby salt licks, eighteen different individuals of *Tapirus terrestris* and nine of *Mazama americana* were identified in the study sites (Table 2.4). The number of *Tapirus terrestris* and *Mazama americana* individuals identified was very similar between natural and oil-polluted salt licks, as well as their visit's frequency and duration, and the percentage of ingestion evidence. In the Wilcoxon test, no significantly differences were found between the median values of any of these variables in both types of salt licks (Table 2.4). However, the number of simultaneous appearances of different species was higher in oil-polluted than in natural salt licks. Additionally, the groups of *Mazama americana* and *Tapirus terrestris* simultaneously visiting the sites were bigger in oil-polluted than in natural salt licks (Table 2.4).

Oil-polluted salt licks

A total of 1,460 videos were recorded during the 345 camera days in oil-spilled salt licks from which 79.93% contained animals. These videos corresponded to 768 visits, with a visit frequency of 222.54 visits/100 camera days. Visit frequency widely varied among sites and species. Site 5 presented the highest visit frequency (510.76), followed by site 3 (459.04), and site 4 (140.73). Site 6 showed the lowest visit frequency with 38.90 visits/100 camera days (Table 2.4).

The fifteen (15) different species recorded in oil-polluted sites (Table 4) are the ungulates *Tapirus terrestris* (accounting for 57.55% of the visits), *Mazama Americana* (33.72%) and *Pecari tajacu* (0.26%), the rodents *Cuniculus paca* (0.91%), *Dasyprocta fuliginosa* (1.17%) and *Coendou prehensilis* (0.78%), the birds *Ara chloropterus* (2.60%), *Ara macao* (1.56%), *Brotogeris sp* (0.52%), *Psophia leucoptera* (0.26%), *Pitangus sulphuratus* (0.13%) and *Cacicus cela* (0.13%), the feline *Leopardus sp*. (0.13%), the squirrel *Sciurus sp*. (0.13%) and the reptile *Kentropyx sp.* (0.13%) .

The following eight (8) species appeared ingesting soil or water: *Tapirus terrestris* (ingestion proofs in 55.20% of the visits), *Mazama americana* (79.54%), *Pecari tajacu* (50.00%), *Cuniculus paca* (14.29%), *Coendou prehensilis* (16.67%), *Ara chloroptera* (85.00%), *Ara macao* (83.33%), and *Brotogeris sp.* (100.00%).

In all the oil-polluted salt licks, ingestion proofs were recorded in more than half of the visits (62.76%). The percentage of ingestion evidence varies from 74.84% in site 5 to 53.73% in site 4.

The species recorded also varied among sites (Fig. 2.5). Oil-polluted salt lick 4 presented the highest gamma-diversity (with nine different species). This site was mainly visited by the ungulates *Mazama Americana* (47.76% of the visits) and *Tapirus terrestris* (35.82%), with some videos showing their simultaneous visit to the site. The rodents *Cuniculus paca* (5.22%) and *Coendou prehensilis* (4.48%) were also important visitors. Other species recorded in site 4 were *Pecari tajacu* (1.49%), *Dasyprocta fuliginosa* (2.24%), *Psophia leucoptera* (1.49%) and *Sciurus sp*. (0.75%) and *Kentropyx sp* (0.75%). With seven different species recorded during the survey, salt lick 6 is the second most biodiverse site. The 73.47% of the visits to this site belonged to the parrots *Ara chloroptera* (40.82%), *Ara macao* (24.49%) and *Brotogeris sp* (8.16%), which often visited simultaneously the site forming big and multi-species groups of parrots. *Mazama americana* (10.20%) and *Dasyprocta fuliginosa* (12.24%) were also frequent visitors to site 6. *Leopardus sp.* and the bird *Cacicus cela* visited this site only once. Oil-polluted salt lick 3 was only visited by *Tapirus terrestris* (55.40%) and *Mazama amerciana* (44.60%) and their simultaneous visit to the site was also reported. Oil-polluted salt lick 5 was almost exclusively visited by *Tapirus terrestris* (99.37%). The other recorded visitor in site 5 was the bird *Pitangus sulphuratus*.

According to the species visiting them, the oil-polluted salt licks can be classified into two categories: those mainly visited by the ungulates *Tapirus terrestris* and *Mazama Americana* (sites 3, 4 and 5) and one that was mainly visited by birds (site 6) (Fig. 2.5).

Up to three individuals of *Tapirus terrestris* were recorded simultaneously visiting sites 3 and 5, while in site 4 two individuals visited the place at the same time. In site 3, three *Mazama americana* individuals were recorded in the same video, while in site 4 the simultaneous visit of two *Mazama americana* individuals was also recorded. The group species *Pecari tajacu* visited site 4 in a 5-individual herd. Parrots visited site 6 in big flocks formed by up to 75 *Brotogeris sp* individuals, 27 *Ara chloropterus,* and six *Ara macao*. Three individuals of *Cacicus cela* were also simultaneously recorded in site 6. Up to two individuals of *Coendou prehensilis* visited site 4 at the same time. *Psophia leucoptera* visited site 4 in groups formed by up to four individuals. The remaining species were always solitary visitors.

Five, four, and two different individuals of *Tapirus terrestris* were identified in sites 5, 3 and 4, respectively. Five, two, and one individuals of *Mazama americana* were identified in sites 3, 4 and 6 respectively. After comparing identified individuals from close study sites, at least nine *Tapirus terrestris* individuals and eight *Mazama americana* individuals were identified in oil polluted salt lick by long-lasting marks.

The duration of the visits was very heterogeneous even for a single species in a specific site (Table 5). The mean value of visit duration in oil-polluted salt licks was 9.47±14.47 min. The species whose biological unities remained longer in the sites were the group species *Brotogeris sp* (20.25±26.42 min) and *Ara chloropetra* (14.20±17.23 min), followed by the ungulates *Tapirus terrestris, Pecari tajacu* and *Mazama Americana*, with visit durations of 11.19±16.30 min, 10.27±0.38 min and 7.27±10.77 min, respectively. Except for *Coendou prehensilis* (3.64±4.19 min), *Cuniculus paca* (2.11±2.95 min) and *Ara maco* (2.02±2.55 min), the remaining species did not visit the site for more than 1 minute.

Natural salt licks

In the three natural salt licks studied, a total of 746 videos were obtained in 106.45 camera days. Animals appeared in 77.88% of them, accounting for 304 visits and a visit frequency of 285.59 videos/100 camera days. The highest visit frequency was found in the natural salt lick 2 (574.48), followed by site 7 (236.13) and finally site 1 (196.74) (Table 2.4).

In total, eleven (11) different species have been recorded in natural salt licks (Table 4): the ungulates *Tapirus terrestris* (74.67%) and *Mazama Americana* (0.99%), the rodents *Cuniculus paca* (2.63%), *Dasyprocta fuliginosa* (0.99%) and *Myoprocta pratti* (0.99%), the birds *Ortalis guttata* (17.43%) and *Momotus momota* (0.66%), the primates *Saguinus sp* (0.33%), the felines *Panthera onca* (0.33%) and *Leopardus sp.* (0.33%) and the mustelid *Eira Barbara* (0.66%).

Five (5) species present ingestion evidences in their visits to natural salt licks: *Tapirus terrestris* (80.62%), *Mazama americana* (66.67%), *Cuniculus paca* (12.5%), *Ortalis guttata* (50.94%), and *Momotus momota* (50%).

As in the oil-polluted salt licks, species appearing in natural salt licks differ from site to site (Table 2.4). Site 1, with 10 different species recorded, is the most gamma-biodiverse natural salt lick, mostly visited by the bird *Ortalis guttata* (56.99%) and *Tapirus terrestris* (23.66%). The three rodents *Cuniculus paca*, *Dasyprocta fuliginosa* and *Myoporcta pratti* account for 12.91% of the visits to site 1 and other visitors include the bird *Momotus momota* (2.15%), the primate *Saguinus sp*. (1.08%), the felines *Panthera onca* (1.08%)

and *Leopardus sp*. (1.08%) and the mustelid *Eira Barbara* (1.08%). *Sanguinus sp*. individuals were recorded playing in the trees nearby site 1. Sites 2 and 7 are mostly frequented by *Tapirus terrrestris*, representing the 95.87% and 98.89% of the visits, respectively. In site 2 *Mazama Americana* (2.48%) and *Cuniculus paca* (1.65%) were also recorded, while *Eira Barbara* visited once site 7. However, there was no simultaneous appearance of multiple species in sites 2 and 7. Salt licks 2 and 7 could be classified as mono-specific sites, both visited almost exclusively by the ungulate *Tapirus terrestris*, while salt lick 1 is more gamma-biodiverse and is principally, although not exclusively, visited by birds.

Up to two *Tapirus terrestris* were simultaneously recorded in sites 2 and 7, while they were always solitary in the salt lick 1. Up to two *Cuniculus paca* simultaneously visited site 1. *Ortalis guttata* was recorded in site 1 in flocks formed by up to 9 individuals and *Momotus momota* visited the same site in pairs. *Saguinus sp.* visited site 1 in a group of 3 individuals.

Five, three, and two individuals of *Tapirus terrestris* were identifiable in natural salt licks 2, 7, and 1, while only one *Mazama americana* individual was identifiable in site 2. Considering animals' mobility and the distance among sites, at least nine individuals of *Tapirus terrestris* and one individual of *Mazama americana* had been identified in oil polluted sites by long lasting marks were also observed in natural salt licks.

Visits' duration values in natural salt licks had a mean value of 11.79 min ±15.09 min (Table 2.5). *Tapirus terrestris* appeared as the species that remained longer in sites 2 and 7, with V.D. of 17.00 min ±17.19 min and 12.83 min ±15.05 min respectively. In natural salt lick 1, the birds *Momotus momota* (9.83 min ±12.48 min) and *Ortalis gutatta* (6.13 min ±9.01 min) had the longer visits.

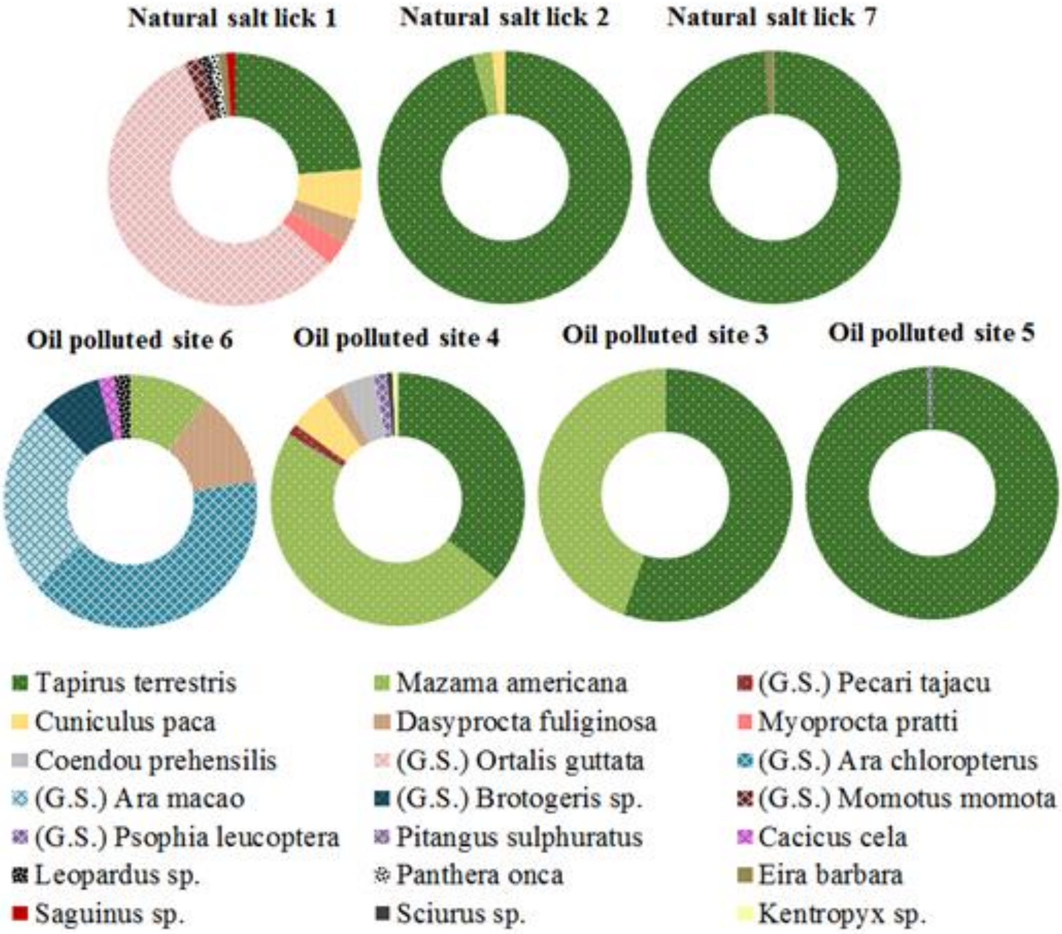


Figure 2.5 | Species visiting the studied salt licks.
Note: Percentage of visits belonging to each species in each salt lick. Dotted texture belongs to ungulates, lineal patterns to rodents, the grid motive represents bird species and black-white spotted textures belong to felines. G.S. states for group species, and refers to species that usually form herds or flocks.

Table 2.4 | Descriptive variables for the salt licks studied.

Study sites:	Natural salt lick 1	Natural salt lick 2	Natural salt lick 7	Oil polluted site 3	Oil polluted site 4	Oil polluted site 5	Oil polluted site 6	Natural Salt lick	Oil-polluted salt lick	TOTAL	NATURAL SALT LICKS Median (IQR)	OIL POLLUTED SALT LICKS Median (IQR)	Wilcoxon test p-value
Survey days	47.27	21.06	38.11	92.80	95.22	31.13	125.95	106.45	345.10	451.55			
Number of species	10	3	2	2	9	2	7	11	15	21	3.00 (4.00)	4.50 (5.50)	0.854
Visitation frequency (V.F.)	196.74	574.48	236.13	459.04	140.73	510.76	38.90	285.59	222.54	237.41	236.13 (188.87)	299.88 (356.70)	0.629
% Ingestion evindence	50.54	83.47	73.33	60.80	53.73	74.84	65.31	70.39	62.76	64.93	73.33 (16.47)	63.05 (8.66)	0.858
Nº identified *Tapirus terrestris*	2	5	3	4	2	5	0	9	9	18	3.00 (1.50)	3.00 (2.75)	0.857
Nº identified *Mazama americana*	0	1	0	5	2	0	1	1	8	9	0.00 (0.50)	1.50 (2.00)	0.267
Average visits duration (V.D.)	5.15 ±8,88	16.35 ±17,13	12.70 ±15,01	8.35 ±12,66	5.36 ±9,71	16.37 ±19,39	8.09 ±14,52	11,79 ± 15,09	9,47 ± 14,47	10,13 ± 14,68	12.70 (5.60)	8.22 (2.95)	1.000

Recorded species

Scientific Name	V.F.	I./V.	V.F.	I./V.	V.F.	I./V.	V.F.	I./V.	V.F.	I./V.	V.F.	I./V.	V.F.	I./V.	V.F.	V.F.	V.F.	Family	Common Name
Tapirus terrestris	46.54	(1)	550.74	(1-2)	233.51	(1-2)	254.30	(1-3)	50.41	(1-2)	507.55	(1-3)			213.25	128.08	148.16	Tapiridae	South American tapir
Mazama americana			14.24	(1)			204.74	(1-3)	67.21	(1-2)			3.97	(1)	2.82	75.05	58.02	Cervidae	South Am. Red brocket deer
(G.S.) *Pecari tajacu*									2.10	(1-5)						0.58	0.44	Tayassuidae	Collared peccary
Cuniculus paca	12.69	(1-2)	9.50	(1)					7.35	(1)					7.52	2.03	3.32	Cuniculidae	Lowland paca
Dasyprocta fuliginosa	6.35	(1)							3.15	(1)			4.76	(1)	2.82	2.61	2.66	Dasyprctidae	Black agouti
Myoprocta pratti	6.35	(1)													2.82		0.66	Dasyprctidae	Green acouchi
Coendou prehensilis									6.30	(1-2)						1.74	1.33	Erethizontidae	Brazilian porcupine
(G.S.) *Ortalis guttata*	112.12	(1-9)													49.79		11.74	Cracidae	Speckled chachalaca
(G.S.) *Ara chloropterus*													15.88	(1-27)		5.80	4.43	Psittacidae	Red-and-green macaw
(G.S.) *Ara macao*													9.53	(1-6)		3.48	2.66	Psittacidae	Scarlet macaw
(G.S.) *Brotogeris sp.*													3.18	(2-75)		1.16	0.89	Psittacidae	Parakeet
(G.S.) *Momotus momota*	4.23	(2)													1.88		0.44	Momotidae	Amazonian motmot
(G.S.) *Psophia leucoptera*									2.10	(2-4)						0.58	0.44	Psophiidae	White-winged trumpeter
Pitangus sulphuratus											3.21	(1)				0.29	0.22	Tyrannidae	Great kiskadee
(G.S.) Cacicus cela													0.79	(3)		0.29	0.22	Icteridae	Yellow-rumped cacique
Leopardus sp.	2.12	(1)											0.79	(1)	0.94	0.29	0.44	Felidae	Cat / Ocelot
Panthera onca	2.12	(1)													0.94		0.22	Felidae	Jaguar
Eira barbara	2.12	(1)			2.62	(1)									1.88		0.44	Mustelidae	Tayra
(G.S.) Saguinus sp.	2.12	(3)													0.94		0.22	Callitrichidae	Tamarin
Sciurus sp.									1.05	(1)						0.29	0.22	Sciuridae	Squirrel
Kentropyx sp.									1.05	(1)						0.29	0.22	Teiidae	Kentropyx

Note: Visit frequency is expressed by nº of visits / 100 hours of survey. The percentage of ingestion evidence is obtained considering the rate of visits in which ingesting proofs were recorded. G.S. indicates group species and refers to species that usually form herds or flocks. The minimum and maximum number of individual per video (I./V.) is shown per species and sites. Species name is underlined when the species was recorded ingesting. The shading indicates simultaneous appearance of two or more species in a site. The number of identified *Tapirus terrestris* and *Mazama americana* reflect the animals showing permanent or long-lasting marks or exceptional body size.

Table 2.5 | Visitation duration (V.D.) and standard deviation (S.D.), in minutes and per species and salt licks.

Study sites	**Natural salt lick 1**	**Natural salt lick 2**	**Natural salt lick 7**	**Oil polluted site 3**	**Oil polluted site 4**	**Oil polluted site 5**	**Oil polluted site 6**	**NATURAL SALT LICKS**	**OIL POLLUTED**	**TOTAL**
Species	**V.D. ± S.D.**	**V.D. ± S.D.**	**V.D. ± S.D.**	**V.D. ± S.D.**	**V.D. ± S.D.**	**V.D. ± S.D.**	**V.D. ± S.D.**	**V.D. ± S.D.**	**V.D. ± S.D.**	**V.D. ± S.D.**
Tapirus terrestris	5,40 ± 11,22	17,00 ± 17,19	12,83 ± 15,05	8,15 ± 13,38	8,74 ± 13,94	16,47 ± 19,41		14,26 ± 16,21	11,19 ± 16,30	12,23 ± 16,32
Mazama americana		1,00 ± 0,00		8,60 ± 11,76	3,70 ± 6,04		1,00 ± 0,00	1,00 ± 0,00	7,27 ± 10,77	7,20 ± 10,72
(G.S.) *Pecari tajacu*					10,27 ± 0,38				10,27 ± 0,38	10,27 ± 0,38
Cuniculus paca	3,06 ± 5,04	1,00 ± 0,00			2,11 ± 2,95			2,54 ± 4,37	2,11 ± 2,95	2,34 ± 3,65
Dasyprocta fuliginosa	1,00 ± 0,00				1,00 ± 0,00		1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00
Myoprocta pratti	1,00 ± 0,00							1,00 ± 0,00		1,00 ± 0,00
Coendou prehensilis					3,64 ± 4,19				3,64 ± 4,19	3,64 ± 4,19
(G.S.) *Ortalis guttata*	6,13 ± 9,01							6,13 ± 9,01		6,13 ± 9,01
(G.S.) *Ara chloropterus*							14,20 ± 17,23		14,20 ± 17,23	14,20 ± 17,23
(G.S.) *Ara macao*							2,02 ± 2,55		2,02 ± 2,55	2,02 ± 2,55
(G.S.) *Brotogeris sp.*							20,25 ± 26,42		20,25 ± 26,42	20,25 ± 26,42
(G.S.) *Momotus momota*	9,83 ± 12,48							9,83 ± 12,48		9,83 ± 12,48
(G.S.) *Psophia leucoptera*					1,00 ± 0,00				1,00 ± 0,00	1,00 ± 0,00
Pitangus sulphuratus						1,00 ± 0,00				1,00 ± 0,00
(G.S.) Cacicus cela							1,00 ± 0,00		1,00 ± 0,00	1,00 ± 0,00
Leopardus sp.	1,00 ± 0,00						1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00
Panthera onca	1,00 ± 0,00							1,00 ± 0,00		1,00 ± 0,00
Eira barbara	1,00 ± 0,00		1,00 ± 0,00					1,00 ± 0,00		1,00 ± 0,00
(G.S.) Saguinus sp.	1,00 ± 0,00							1,00 ± 0,00		1,00 ± 0,00
Sciurus sp.					1,00 ± 0,00				1,00 ± 0,00	1,00 ± 0,00
Kentropyx sp.					1,00 ± 0,00				1,00 ± 0,00	1,00 ± 0,00
Total	5,15 ± 8,88	16,35 ± 17,13	12,70 ± 15,01	8,35 ± 12,66	5,36 ± 9,71	16,37 ± 19,39	8,09 ± 14,52	11,79 ± 15,09	9,47 ± 14,47	10,13 ± 14,68

Species composition and mineral concentrations

Study sites mostly visited by birds -salt licks 1 and 6- showed significantly higher median concentrations of Na, Fe and P than sites primarily visited by ungulates –sites 2,3,4,5 and 7 (Table 2.6). Although the remaining minerals did not present significantly different median concentrations between site types, median concentrations of Ca, Mg, K, Mn were indeed higher in sites commonly visited by birds than in sites mostly visited by ungulates. When assessing the relation between the visit frequency of bird species and the concentrations of minerals, positive and significant correlations have been detected for Ca, Mg, Na, P and Fe (Table 2.6). Contrastingly, only P concentrations significantly –and negatively- correlated with ungulate species' visit frequency (Table 2.6).

Table 2.6 | Statistical relation between mineral content, species' composition, and visit frequency of the studied salt licks.

Sites		Ca (mg/g)~^	Mg (mg/g)~^	K (mg/g)~^	Na (mg/g)~^	P (mg/g)^	Mn (µg/g)~^	Fe (mg/g)~^
BIRDS - Natural salt lick 1		30.32	18.80	4.06	18.63	0.85	792.06	46.06
BIRDS - Oil-polluted site 6		2.19	0.75	5.65	2.59	0.85	414.60	38.22
UNGULATES - Natural salt lick 2		< 1	0.53	1.36	< 1	< 0,1	295.81	23.41
UNGULATES - Natural salt lick 7		2.65	0.39	2.00	< 1	0.23	872.42	19.01
UNGULATES - Oil-polluted site 3		< 1	1.12	4.25	1.17	0.28	511.58	33.78
UNGULATES - Oil-polluted site 4		< 1	0.93	4.63	1.18	0.12	387.69	30.99
UNGULATES - Oil-polluted site 5		< 1	0.16	2.79	< 1	0.10	111.67	17.32
Birds licks		16.26 (14.06)	9.77 (9.03)	4.85 (0.80)	10.61 (8.02)	0.85 (0.00)	603.33 (188.73)	42.14 (3.92)
Ungulates licks		0.5 (1.05)	0.53 (0.54)	2.79 (2.25)	0.50 (0.67)	0.12 (0.13)	387.69 (215.77)	23.41 (11.98)
Wilcoxon test - p-value		0.160	0.381	0.381	0.071*	0.095*	0.571	0.095*
Birds' VF (correlation test)	r	0.974	0.969	0.310	0.988	0.784	0.466	0.770
	p-value	<0.001*	<0.001*	0.498	<0.001*	0.037*	0.292	0.043*
Ungulates' VF (correlation test)	r	-0.464	-0.449	-0.690	-0.508	-0,770	-0.504	-0.665
	p-value	0.295	0.313	0.133	0.245	0.043*	0.249	0.103

Note: The symbol (~) indicates the association of the metal to oil activities and (^) indicates a metal allegedly sought by wildlife in salt licks. Values <1 and <0.1 are considered equivalent to 0.5 and 0.05 when performing statistical tests. Median (IQR) values of minerals concentrations are given by both types of licks. Symbol (*) indicates a significant p-value < 0.10.

HUNTING GROUNDS AFFECTED BY OIL POLLUTION

Considering animals' home ranges and mobility, the area indirectly exposed to oil pollution varies among species. For the species recorded ingesting oil-polluted soil and water, home ranges vary from 0.02 to 11.7 Km^2 (Table 2.7 and Fig. 2.6). However, it has been documented that *Tapirus terrestris* may walk over 10 Km to visit a salt lick -314.16 Km^2 (Tobler, 2008), exceeding its own home range, which typically would range from 0.5 Km^2 to 5.78 Km^2 (Noss *et al.*, 2003). Therefore, for *Tapirus terrestris* the area exposed to oil pollution from oil infrastructure in block 1AB/192 may be very wide (up to 5,820 Km^2). *Pecari tajacu* is the recorded species with the highest home range (from 6.85 Km^2 to 11.7 Km^2 Taber, Doncaster, Neris, & Colman, 1994; Vieira-Fragoso, 1999). Thus, the area exposed to oil pollution for this species may be also very broad (up to 2,231 Km^2). Contrastingly, *Cuniculus paca, Coendou prehensilis* and *Mazama americana* have home ranges smaller than 1 Km^2 and the area exposed to oil pollution through their mobility may be 117 Km^2, 400 Km^2 and 467 Km^2, respectively. To our knowledge, there is no data available about home ranges or mobility values of *Dasyprocta fuliginosa, Myoprocta pratti, Ara chloropterus, Ara macao, Brotogeris sp.* and *Psophia leucoptera*, for which the same calculations cannot be made. In sum, the data available suggest that the area exposed to oil pollution would overlap a very different percentage of the oil concession 1AB/192 depending on the species studied: from 1.87% to 83.47% of the concession area for the species for which data are available.

Table 2.7 | Mobility to salt licks (MSL) and home ranges (HR) for the species visiting oil-polluted salt licks and the area exposed to oil pollution considering animals' mobility. MSL were used in the calculations.

Species	Mobility* (Km^2-HR) (Km-MSL)	Source	Area exposed to oil-pollution (Km^2)	% of the Block 1ab/192 affected
Pecari tajacu	6,85 - 11,7 (HR)	Taber et al. 1994 Fragoso et al. 1999	1691 - 2231	31.17 - 40.17
Tapirus terrestris	0,5 - 5,78 (HR) 10 (MSL)	Noss et al. 2003 Tobler et al. 2008	459 - 5820	8.82 - 83.47
Mazama americana	0,52 (HR)	Maffei and Taber2003	467	8.98
Coendou prehensilis	0,08 - 0,38 (HR)	Montgomery and Lubin 1978	189 - 400	3.67 - 7.71
Cuniculus paca	0,02 - 0,03 (HR)	Marcus 1984 and Smythe et al. 1985 consulted in Beckking et al. 1999	96-117	1.87 - 2.26

* The lower and the higher values found in the literature has been used in this analysis

Note: For *Tapirus terrestris*, superindex [a] and [b] indicate that the lowest HR value and the highest MSL were used in the calculations.

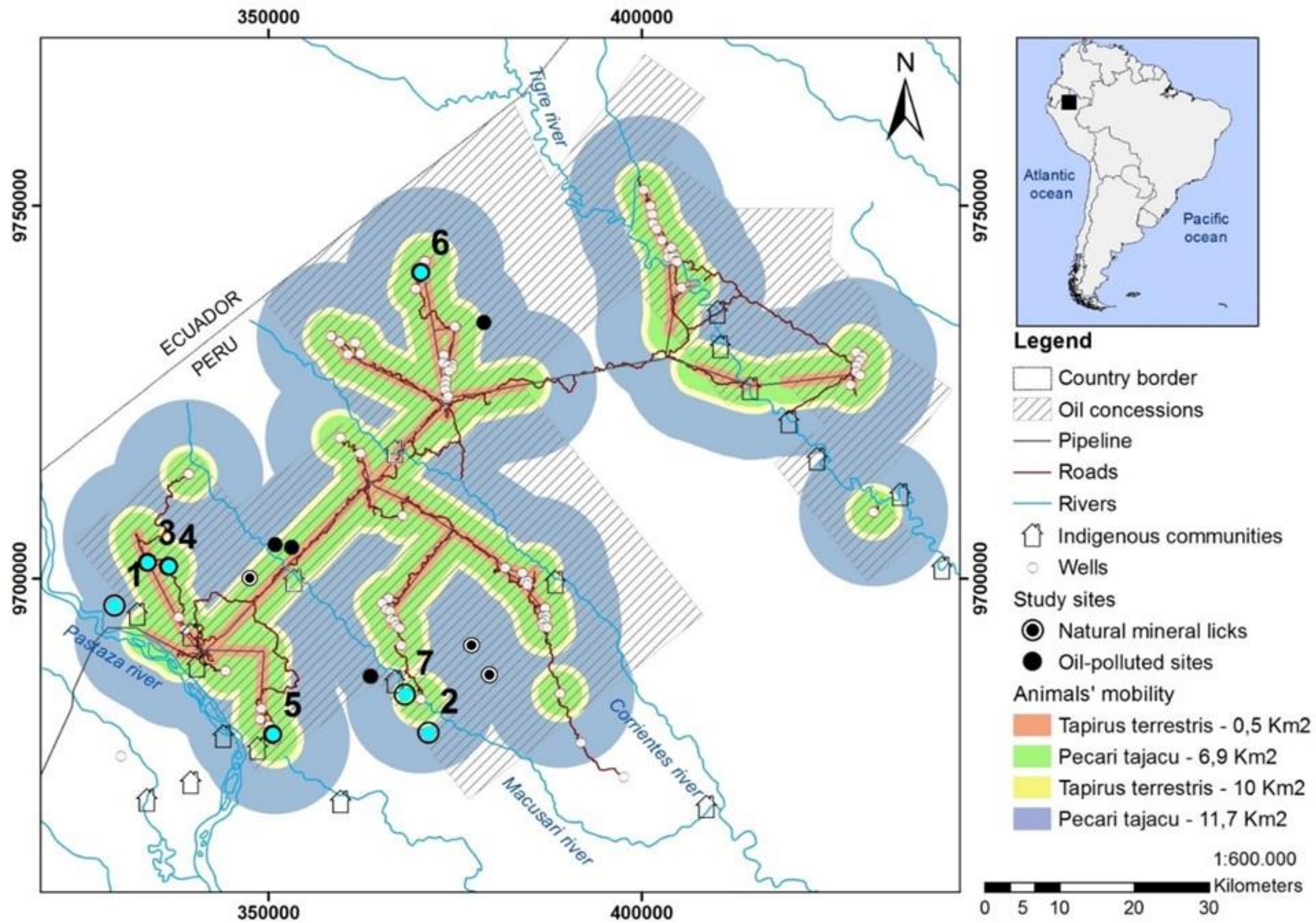


Figure 2.6 | Study area and area exposed to oil pollution considering species' mobility.

Some species presented a clear and homogeneous activity pattern across sites (Table 2.8). For instance, the rodents *Cuniculus paca* (n=15) and *Coendou prehensilis* (n=6) were nocturnal, while *Dasyprocta fuliginosa* (n=12) and *Myoprocta pratti* (n=3) visited the sites during the day. The birds *Ortalis guttata* (n=53), *Ara chloropoterus* (n=20), *Ara macao* (n=12) and *Brotogeris* sp.(n=4) visited the sites during the day and *Psophia leucoptera* (n=2) in the twilight. However, the most frequent visitors, *Tapirus terrestris* (n=669) and *Mazama americana* (n=262), showed different activity patterns depending on the studied site (Table 2.9). This is why they are classified as a cathemeral species in Table 2.8.

Table 2.8 | Activity patterns (AP) of the recorded species are based on their diurnal (%D), crepuscular (%CR) and nocturnal (%N) visitation percentage.

Study sites	N visits	TOTAL			
Species		%D	%CR	%N	AP
Tapirus terrestris	669	17	3	79	CA
Mazama americana	262	40	8	52	CA
(G.S.) *Pecari tajacu*	2	100	0	0	D
Cuniculus paca	15	0	0	100	N
Dasyprocta fuliginosa	12	92	0	8	D
Myoprocta pratti	3	100	0	0	D
Coendou prehensilis	6	0	0	100	N
(G.S.) *Ortalis guttata*	53	96	4	0	D
(G.S.) *Ara chloropterus*	20	100	0	0	D
(G.S.) *Ara macao*	12	100	0	0	D
(G.S.) *Brotogeris sp.*	4	100	0	0	D
(G.S.) *Momotus momota*	2	100	0	0	D
(G.S.) *Psophia leucoptera*	2	0	100	0	CA
Pitangus sulphuratus	1	100	0	0	D
(G.S.) Cacicus cela	1	100	0	0	D
Leopardus sp.	2	0	0	100	N
Panthera onca	1	100	0	0	D
Eira barbara	2	100	0	0	D
(G.S.) Saguinus sp.	1	100	0	0	D
Sciurus sp.	1	100	0	0	D
Kentropyx sp.	1	100	0	0	D
Total	1072	31	4	64	

Note: The set of initials N, D, and CA stands for “nocturnal”, “diurnal” and “cathemeral”.

Tapirus terrestris' nocturnal visits predominated in all the sites (79%), but this dominance differs among sites (Table 2.9). The highest percentages of *Tapirus terrestris*' diurnal visits were reported in sites 2, 5 and 1 which are classified as exposed to noise from ongoing oil activities and expected to show a lower hunting pressure (Table 2.9). Moreover, in sites 2, 5 and 1 the number of *Tapirus terrestris* visits was similar during the whole day, while in sites 3, 7 and 4, visits were almost exclusively reported during the night and twilight (Fig. 2.7). Sites 3, 7 and 4 have in common that both are highly exposed to hunting pressure, while sites 5, 2 and 1 are much less exposed to hunting and noise. However, when the percentage of *Tapirus terrestris*' diurnal visits was compared between sites differently exposed to noise and hunting pressure, no significantly difference was found (Fig. 2.8). Similarly, no significant differences between sites expected to be differently affected by noise and hunting pressure were found neither for *Tapirus terrestris*' visit duration or frequency nor for percentage of ingestion evidence (Fig. 2.8). However, all studied variables (visit duration and frequency, percentage of diurnal visits and percentage of ingestion evidence) showed higher median values in sites expected to be less exposed to noise than in sites with higher noise exposure. The same is true when comparing sites expected to be less exposed to hunting pressure with more exposed sites.

Similarly, *Mazama americana* showed a very heterogeneous behaviour among sites (Table 2.9). It only showed a diurnal pattern in the site 2 (n=3), which had a low expected exposure to hunting pressure and noise. In sites located close to oil infrastructure and expected to be exposed to a high hunting pressure *Mazama amerciana* showed a cathemeral pattern (sites 3 (n=190) and 4 (n=64)). The same species showed a nocturnal pattern in site 6 (n=5) (Table 2.9), which is not expected to be exposed to high hunting pressure but it was highly exposed to noise from oil extraction activities.

Table 2.9 | Activity patterns of *Tapirus terrestris* and *Mazama americana* are based on their diurnal (%D), crepuscular (%CR) and nocturnal (%N) visitation percentage in each site.

	Activity pattern									
	Tapirus terrestris					*Mazama americana*				
Study sites	**N visits**	**%D**	**%C**	**%N**	**AP**	**N visits**	**%D**	**%C**	**%N**	**AP**
Natural salt lick 1	22	14	5	82	N					
Natural salt lick 2	116	42	3	54	C	3	100	0	0	D
Natural salt lick 7	89	0		100	N					
Oil polluted site 3	236	9	4	87	N	190	41	8	52	C
Oil polluted site 4	48	2	0	98	N	64	37	8	56	C
Oil polluted site 5	158	27	6	68	C					
Oil polluted site 6						5	20		80	N
NATURAL SALT LICKS	227	23	2	75	C	3	100	0	0	D
OIL POLLUTED SITES	442	14	4	81	N	259	39	8	53	C
GENERAL	669	17	3	79	C	262	40	8	52	C

Note: The set of initials N, D, and CA stands for “nocturnal”, “diurnal” and “cathemeral”.

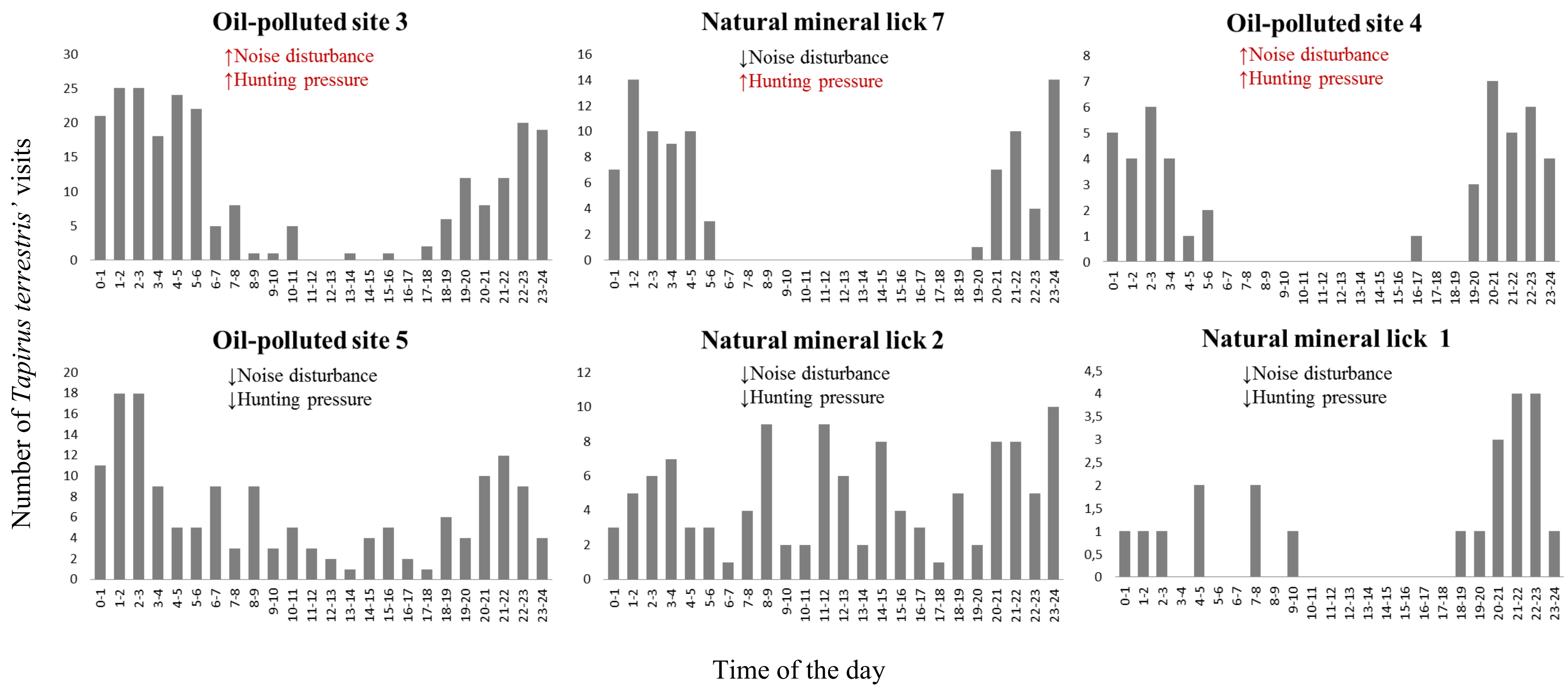


Figure 2.7 | Activity patterns for *Tapirus terrestris* in natural and oil-polluted salt licks with different expected exposure to hunting and noise from ongoing oil activities.

	Noise disturbance			
	Sites	*No*	**Sites**	*Yes*
VD	**1**	5.4	**3**	8.15
	2	17	**4**	8.75
	7	12.83		
	5	16.47		
	M (IQR)	14.65 (5.63)		8.45 (0.30)
	P-value	0.533		
%D	**1**	14	**3**	9
	2	42	**4**	2
	7	0		
	5	27		
	M (IQR)	20.50 (20.25)		5.50 (3.50)
	P-value	0.533		
%I	**1**	50	**3**	60.8
	2	83.47	**4**	0
	7	73.33		
	5	74.84		
	M (IQR)	74.09 (9.50)		57.27 (2.54)
	P-value	0.533		
VF	**2**	550.74	**3**	254.3
	7	233.51		
	5	507.55		
	M (IQR)	507.55 (158.62)		254.30 (0.00)
	P-value	1.000		

	Hunting pressure			
	Sites	*Low*	**Sites**	*High*
VD	**1**	5.4	**7**	12.83
	2	17	**3**	8.15
	5	16.47	**4**	8.75
	M (IQR)	16.47 (5.80)		8.75 (2.34)
	P-value	0.700		
%D	**1**	14	**7**	0
	2	42	**3**	9
	5	27	**4**	2
	M (IQR)	27.00 (14.00)		2.00 (4.50)
	P-value	0.100		
%I	**1**	50	**7**	73.33
	2	83.47	**3**	60.8
	5	74.84	**4**	0
	M (IQR)	74.84 (16.74)		60.80 (9.80)
	P-value	0.700		
VF	**2**	550.74	**7**	233.51
	5	507.55	**3**	254.3
	M (IQR)	529.15 (21.60)		243.91 (10.40)
	P-value	0.333		

Figure 2.8 | *Tapirus terrestris'* visit duration (VD, in minutes), percentage of diurnal visits (%D), percentage of the visits showing ingestion proofs (%I), and visit frequency (VF), by site exposure to noise disturbance and hunting pressure.
Note: Medians (M) and interquartilic ranges (IQR) are given. P-value from a Wilcoxon test.

The composition of species visiting the licks does not seem to depend on the proximity of human communities nor of ongoing oil activities, since both types of sites (those mainly visited mainly by birds and those mainly visited by ungulates) were found in very diverse scenarios in terms of human and noise exposure. For instance, one site mostly visited by birds was located close to an active and noisy oil battery, while the other was located very far from Indigenous communities and oil activities. Sites mostly visited by ungulates were also found in very diverse scenarios in terms of hunting pressure and noise. However, no statistical test has been conducted to analyse the association between the composition of species and the proximity of the salt licks to human communities and ongoing oil activities.

DISCUSSION

CAVEATS AND LIMITATIONS

Before discussing the main results of this chapter, a number of caveats and limitations that might apply to the findings presented here are exposed. Caveats and limitations are organised in relation to the main finding they might affect.

<u>Oil-polluted soil and water ingestion by wildlife: description and causes</u>

A first caveat of this study that might affect the description of wildlife oil-polluted soil and water ingestion is that survey time was fragmented and differed from one site to another. Thus, depending on the salt lick, videos were collected in different months (from February to September). Although most videos were collected in the humid season[9], a little proportion of the videos were collected at the end of the dry season. Consequently, some of the variation found across sites could be, indeed, due to seasonal variation in data collection rather than to other factors. In fact, season variability on animals' and hunters' visits to salt licks has been reported in the literature (Parish, 2001; Blake *et al.*, 2011). Unfortunately, the sample size used in this chapter is too small to detect the potential effect of seasonal variation.

Second, the number of individuals of *Mazama americana* and *Tapirus terrestris* identified with certainty is considerably lower than the number of individuals of these species recorded, as only individuals with permanent or long-lasting marks were identified. Consequently, the real number of individuals showing ingestion of oil-polluted soil and water cannot be estimated. However, assuming a homogeneous distribution of permanent and long-lasting marks among the populations of these species and a uniform population density in all the study area allows the comparison between artificial and natural salt licks to assess whether one type of salt licks has a higher pull effect then the other.

The third potential bias relates to intercoder reliability. In an analysis not reported in the results, 5% of the visits of each salt lick were randomly selected and re-analysed to test for intercoder reliability. In this control analysis, most of the variables obtained the same

[9] The humid season ranges from March to the September and the dry season from October to February, approximately (Carranza, 2011).

values across coders. However, the variable that recorded whether the animal ingested soil/water in a visit or not showed differences across coders, with 2.35% of the visits being differently classified by different coders. Similarly, in 3.5% of the visits sex determination values did not coincide between coders. So, a bias related to the intercoder reliability might be affecting these two variables.

Fourth, the variable visit frequency might not be very accurate. Several criteria that have been used by other camera trapping specialists and that are accepted in the academic literature as reasonably accurate (Srbek-araujo and Chiarello, 2005; Rovero and Marshall, 2009; Tobler *et al.*, 2009; Blake *et al.*, 2011; Carvalho *et al.*, 2011) were used to construct this variable (i.e., videos must be consecutive and from the same species, the individual must look similar, time span between videos must be lower than 30minuts and with a 60minuts cut-off for visits' length). However, it could still happen that diverse individuals might have been included in the same visit, or that the 60minuts cut-off criterion might have split visits of a single individual.

Fifth, since samples from adjacent soils to salt licks were not collected, it is not possible to compare the salt licks' mineral composition with that of other nearby soils. Thus, the null hypothesis that animals ingest soil and water in oil-polluted salt licks for mineral supplementation cannot be tested.

Finally and more important, the analyses presented here are based on videos and soil samples collected in seven study sites: three natural salt licks and four oil-polluted salt licks. For many statistical tests, this sample is just too small to obtain reliable p-values. Therefore, the statistical significance of results must be considered with caution.

<u>Hunting grounds affected by oil pollution</u>

The results on hunting grounds affected by oil pollution might also be biased for a couple of reasons. First, the analysis of the hunting grounds affected considering species' mobility was restricted to the oil concession limits. Abandoned wells located outside the current oil concession limits and the 802 Km of the North Peruvian pipeline (from the oil concession to the Bayóvar harbour terminal, in the Peruvian Pacific coast) were not included in the analysis. However, these abandoned oil wells may also be leaking (Davies *et al.*, 2014; King and Valencia, 2014; O'Callaghan-Gordo *et al.*, 2018) and be visited by the same species or even individuals observed in this study. Thus, the estimation of hunting grounds affected by oil pollution is conservative.

Second, very little is known on Amazonian species' mobility and the extension of their home range. Data on the distances that these species may walk to visit salt licks is even rarer in the scientific literature. Lack of information on species mobility limits the capacity of this study to assess the geographical impact of the ingestion of oil-polluted sites by all the species. In other words, the findings presented here only reflect the hunting ground for some of the species ingesting oil-polluted soils and waters.

Third, in the spatial analysis of the hunting grounds affected considering species' mobility it was assumed that oil-pollluted salt licks were homogeneously distributed along the infrastructure (i.e., wells and pipelines).

The first two caveats affect the estimation of the extension hunting grounds affected by oil-polution in a conservative way. However, the third caveat might overestimate the exposure of wildlife to oil-polluted sites. Thus, the results presented in this chapter of the actual hunting grounds affected by oil pollution should be considered wth caution.

Activity patterns and human influence on wildlife behaviour

Two additional caveats affect results on the association between wildlife behaviour and human influence. First, distance from the salt licks to ongoing oil activities (including roads, oil wells and central production facilities) are used as a proxy for noise disturbance. However, this proxy might not be completely accurate and a proper noise study (e.g., including decibels measurement) should be conducted. Similarly, travel distance from the Indigenous communities to the salt licks is used as a proxy for hunting pressure. Although previous studies have reported the strong relation between these two variables (Vickers, 1991; Parish, 2001), hunting pressure depends on many other factors than are not considered in this proxy. Future work should find more accurate measures of hunting pressure.

Finally, the calculation of activity patterns and human influence on wildlife behaviour might also be biased by the small number of sites from which data is available (n=7), for which statistical results must be considered with caution. In the same line, since the effects on behaviour of the variables that proxy for noise of oil operations and hunting exposure may be similar, a bigger sample size would be required to identify their effects separately.

Keeping these caveats in mind, the main findings from the work presented in this chapter are discussed below.

OIL-POLLUTED SOIL AND WATER INGESTION BY WILDLIFE: DESCRIPTION AND CAUSES

The results of this research provide robust data to confirm geophagy by Amazonian fauna in oil-polluted sites. Taken together, the data presented show that geophagy of oil-polluted soil is not an unusual phenomenon, but rather a widespread behaviour in oil extraction areas of the Peruvian Amazon.

Eight (8) species have been recorded ingesting oil-polluted soil and water: *Tapirus terrestris*, *Mazama Americana*, *Cuniculus paca*, *Pecari tajacu*, *Coendou prehensilis* and three parrot species (*Ara chloropterus*, *Ara macao* and *Brotogeris sp.*). The presence of *Dasyprocta fuliginosa, Psophia leucoptera* and *Saguinus sp.* in the oil-polluted sites has also been recorded. Although these species have not been recorded ingesting soil or water, it is well known that these species, or very close ones (*Psophia crepitans, Saguinus mystax*), are also geophagous. Thus, chances are that they might also display this behaviour in oil-polluted salt licks (Heymann and Hartmann, 1991; Blake *et al.*, 2011; Molina *et al.*, 2014).

It is also worth noticing that, although up to fifteen species have been recorded in oil-polluted salt licks, 91.27% of visits belong to the ungulates *Tapirus terrestris* and *Mazama americana*, which are the main visitors in three thirds of the oil-polluted salt licks studied. These two species would be those more exposed to oil pollution through geophagy.

<u>Why geophagy in oil-polluted sites?</u>

All the species recorded ingesting soil and water in the oil-polluted sites are frugivorous and herbivorous. Indeed, the literature on geophagy in natural salt licks from the Neotropics and elsewhere shows evidence of intentional geophagy for frugivorous and herbivorous species (Blake *et al.*, 2011; Varanashi, 2014). Different non-excluding explanations have been suggested to explain geophagy in natural salt licks, including species need to overcome nutritional deficiencies, to alleviate digestive disorders, and/or to increase their buffering capacity (Emmons and Stark, 1979; Kreulen, 1985; Klaus and Schmidg, 1998; Blake *et al.*, 2011). Here it is argued that geophagy in oil-polluted sites might serve the same purpose than it serves in natural sites. Thus, the same explanations used to explain geophagy in natural salt licks might also apply to explain geophagy in oil-polluted salt licks.

Data collected in this work show that most minerals allegedly sought by wildlife have similar concentrations in the studied natural and oil-polluted salt licks, suggesting that the redirection of intentional geophagy to oil-polluted sites may be driven by the concentration of these minerals. Indeed, probably because the very high salinity of the produced water dumped, and mixed with crude oil in the oil-polluted sites (up to 300,000 mg of salt/L, (Fakhru'l-Razi *et al.*, 2009), Na, Mg, K, Fe, P and Mn show similar concentrations in natural and oil-polluted salt licks. Differently, Ca presented a significantly higher median concentration in natural than in oil-polluted salt licks. While further test require the comparison of mineral concentration with soils sampled in areas not considered salt licks, data presented here showing the similar levels of minerals allegedly sought by wildlife found in both natural and oil-polluted salt licks suggest a similar pull-effect for wildlife of the oil-polluted sites when compared to the natural salt licks. Moreover, although not significantly, median concentrations of Mg, K, Na, Fe, Cu and Zn were higher in the apparently oil-polluted salt licks than in natural ones.

Concordantly, analysis of videos from the camera trap also attributed a similar pull-effect to both natural and oil-polluted salt licks. Visit frequency, duration, and ingestion evidence ratios in oil-polluted sites were similar to those in natural salt licks. The number of identified individuals of *Tapirus terrestris* and *Mazama americana* was also very similar between natural salt licks and oil-polluted sites and no significant differences were found for these variables between natural and oil-polluted salt licks. However, the number of simultaneous appearances of different species was higher in oil-polluted sites. Also, the groups of *Mazama americana* and *Tapirus terrestris* simultaneously visiting the sites were bigger in oil-polluted sites than in natural salt licks.

<u>Consequences and relevance of oil-polluted soil ingestion</u>

The presence of hydrocarbon petroleum biomarkers (hopanes and steranes) in the apparently oil-polluted sites confirms the actual occurrence of oil pollution. Moreover, the presence of these biomarkers also suggests the pollution of the apparently oil-polluted sites by oil-related heavy metals or other hydrocarbon associated to oil activities at some point in time[10]. It is important to say that among the oil-related heavy metals or other

[10] Some of them might have been washed away by the heavy rains of the rainforests.

hydrocarbon associated to oil activities there could be toxic and carcinogenic compounds (see *Introduction: Oil pollutants*).

When comparing the metal levels found in soil samples from the study sites with Peruvian and Canadian MPLs of Ba for agricultural and residential/natural protected areas, levels of pollution were exceeded in one oil-polluted salt lick. Similarly, the four categories of Canadian MPLs for V were also exceeded in three oil-polluted salt licks. So, the four oil-polluted sites exceed one or another of the MPLs. It should be noted that soil samples from one natural salt lick also exceeded the Canadian MPLs of V, Cr (total) and Ni, although the reasons behind these high concentrations of metals in this natural site have not been addressed in this study. Soil ingestion differs between species, however some vertebrate species visiting salt licks do ingest large quantities of soil (up to ~30% of digesta) (Beyer *et al.*, 1994; Hui, 2004); chronic exposure via such high amounts of ingesta may result in important bioaccumulation of certain pollutants. Moreover, as both natural and oil-polluted salt licks are also visited by predators, such as the mustelid *Eira barbara* or the felines *Leopardus sp*. and *Panthera onca*, a phenomena already reported in the literature for natural salt licks (Montenegro, 2004; Blake *et al.*, 2011; Varanashi, 2014), the consumption of oil polluted soil might cause the entrance of oil-related pollutants into the food chain beyond herbivorous and frugivorous species. In other words, herbivorous' and frugivorous' soil and water ingestion in oil-polluted salt licks might have an impact to the whole ecosystem, including species from higher trophic levels. The effect might be stronger for Pb, which has been found in higher concentrations in oil-polluted salt licks.

Furthermore, human populations might be also exposed to these pollutants, since the use of oil-polluted salt licks for hunting purposes is frequent among Indigenous Peoples in the study area. The relative importance of biomass captured in oil-polluted salt licks in relation to the total hunted biomass is unknown but, since oil-polluted sites are normally located in more accessible sites than natural salt licks (because of the road system opened by the oil company), oil-polluted sites might be visited by hunters with a similar or even higher frequency than natural salt licks. Indeed, at least one study suggests that Indigenous communities of the Colombian Amazon and those in Yavarí-Miri River in Northeastern Peru obtain about 25% and over 30% of consumed meat from salt licks (Montenegro, 2004). Moreover, the species recorded ingesting oil-polluted soil and water play an important role in Amazonian Indigenous Peoples' diet, accounting for a high percentage of daily protein intake. Indeed, a 12-month study conducted among Shuar native communities in the Ecuadorian Amazon (Zapata-Ríos *et al.*, 2009) concluded that species[11] here recorded in the oil-polluted salt licks represent more than 66% of the biomass hunted. Other studies from the Northern Peruvian Amazon find similar percentages for the same species, ranging from 49.3-68.2% of total hunted biomass (Bodmer and Lozano, 2001). Moreover, according to Zapata-Ríos *et al.* (2009), the two most frequent visitors to oil-polluted salt licks –*Tapirus terrestris* and *Mazama americana*- account for 11.05% and 14.28% of the hunted biomass, respectively, and for 23.93-33.60% and 1.73-33.60% according to Bodmer & Lozano (2001).

Furthermore, impacts derived from human ingestion of animals practicing geophagy in oil-polluted sites might reach beyond the local communities. Wild meat trade to urban markets has been reported in the oil concession (Orta Martínez *et al.*, 2007). Probably, and similarly as in other oil concessions (Suárez *et al.*, 2009, 2013), oil roads and the transportation subsidies provided by the oil companies to Indigenous hunters have promoted a wild meat market that boosts wild meat trade from oil concessions. In the market of Iquitos (the biggest city of the Peruvian Amazon), species[12] that have been reported as consuming oil-polluted soils in this chapter represent as much as 51.80% of the wild meat sold (Moya, 2011). *Tapirus terrestris* and *Mazama americana* account for the 2.24 and 4.52% of the wild meat sold in the market of Iquitos (Moya, 2011). Therefore, the consumption of wild animals who have consumed oil-polluted soil and water might go well beyond the local communities and risk to become a public health problem beyond the local level.

Salt lick ecology and conservation in the Northern Peruvian Amazon

Although some studies have generally suggested that mineral requirements of birds may be different from those of mammals (e.g., more calcium might be needed for egg laying) (Emmons & Stark 1979), scant work has been done to study the link between the specific mineral content of the salt lick and the particular species visiting these sites.. The only studied located addressing this topic (Molina *et al.*, 2014) finds an association between the physicochemical properties of the licks (e.g., contents of Mg, K, Na, P, Zn, Mn and

[11] The calculations include the species *Pecari tajacu, Cuniculus paca, Dasyprocta fuliginosa, Mazama americana* and *Tapirus terresris*.
[12] The calculations include the species *Pecari tajacu, Cuniculus paca, Dasyprocta fuliginosa, Mazama americana* and *Tapirus terresris* and *Psophia Leucoptera*.

sand) and the visit frequencies of three rodents and one ungulate species, thus suggesting that the topic is worth further examination.

In this line, results from this work also point at differences on sites' most common visitor species related to site's minerals concentrations. Thus, the sites mostly frequented by birds had significantly higher concentrations of Na, P and Fe than the sites mostly used by ungulates. Sites commonly visited by birds also presented higher median concentrations in the remaining minerals (Ca, Mg, K and Mn), although these differences were not statistically significant. These results suggest that mineral composition may influence the species visiting the sites, although further research on the topic should take into consideration other aspects such as site accessibility (e.g., the size of the site, the likelihood of floods, soil properties, etc.), hunting pressure, or noise. Indeed, the complete analysis of all the videos recorded in 22 sites in the frame of this work will contribute to fill this knowledge gap on the relationship between species composition and mineral content.

Visit frequencies found in this study were very high, exceeding by far the values reported in previous studies in natural salt licks in Ecuador and Peru (Tobler, 2008; Tobler *et al.*, 2009; Blake *et al.*, 2011). For example, visit frequencies for *Tapirus terrestris* in sites 2 and 5 were higher than 500 visits/100 camera days, while the highest value reported in previous studies was 97.7 visits/100 camera days (Blake et al., 2011). Considering that Blake *et al.* (2011) used a cut-off of 30minutes for visits' length, the difference between both studies may be even higher. In the same vein, *Mazama americana*'s visit frequency to site 3 was higher than 200visits/100 camera days, while the highest value reported in previous studies analysing visit frequency to natural salt licks was 161.9 visits/100 camera days (Blake et al., 2011).

High visit frequency of *Tapirus terrestris* and *Mazama americana* might indicate (a) the existence of a higher ungulate population density in the region or (b) a higher pull-effect of the studied salt licks than previously studied sites. Given the low human population density and the long distance to the local markets, the area might indeed display a low hunting pressure, thus resulting in a higher ungulate population density. Moreover, none of the two species, tapir and red brocket deer, was consumed until recently by the Achuar, who had some hunting taboos around these species (Ross, 1976; Descola, 1996). Considering that *Tapirus terrestris* is Vunerable by the IUCN's Red List of Threatened

Species, listed as Endangered by the United States Fish & Wildlife Service (Naveda *et al.*, 2008), listed under Appendix II by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and very vulnerable to over-hunting, habitat loss and fragmentation (Bodmer *et al.*, 1997), data here presented might indeed indicate the preservation of a vulnerable species in the study area, which in turn highlights the conservation importance of the area.

This thesis also reports for the first time a group visit of *Tapirus terrestris* and *Mazama americana* to salt licks. Up to three *Tapirus terrestris* individuals were recorded simultaneously in an oil-polluted sites, as well as up to three individuals of *Mamazama americana.* The results presented here also show for the first time the simultaneous visit of *Mazama americana* and *Tapirus terrestris* individuals to a salt lick. Considering that both species are solitary, except during the reproductive season (Brooks *et al.*, 1997), the findings might point, again, to the existence of very high population densities in the study area or a very strong pull effect of oil-polluted salt licks.

Visit durations reported in this study are comparable to those reported in the literature (Montenegro, 1998; Lizcano and Cavelier, 2000; Tobler, 2008). *Tapirus terrestris* presented the highest median visit duration (21.23±16.32 min) among individual species. The range of observed visit durations for this species went from less than 1minute to 60minutes (the established length cut-off), indicating a high heterogeneity in visits durations. These results are similar to those of Montenegro (1998), who reported that individuals of this species stayed at the lick for a few minutes or up to one hour. Contrastingly, Tobler (2008) found that *Tapirus terrestris* stayed less than 10 minutes per visit in the licks.

HUNTING GROUNDS AFFECTED BY OIL POLLUTION

Over the last decade, there has been a huge controversy in trying to estimate the area affected by oil extraction activities in the western Amazon. The Peruvian Ministry of Mines and Energy (MEM, 1998) published a report claiming that only 105.38 Km^2 (2.06%) of the oil concession had been impacted by oil activities. This calculation included only the total deforested areas and areas covered by oil spills. In 2010, the Peruvian Ministry of Environment declared that only 20.37 Km^2 (0.40%) of the 1AB/192 oil concession) was impacted by oil activities. In the calculations, MINAM only took into account the area where the installations were placed (MINAM, 2010). Similar debates have taken place in Ecuador, where the former Prime Minister, Mr. Rafael Correa, stated that the ITT oil block was going to affect only 0.2% of the Yasuní National Park (El Universo, 2014).

Considering the mobility of the species recorded in oil-polluted salt licks, it can be argued that the area exposed to oil pollution is much large. Thus, for some species, especially those with high mobility, a high percentage of the territory overlapped by the oil concession might be threatened by the existence of potential oil-polluted sites. Consequently, the portion of hunting grounds of the Indigenous populations of the study area -whose ancestral lands now overlap with oil concession- that are potentially threatened by the existence of meat from animals practicing geophagy in oil-polluted sites might also be very high. Indeed, considering the mobility of the most frequent visitor to the studied sites –*Tapirus terrestris*-, up to 5,820 Km^2 and up to 83.47% of the area occupied by the oil block might be exposed to oil pollution. Thus, this research provides novel data that call into question established consensus on how the area indirectly affected by oil extraction activities is calculated.

HUMAN INFLUENCE: HUNTING PRESSURE AND NOISE

The data presented show different activity patterns depending on species and taxon: Birds and *Pecari tajacu* were recorded during the day, *Coendou prehensilis* at night, and *Mazama americana* presented a cathemeral pattern. These patterns dovetail with the behaviour of these species reported in previous studies (Montenegro, 1998; Tobler, 2008; Blake *et al.*, 2011; Hon and Shibata, 2013; Molina *et al.*, 2014).

The ecologically similar species *Cuniculus paca* and *Dasyprocta fuliginosa* showed a mostly non-overlapping activity pattern, in which the first species is nocturnal while the second is mainly diurnal. As Tobler (2008) has suggested, this might illustrate the temporal division of resources. However, a more detailed knowledge of the diets and patterns of habitat use of these species is still needed to better understand differences in behavioural patterns (Blake et al., 2011).

Tapirus terrestris presented a cathemeral behaviour, showing different activity patterns depending on the studied salt lick. These results differ from those of Blake et al., (2011) and Tobler et al., (2009), which report a nocturnal pattern for this species. However, data presented here is consistent with earlier studies which suggest that *Tapirus terrestris* can be active both during the day and during the night (van Schaik and Griffiths, 1996; Lizcano and Cavelier, 2000). In fact, the results of this chapter show that *Tapirus terrestris* presented a cathemeral pattern in the two sites located far away from human communities and active oil infrastructure and, therefore, less exposed to hunting pressure and noise disturbance. On the contrary, the same species presented a nocturnal pattern in sites located close to Indigenous communities and oil infrastructure. Similar changes on the pattern activity were documented for *Mazama americana*. Thus, data suggest that human activities, and particularly hunting pressure and industrial noise, might affect animal behaviour.

This is not the first study pointing out that wildlife diurnal activity pattern might change in response to the proximity of human activities. For instance, a comparative study performed in Yasuní National Park between salt licks affected and largely unaffected by hunting detected a clear decrease in diurnal activity of *Mazama americana* at sites with a higher hunting pressure (Blake et al 2012). In line with these findings, Bitetti et al., (2008) reported a more diurnal activity pattern of *Mazama americana* in best-protected areas, denoting that the species may adjust its activity pattern to local hunting pressure. The data presented in this chapter suggest, for the first time, a similar shift on the diurnal activity of *Tapirus terrestris* due to hunting pressure and noise disturbance.

CONCLUSIONS

In this chapter the ingestion of oil-polluted soils by wild Amazonian species in a remote oil concession in the Peruvian Amazon is reported. The chapter also shows that this new-to-science behaviour is not performed by isolated individuals but it is common among individuals from at least eight wild species. Moreover, the behaviour is not limited to a particular site, since it has been documented in all the studied oil-polluted salt licks.

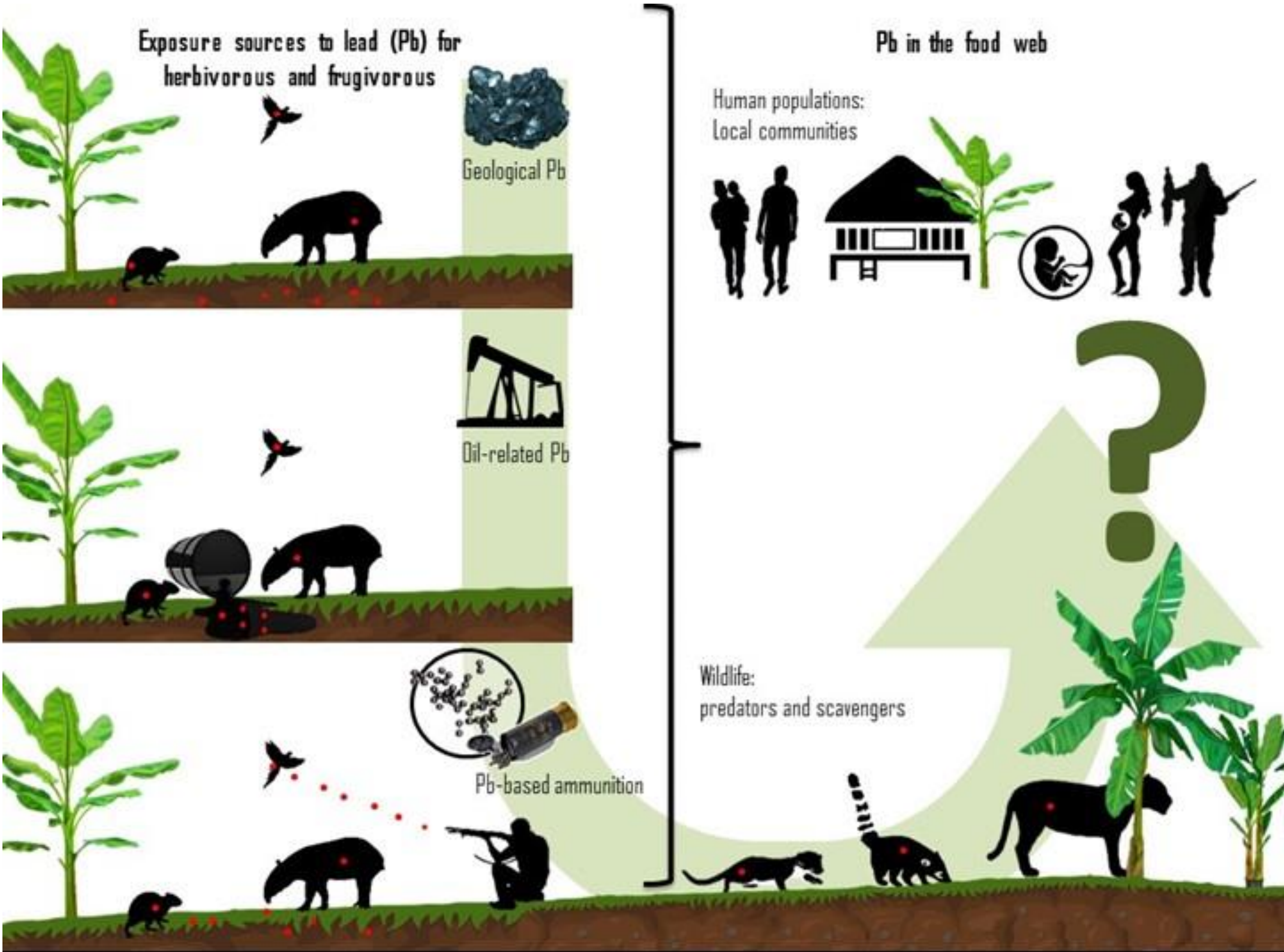
Soil ingestion emerges, therefore, as an important exposure way to many pollutants associated with the oil industry. Among these pollutants there are highly toxic, carcinogenic, and mutagenic compounds. Some of them might bioaccumulate in animals' tissues and some might even biomagnificate[13] along the food chain. Thus, geophagy, far

[13] When biomagnification occurs, there is an increment of Pb levels from one link in a food chain to another.

from being a low impact behaviour, might indeed expose the whole ecosystem, including top predators, to oil-related pollutants.

Moreover, the oil concession overlap with Indigenous communities who largely depend on subsistence hunting, and whose hunting grounds are mostly located inside the oil concession limits. Considering the mobility of the species that have been recorded ingesting oil-polluted soils and water, up to 80% of the oil concession territory might be considered unsafe hunting grounds, since polluted animals can be hunted far away from the oil infrastructure. In addition, and worrisomely, oil-polluted salt licks are important hunting sites for local hunters, a fact that increase even more the probabilities of local people to ingest polluted game meat.

Importantly, results of this study illustrate the existing knowledge gaps regarding oil extraction impacts and thus represent an important step in the quest to fill them. Although the ingestion of soils has already been pointed out as an increasingly important route for contaminant exposure for both livestock and free-ranging wildlife in industrialized countries (Weeks and Kirkpatrick, 1976; Weeks, 1978; Arthur and Alldredge, 1979; Fries, 1982; Fries *et al.*, 1982), it was never considered as a potential exposure route to petrogenic pollutants for both fauna and human population in remote and biodiverse rich areas, like tropical rainforests. Considering that oil hydrocarbon reservoirs overlap with 30% of world tropical forests (Orta-Martínez et al., 2018), the relevance of the behaviour presented here exceed the frontiers of the study region. For this reason and considering the potential negative impacts of geophagy in the vulnerable rainforest ecosystems and Indigenous Peoples, further research on oil-polluted soil and water ingestion is urgent and needed and should include the analyses of oil-related compounds in wildlife and human population blood or tissues as well as the conduction of camera trap surveys in oil-polluted sites in other regions and even other ecosystems.



CHAPTER III

ANTHROPOGENIC LEAD IN AMAZONIAN WILDLIFE

Picture in the previous page: *Exposure Pb sources for frugivorous and herbivorous species and its entrance in the food web*.
Source: Own elaboration.
Vector images obtained in pixabay.com, welovesolo.com, supercoloring.com, moziru.com, sp.depositphotos.com, hddfhm.com, es.vecteezy.com, 123rf.com, freedesignfile.com, hyperphysics.phy-astr.gsu.edu, clker.com and dreamstime.com

CHAPTER III • ANTHROPOGENIC LEAD IN AMAZONIAN WILDLIFE

ABSTRACT

Lead (Pb) levels and isotopic fingerprints in 315 free-ranging animals belonging to 18 wild game species in four remote areas of the Peruvian Amazon provide a comprehensive picture of anthropogenic Pb pollution in tropical rainforests. The high average concentration of Pb (0.49 µg/g wet weight) in livers from Amazonian wild game is comparable to values from industrialized countries and mining areas. Hunting ammunition and, to a lower extend, oil-related pollution are identified as the major sources of Pb in wildlife hunted in oil extraction areas. Potential exposure pathways to Pb for wildlife include the ingestion of Pb (probably by the ingestion of lead-polluted water, soil, vegetation and animals and the mistaken of ammunition for grit and food items), as well as the deposition of lead-based ammunition fragments in the animal tissues during the shot. Due to the extended worldwide use of lead-based ammunition in subsistence hunting and the ever-encroaching oil extraction in tropical rainforests, these results uncover important health risks to tropical wildlife and local human communities that rely on subsistence hunting.

Keywords: Amazon; Ammunition; Lead (Pb); Subsistence hunting; Oil extraction; Wildlife.

INTRODUCTION

On a global perspective, the accumulation of pollutants in a given ecosystem largely depends on the history of industrialization across continents and the distance from the area to pollutants emissions, and less so on ecosystem type and climatic variables (Bartrons *et al.*, 2016). Arguably, and given its remoteness from the putative sources, Amazonian wildlife should not be largely affected by industrial and urban pollutants. Nevertheless, some studies indicate a general accumulation of toxic pollutants in most ecosystems across the world (Bartrons *et al.*, 2016). This is, for example, the case for Pb, a cumulative toxic and non-essential metal that may cause deleterious effects over most body systems (Komárek *et al.*, 2008). Despite being a natural component of the earth's crust, normally found in nature at trace levels, Pb is the most widespread toxic metal in the world, largely as a result of human activities (Cheng and Hu, 2010). Exposure to Pb is associated with neurological and kidney damage, hypertension, cardiac disease, and low levels of fertility (Hu *et al.*, 2007). Lead-related health effects have been demonstrated even at very low levels of exposure (around 3 μg/dl), and there is widespread consensus that there is no threshold for innocuous levels of Pb in blood (Gilbert and Weiss, 2006). Recently, the World Health Organisation and the European Food Safety Authority stated that there are no safe levels of Pb intake in humans (EFSA CONTAM, 2010; World Health Organization (WHO), 2017).

Nowadays, Pb is released into the environment from multiple anthropogenic sources, which account for 95-99% of the total atmospheric Pb deposition. For several decades, leaded gasoline has been the dominant source of human exposure to Pb (United Nations Environmental Porgramme (UNEP), 2010). In many countries, lead-containing paints, gasoline, and diverse household objects are outlawed, although Pb is still allowed in ammunition, which is extensively used by hunters (Arnemo *et al.*, 2016). Consequently, the most important source of direct Pb release to soil at the global level is the use of lead-based ammunition, which in 2003 had a total global consumption of 120,000 tons (United Nations Environmental Porgramme (UNEP), 2010).

Most research on Pb impacts has been conducted in areas susceptible of being polluted by Pb, and notably in industrialized countries (Nawrot and Staessen, 2006). Results of this research shows that environmental Pb exposure, also called the "silent killer", presumably persists in low but risky/harmful levels in the United States and in many European countries (Nawrot and Staessen, 2006), despite most lead-based products being not commercialized anymore. Not much research has been conducted in areas with presumable scarce anthropic sources of Pb, but these few studies have found high levels of Pb in blood of Indigenous Peoples living in remote Amazonian communities (DIGESA, 2006; Barbosa *et al.*, 2009; Anticona, Ingvar A Bergdahl, *et al.*, 2012). There are several potential pathways for Pb exposure among Amazonian human populations. First, daily practices such as the artisanal transformation of manioc to flour (*farinha*) in large metal pans or the chewing of Pb scraps to manufacture fishing sinkers have been suggested as possible exposure ways (Barbosa *et al.*, 2009; Anticona *et al.*, 2011; Anticona, Ingvar A Bergdahl, *et al.*, 2012; Anticona, Ingvar A. Bergdahl, *et al.*, 2012). A second pathway for Pb exposure could be lead-based shot in game hunting (Arnemo *et al.*, 2016), which represents one of the main source of protein intake for rural communities in most tropical forests, including the Amazon (Robinson and Bennett, 2000). A third pathway could be oil extraction (Neff, 2002). In 2008, 733,414 Km^2 of the western Amazon in Bolivia, western Brazil, Colombia, Ecuador and Peru were covered by oil extraction and exploration concessions (Finer *et al.*, 2015). The main waste product of oil extraction operations is produced water, which contains a complex mixture of dispersed hydrocarbons and metals, including Pb ($<1 \cdot 10^{-6}$ – 18 mg/L). The dumping of produced water has been pointed out as the cause of the increase of Pb concentration levels in the Amazon rivers (Yusta-García *et al.* 2017). Finally, long-range atmospheric transport of anthropogenic Pb emissions has also been proven to increase Pb natural content in soils in remote locations, from the tropics to the polar latitudes (Candelone *et al.*, 1995; Ikegawa *et al.*, 1999; Halstead *et al.*, 2000; Correia *et al.*, 2003).

The three last afore mentioned putative sources would be the most relevant additional Pb inputs to Amazonian soils' Pb natural content. Pb in soils can then reach wildlife through geophagy, the deliberate ingestion of soil by wildlife. Indeed, geophagy has been proved to be an important route for contaminant exposure in industrial areas, posing a risk to animal's health (Beyer and Fries, 2002),. Similarly, it may also be an important route for contaminant exposure for Amazonian wildlife, where redirected geophagy from salt licks to oil-polluted sites has been documented (see *Chapter II*).

In this chapter, Pb levels and their isotopic fingerprints in 18 free-ranging wild game species of the Amazon, hunted by Indigenous hunters for local consumption, are evaluated with the aim to provide a comprehensive picture of Pb pollution and anthropogenic disturbances in a remote tropical rainforest region of the world. To precisely determine the sources of Pb, the study focuses on isotopic fingerprints, and not only on Pb levels (Hopper *et al.*, 1991; Komárek *et al.*, 2008).

METHODOLOGY

This chapter is divided into two parts: a) Subchapter A: identification of the main sources of Pb found in wild game species through the analysis of Pb isotopic ratios and Pb concentrations, and b) Subchapter B: variability of Pb concentrations in Amazonian animals' tissues according to explanatory variables, such as oil-activity, basin, species (interspecies differences in metabolism and kinetics), body mass, habitat and trophic level. A general methodology for both subchapters is presented below but the Data analysis, Results, Discussion and Conclusions of each subchapter are presented separately.

STUDY AREA

The four study areas are located in the region of Loreto, in Northern Peruvian Amazon. All of them are remote areas, still considered relatively well-conserved, for which the effect of the few extant human activities can accurately be measured. All sampling areas are inhabited by indigenous communities that depend on their territory for their livelihoods, and whose subsistence activities include hunting, fishing and small-scale agriculture. As oil extraction is a potential pathway for human Pb contamination, two areas devoid of any oil extraction activity (Yavarí-Mirin River basin and the Pucacuro Natural Protected Area) and two areas within oil extractive block 192/1AB (Pastaza and Corrientes River basins) were selected (Fig. 3.1).

The Yavarí-Mirin River basin is close to the Brazilian border. This area spans 1,070 Km^2 of continuous forest, predominantly non-flooded *terra firme* forest. Within this area there is only one Yagua village, Nueva Esperanza. All samples from this basin were collected by local hunters in the ancestral territory of the aforementioned indigenous community. The only cash economic activity in the area is selective logging.

The Pucacuro National Reserve is located close to the Ecuadorian border. The reserve covers an area of 6,379.53 Km^2 and aims to protect a worldwide important rich biodiversity area. At the time of data collection, there was no oil extraction or logging activity in the area. Samples from this national reserve were collected by the park rangers.

The Pastaza and Corrientes River basins are located inside an oil concession called block 192 (formerly, block 1AB), the oldest and most productive oil concession in the Peruvian Amazon covering 5,123.47 Km^2. Severe oil pollution has been documented in the area and, consequently, the Peruvian government declared the environmental and health states of Emergency due to oil pollution in both basins in 2013 and 2014 (see *Chapter I: Study Area*). Pastaza samples were collected in four indigenous communities close to the Ecuadorian border inhabited by Quechua and Achuar people (Andoas Nuevo, Andoas Viejo, Los Jardines and Titiyacu). Corrientes samples were collected in two Achuar communities (Nueva Jerusalén and José Olaya).

All studied sites are located far from Peru's main Pb sources, as identified by the Health Ministry (MINSA, 2007). In all studied basins, beside natural Pb in soils, the use of lead-based ammunition for subsistence hunting purposes is also documented. Oil extractive activities were only taking place in the Pastaza and Corrientes River basins.

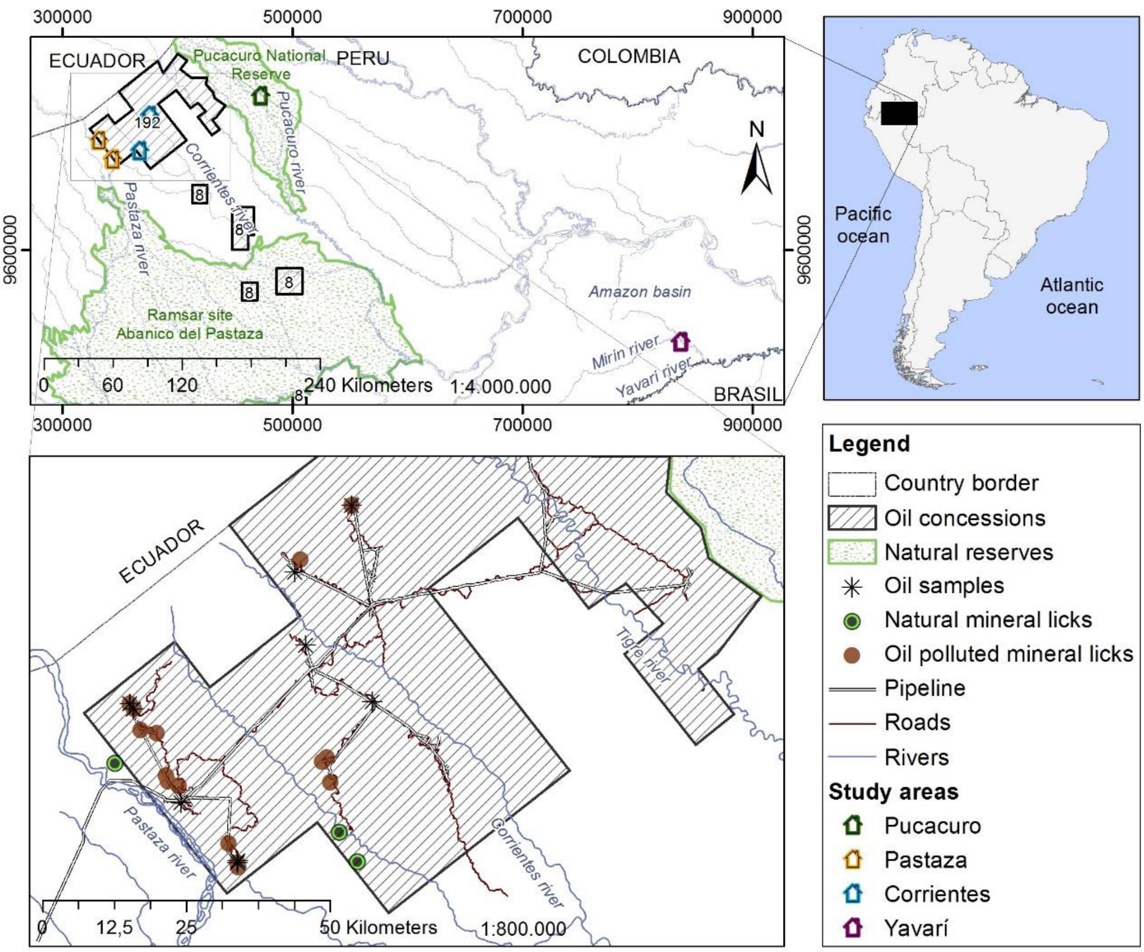


Figure 3.1 | Map of the study area including the sampling areas.

BIOLOGICAL SAMPLING

Between May 2013 and August 2015, liver[14] tissues from 315 individuals from 18 wild species (Table 3.1) were collected in the four study areas. Samples were preserved in buffered 4% formaldehyde solution (v/v). The research protocol was approved by the *Dirección General de Flora y Fauna Silvestre* from Peru (0350-2012-AG-DGFFS-DGEFFS) and by a Resolution of the Head of the National Reserve of Pucacuro (nº 03-2012-SERNANP-RN Pucacuro). All liver samples were from animals that were hunted

[14] Although samples from other tissues have also been collected, the collection was mostly focussed on liver for several reasons. First, Liver tissues are ingested by both wildlife predators and human people, which allows the exploration of the lead exposure associated to the ingestion of lead polluted meat for the ecosystem (predators) and human populations. Second, lead bioaccumulates in liver tissues much more than it does in muscle tissues, so the lead concentrations and fingerprints found in liver tissues are very useful for studying the recent (last days-weeks) exposition to lead for the collected animals (Ma, 1996). Moreover, previous literature in the field usually collected data on liver lead levels, which allows the comparison of the findings of this thesis with that of other previous studies. Finally, liver tissues also accumulate other heavy metals related with oil extraction activities, which will be evaluated in the near future.

for household consumption and using shotguns. No animal was killed solely for this study. Each sample was identified with a code. Date and place of collection, species, and sex of the animal were saved for each sample.

SAMPLING OF PUTATIVE SOURCES

To investigate the origin of Pb in animal livers, the Pb isotopic ratios were compared with those analysed from samples representative of potential sources (ammunition, oil pollution, and natural Pb content of soils).

Soil samples (n=9) in natural salt licks from the Yavarí-Mirin (n=4), Pastaza (n=2) and Corrientes (n=3) River basins were collected. Soil samples in 15 oil-polluted sites allegedly used by wildlife as salt licks (10 in the Pastaza and five in the Corrientes River basins) were also collected. Soil samples resulted from the combination and homogenization of three samples taken from the superficial soil (between 0 and 20 cm-depth), separated by at least 1.5 meters in each site and collected using a methacrylate tube (7 cm ø) (Table 3.1). Soil samples were stored in plastic bags, transported at ambient temperature, and placed in a freezer once arrived at the laboratory.

Ammunition samples (n=2) of the two most cited ammunition used by local hunters (Armusa® and Saga®) were obtained from local markets for analysis. Similarly, shot pellets (n=3) found during the manipulation of hunted animal tissues were also analysed.

Oil samples were collected in oil spills (n=4) and abandoned wells (n=1). They were all collected using crystal jars. Four samples of liquid arising from oil infrastructure were also collected; these include samples from sump tanks (n=2) and abandoned wells (n=2) that were still leaking when this study was carried out. These petrogenic samples were collected in seven different oil fields of the block 192/1AB by the indigenous environmental monitors (Fig. 3.1).

The isotopic signature from the potential atmospheric sources of Pb in South America was retrieved from scientific literature (Bollhöfer and Rosman, 2000).

Table 3.1 | Location of oil and soil samples analysed in this study.

Type	UTM Coordinates	Study area
Oil	18M 374014 9707036	Corrientes
Oil	18M 370415 9741077	Corrientes
Oil	18M 350696 9679430	Pastaza
Oil	18M 0332491 9705816	Pastaza
Oil	18M 360588 9729502	Corrientes
Natural salt licks	18M 371420 9679240	Corrientes
Natural salt licks	18M 368240 9684409	Corrientes
Natural salt licks	18M 329303 9696322	Pastaza
Natural salt licks	18M 859309 9508632	Yavarí-Mirin
Natural salt licks	18M 860691 9507627	Yavarí-Mirin
Natural salt licks	19 M 178956 9517955	Yavarí-Mirin
Natural salt licks	18 M 370837 9737948	Corrientes
Natural salt licks	18 M 770544 9492748	Yavarí-Mirin
Natural salt licks	18 M 350915 9677744	Pastaza
Oil-polluted salt lick	18M 365284 9696617	Corrientes
Oil-polluted salt lick	18M 361544 9731654	Corrientes
Oil-polluted salt lick	18M 366770 9693025	Corrientes
Oil-polluted salt lick	18M 370415 9741077	Corrientes
Oil-polluted salt lick	18M 366141 9697346	Corrientes
Oil-polluted salt lick	18M 336596 9701584	Pastaza
Oil-polluted salt lick	18M 338569 9693173	Pastaza
Oil-polluted salt lick	18M 332557 9705779	Pastaza
Oil-polluted salt lick	18M 350777 9678313	Pastaza
Oil-polluted salt lick	18M 340409 9692361	Pastaza
Oil-polluted salt lick	18M 331902 9706704	Pastaza
Oil-polluted salt lick	18M 333793 9702125	Pastaza
Oil-polluted salt lick	18M 338129 9694197	Pastaza
Oil-polluted salt lick	18M 349006 9682466	Pastaza
Oil-polluted salt lick	18M 350590 9679070	Pastaza
Liquid from oil structures	18M 362454 9716821	Corrientes
Liquid from oil structures	18M 331902 9706704	Pastaza
Liquid from oil structures	18M 350590 9679070	Pastaza
Liquid from oil structures	18M 340780 9689542	Pastaza

LEAD ISOTOPIC FINGERPRINT FOR THE IDENTIFICATION OF LEAD SOURCES

Pb isotopic fingerprint is a very powerful and frequently used tool for the identification of Pb pollution sources and pathways, both at local and global scales (Chow and Johnstone, 1965; Rabinowitz and Wetherilll, 1972; Gulson *et al.*, 1981; Nakata *et al.*, 2015). Many sources of Pb have distinct isotopic fingerprint and the isotopic composition of Pb in a sample shows the mixture of Pb sources (Chiaradia *et al.*, 1997; Gulson, 2008; Komárek *et al.*, 2008; Álvarez-Iglesias *et al.*, 2012). Isotopic composition in environmental sciences is usually expressed as ratios between the four main Pb isotopes: the radiogenic isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb and the stable isotope ^{204}Pb. Pb fingerprint depends on the concentration of primordial Pb, U and Th (the radioactive parents of the aforementioned isotopes) and the duration of the decay time (Komárek *et al.*, 2008; Cheng and Hu, 2010). Since Pb isotopic fingerprint does not significantly changes during physico-chemical fraction processes but only over geological time, it provides a tool for identifying Pb sources and transport pathways. Pb isotopic fingerprint has been recently used in diverse disciplines to study, for example, human health and exposure risk (Chiaradia *et al.*, 1997; Patel *et al.*, 2008; Tsuji *et al.*, 2008; Oulhote *et al.*, 2011; Cao *et al.*, 2015), wildlife exposure to pollutants (Rabinowitz and Wetherilll, 1972; Gulson *et al.*, 2012; Nakata *et al.*, 2015), atmospheric transport patterns (Grousset and Biscaye, 2005; Félix *et al.*, 2015), and anthropogenic activity impacts on the ecosystem (Gulson *et al.*, 1981; Ferrand *et al.*, 1999; Duzgoren-Aydin, 2007; Zhu *et al.*, 2010; Álvarez-Iglesias *et al.*, 2012; Adánez Sanjuán *et al.*, 2015). The main limitations of this technique are the high cost of the measurement equipment, the costly and labour-intensive sample preparation, and the fact that its use should be limited to cases where only few quantitative dominant sources exist and have widely differing isotopic fingerprints. Moreover, since there is a potential overlap of Pb fingerprints from different sources (Cheng and Hu, 2010), Pb isotope data must be always interpreted with caution and considering the context.

LEAD CONCENTRATION AND ISOTOPIC ANALYSIS

Pb concentration and isotopic analyses of biological, soil, ammunition and oil samples were carried out in the *Servei d'Anàlisi Química* from the UAB (Export permits: 000605-CITES-Peru and 003106-CITES-Perú, 001309-MINAGRI-DGFFS and 003005-SERFOR).

Liver samples were peeled 1 cm from the surface to remove the mass exposed to the formaldehyde solution and discard external contamination by contact. Liver samples were freeze dried, and finally pulverized using a porcelain mortar. Soil samples were freeze dried, sieved (0.6 mm), homogenized and grounded.

An aliquot of each sample (0.1 g for liver samples, 0.1-0.2 g and 10 mL for the lipophilic and hydrophilic phases of oil samples, respectively, 0.25 g for soil samples and 10 mL for not clear liquid) was digested with HNO_3, HCl and HF in a microwave (Ultrawave, Milestone), at 240 ºC for 15 minutes for liver and oil samples, at 240 ºC for 30 minutes for soil samples and at 190 ºC for 10 minutes for water samples. Digestion blanks were created simultaneously. Aliquots of clear water samples (5 g) were not digested but acidified with concentrated HCl, bringing the sample to a pH of ~1, and blanks of this procedure were created simultaneously.

Ammunition samples were treated with concentrated HNO_3 during 48 h at room temperature. All samples were diluted with HCl 1% (v/v) before being injected in an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (7500ce, Agilent Technologies). Pb concentration was determined by ICP-MS spectrometry. Every 15 samples, a reference standard created in the laboratory was injected to measure stability and accuracy. All ICP-MS injections were performed using a concentric nebulizer and a double-pass propylene chamber.

A sample of the formaldehyde solution (0.1 g aliquot) was also digested with HNO_3, HCl and HF in a microwave (Ultrawave, Milestone) at 240 ºC for 15 minutes and analysed to assure no external contamination. Pb concentrations in biological samples are expressed using the median and the interquartile range (IQR), and Pb concentrations found in oil and soils are expressed by the mean and the standard deviation. Pb concentration values are expressed in relation to the sample's fresh weight (also called wet weight) (WW) or dry weight (DW). Outliers (n=28) were defined as values larger than Q3 + 1.5 * IQR.

Signals for ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb lead isotopes were determined using quadrupole ICP-MS. Every five samples, the correction for the mass discrimination effect was performed using the lead standard reference material NIST 981 (National Institute of Standards and Technology (NIST), USA).

To avoid isotopic determination error, only samples with Pb concentrations over the quantifying limit have been used. Pb isotopic composition was presented in terms of concentration ratios, showing the relative abundances of $^{207/206}Pb$ and $^{208Pb/206}Pb$. Since isobaric interferences of ^{204}Hg were detected on the ^{204}Pb isotopic signal, isotopic ratios involving ^{204}Pb isotope were not used.

SUBCHAPTER A • SOURCES OF LEAD EXPOSURE FOR AMAZONIAN WILDLIFE

DATA ANALYSIS

In subchapter A, Pb levels in Amazonian wildlife are assessed. Additionally, the main sources of Pb found in Amazonian wildlife through the analysis of Pb concentrations and isotopic ratios are identified.

Plots of $^{207/206}$Pb and $^{208/206}$Pb isotopes ratios were used to pinpoint the source of Pb by comparing the isotopic ratios found in the samples with those of the potential sources.

The area of $^{207/206}$Pb and $^{208/206}$Pb isotopic fingerprint of the ammunition at a 99% confidence interval (mean ± 2.58*SD) was estimated to determine a square area of the Pb isotopic fingerprint of the ammunition and to estimate the proportion of liver samples whose Pb source was most influenced by lead-based shot. The mean and SD of the ratios $^{207/206}$Pb and $^{208/206}$Pb was used to estimate this area. A Chi-square test was used to compare the percentage of biological samples within the influence area of lead-ammunition isotopic fingerprint according to sampling area.

The average distance of the Pb isotopic fingerprint in liver samples with respect to the mean fingerprint of ammunition for the ratios $^{207/206}$Pb and $^{208/206}$Pb separately (here called isotopic displacement) was calculated. A one-way ANOVA and a Tukey–Kramer Multiple Comparisons test were used to compare the isotopic displacement of liver samples according to study area. Only in livers from areas with oil activities, the Spearman's correlation was used to assess the association between Pb concentration and the isotopic ratios $^{207/206}$Pb and $^{208/206}$Pb.

Given that animal tissues isotope composition show exposure to Pb sources with a different isotopic ratio (Rabinowitz and Wetherilll, 1972), to identify the most likely pollution sources in animal tissues, three lineal regressions with Pb isotopic fingerprints of (1) soils, (2) anthropic Pb sources (oil and ammunition), and (3) liver collected in the oil extraction area were performed.

Logarithmic transformations of Pb concentrations were performed to fit the assumptions of normality and homoscedasticity. Comparisons of Pb concentrations in soils from natural salt licks according to the study area and between natural salt licks and oil-polluted soils were tested by one-way ANOVA and Tukey tests.

RESULTS

Liquid samples (n=5) dropping from oil wells and arising from other oil infrastructure presented Pb concentration values of 3.34 ± 4.86 µg/l. Oil samples (n=5) from the abandoned well and from oil spills showed concentrations of 0.86 ± 0.96 µg/g and 0.04 ± 0.06 µg/g in the lipophilic and hydrophilic phases, respectively.

In oil extraction areas, soil samples from oil-polluted sites used by wildlife as salt licks had higher Pb concentrations than soil samples from natural salt licks (25.32 ± 8.07 µg/g, n=15 vs. 10.21 ± 3.96 µg/g, n=5; $P<0.001$). Soil from natural salt licks from both oil extraction and non-extraction areas showed the presence of Pb (at concentrations of 11.39 ± 4.50 µg/g, n=9) and no significant difference was detected in Pb concentration from soils taken in areas with or without oil extraction (10.21 ± 3.96 µg/g, n=5 vs. 12.86 ± 5.28 µg/g, n=4, respectively; $P=0.275$).

Liver samples collected in the four study areas had median Pb concentrations of 2.00 µg/g DW (Table 3.2) and 0.49 µg/g WW (n=315). Pb concentrations among the twenty-nine (9.2%) outliers ranged from 8.46 µg/g to 375.0 µg/g (Table 3.3).

Table 3. 2 | Lead concentrations (in µg/g DW) in livers (n=315), by species and sampling area.

	Corrientes				Pastaza				Pucacuro				Yavarí-Mirin				All basins			
	N	*Q1*	Median	*Q3*	N	*Q1*	Median	*Q3*	N	*Q1*	Median	*Q3*	N	*Q1*	Median	*Q3*	N	*Q1*	Median	*Q3*
Anura	2	*0.8*	0.86	*0.92*	2	*4.6*	5.09	*5.58*									4	*0.92*	2.55	*4.6*
Leptodactylus pentadactylus	2	*0.8*	0.86	*0.92*	2	*4.6*	5.09	*5.58*									4	*0.92*	2.55	*4.6*
Artyodactyla	5	*0.27*	0.72	*0.93*	26	*0.52*	0.81	*1.25*	38	*1.17*	2.55	*4.56*	19	*0.99*	1.3	*2.82*	88	*0.73*	1.21	*2.93*
Mazama americana	1	*0.1*	0.1	*0.1*	10	*0.4*	0.84	*1.05*	2	*2.94*	4.06	*5.18*	11	*1.8*	2.8	*3.21*	24	*0.82*	1.25	*2.81*
*Tayassu pecari**	1	*0.93*	0.93	*0.93*	15	*0.59*	0.78	*1.63*	28	*1.05*	2.96	*4.53*	2	*1.14*	1.48	*1.82*	46	*0.65*	1.68	*3.62*
Pecari tajacu	3	*0.49*	0.72	*0.9*	1	*0.83*	0.83	*0.83*	8	*0.97*	2.04	*3.39*	6	*0.95*	1.05	*1.17*	18	*0.84*	1.09	*1.67*
Carnivora	1	*29.58*	*29.58*	*29.58*	7	*1.75*	3.53	*6.5*					5	*3*	3.2	*3.4*	13	*2.7*	3.4	*5.9*
Nasua nasua	1	*29.58*	*29.58*	*29.58*	2	*0.84*	1.05	*1.27*					5	*3*	3.2	*3.4*	8	*2.4*	3.1	*4.02*
Potos flavus					5	*3.53*	4.1	*8.9*									5	*3.53*	4.1	*8.9*
Crocodilia	6	*0.61*	1.82	*2.29*	4	*1.23*	2.6	*3.66*					3	*3.45*	3.5	*4.47*	13	*1.6*	2.39	*3.61*
Caiman crocodilus	6	*0.61*	1.82	*2.29*	4	*1.23*	2.6	*3.66*					3	*3.45*	3.5	*4.47*	13	*1.6*	2.39	*3.61*
Galliformes					7	*1.7*	2.04	*4.6*					6	*11.51*	21.43	*39.75*	14	*2.42*	7.06	*19.05*
Penelope jaqcuaqu					7	*1.7*	2.04	*4.6*					6	*11.51*	21.43	*39.75*	14	*2.42*	7.06	*19.05*
Gruiformes	2	*6.32*	10.53	*14.73*	4	*1.17*	2.04	*4.6*					1	*7.56*	7.56	*7.56*	7	*1.66*	4.37	*13.25*
Psophia leucoptera	2	*6.32*	10.53	*14.73*	4	*1.17*	2.78	*9.83*					1	*7.56*	7.56	*7.56*	7	*1.66*	4.37	*13.25*
Perissodactyla					1	*1.49*	1.49	*1.49*									1	*1.49*	1.49	*1.49*
*Tapirus terrestris**					1	*1.49*	1.49	*1.49*									1	*1.49*	1.49	*1.49*
Primatia	11	*1.45*	1.85	*2.4*	10	*1.13*	2.5	*4.35*					28	*1.71*	2.24	*4.12*	49	*1.6*	2.06	*4.1*
*Alouatta seniculus**	1	*2.22*	2.22	*2.22*	1	*6.79*	6.79	*6.79*									2	*3.36*	4.5	*5.65*
Cebus albifrons					1	*3.04*	3.04	*3.04*					6	*2.37*	4.57	*6.51*	4	*2.4*	4.1	*6.02*
Sapajus macrocephalus	2	*1.5*	1.6	*1.7*	2	*0.9*	1.27	*1.63*					7	*0.96*	1.5	*2.1*	11	*0.96*	1.5	*1.9*
*Lagothrix sp.**	8	*1.47*	1.96	*3.01*	6	*1.13*	2.51	*4.35*					15	*1.84*	3.41	*3.84*	29	*1.72*	2.08	*4.17*
Psittaciformes	1	*1.3*	1.3	*1.3*	2	*2.71*	3.31	*3.91*									3	*1.7*	2.11	*3.31*
Ara sp.	1	*1.3*	1.3	*1.3*	2	*2.71*	3.31	*3.91*									3	*1.7*	2.11	*3.31*
Rodentia	19	*0.35*	0.46	*1.43*	30	*1*	2.25	*5.78*	21	*2.17*	3.06	*4.99*	30	*1.68*	1.91	*3.3*	101	*1.2*	2.04	*4.17*
Cuniculus paca	16	*0.3*	0.44	*1.2*	22	*0.87*	1.55	*4.41*	20	*2.17*	2.86	*5.01*	20	*1.5*	1.8	*2.03*	78	*0.97*	1.85	*2.94*
Dasyprocta fuliginosa	3	*1.35*	2.32	*2.88*	8	*3.17*	5.57	*9.65*	1	*3.13*	3.13	*3.13*	10	*2.52*	3.75	*6.82*	23	*2.26*	3.6	*6.86*
Tinamiformes	7	*0.5*	1.6	*2.96*	7	*0.8*	0.98	*2.45*					10	*1.7*	1.77	*8.02*	24	*0.95*	1.71	*3.11*
Tinamus major	7	*0.5*	1.6	*2.96*	7	*0.8*	0.98	*2.45*					10	*1.7*	1.77	*8.02*	24	*0.95*	1.71	*3.11*
Total	54	*0.43*	1.35	*2.19*	100	*0.83*	1.67	*3.88*	59	*1.82*	2.78	*4.64*	102	*1.62*	2.21	*4.08*	315	*1.03*	2	*3.97*

Note: * Endangered or vulnerable species according to the IUCN red list of threatened species (International Union for Conservation of Nature and Natural Resources, 2016).

Table 3.3 | Description of biological samples with lead concentrations (in µg/g DW) larger than Q3 + 1.5 * IQR (n=29).

Species	Order	Basin	Lead concentrations (µg/g dry weight)
Dasiprocta fuliginosa	Rodentia	Yavari-Mirin	375.00
Mazama americana	Artyodactyla	Yavari-Mirin	199.45
Penelope jaqcuaqu	Galliformes	Yavari-Mirin	89.73
Tayassu pecari	Artyodactyla	Pucacuro	46.00
Penelope jaqcuaqu	Galliformes	Yavari-Mirin	45.61
Tayassu pecari	Artyodactyla	Pucacuro	42.88
Tinamous major	Tinamiformes	Corrientes	42.00
Nasua nasua	Carnivora	Corrientes	29.58
Tayassu tajacu	Artyodactyla	Pucacuro	28.18
Psophia leucoptera	Gruiformes	Pastaza	26.21
Tayassu pecari	Artyodactyla	Pucacuro	25.00
Penelope jaqcuaqu	Galliformes	Yavari-Mirin	22.20
Caiman crocodilus	Crocodilia	Corrientes	21.85
Tinamous major	Tinamiformes	Yavari-Mirin	21.74
Mazama americana	Artyodactyla	Yavari-Mirin	21.00
Penelope jaqcuaqu	Galliformes	Yavari-Mirin	20.66
Dasiprocta fuliginosa	Rodentia	Pastaza	20.40
Psophia leucoptera	Gruiformes	Corrientes	18.94
Tinamous major	Tinamiformes	Yavari-Mirin	17.00
Dasiprocta fuliginosa	Rodentia	Pastaza	15.00
Penelope jaqcuaqu	Galliformes	Pastaza	14.21
Cuniculus paca	Rodentia	Pastaza	13.85
Cebus albifrons	Primatia	Yavari-Mirin	13.18
Cuniculus paca	Rodentia	Pastaza	10.40
Tinamous major	Tinamiformes	Yavari-Mirin	9.90
Potos flavus	Carnivora	Pastaza	9.23
Dasiprocta fuliginosa	Rodentia	Yavari-Mirin	9.00
Potos flavus	Carnivora	Pastaza	8.90
Penelope jaqcuaqu	Galliformes	Yavari-Mirin	8.46

The plots of isotope ratios of ^{206}Pb, ^{207}Pb and ^{208}Pb showed a homogeneous Pb isotopic fingerprint for the ammunition samples (Fig 3.2). In contrast, crude oil samples presented a diverse isotopic fingerprint according to the oil field. Crude oil and ammunition isotopic fingerprint were well differentiated.

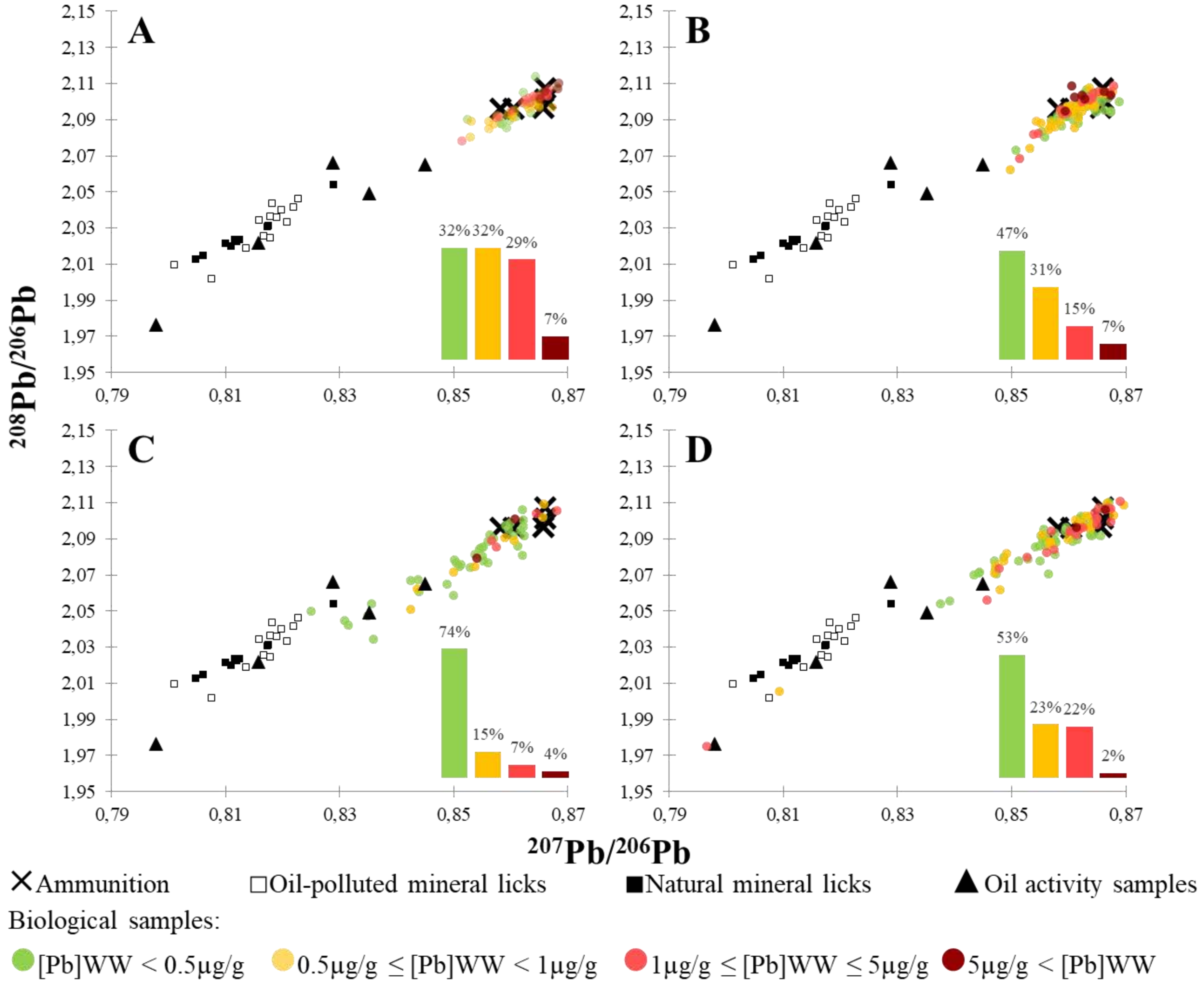


Figure 3.2 | Lead isotopic distribution of putative lead sources (ammunition, oil, and soils) and liver samples from Pucacuro (A), Yavarí (B), Corrientes (C) and Pastaza (D).

Most liver samples (72.1%) matched with the ammunition isotopic area, with variations according to the area studied (P=0.0001; Table 3.4), ranging from 57% matching in oil extraction areas to 86% in areas without oil extraction. In the oil extraction areas, Corrientes and Pastaza River basins, 61.1% and 33.0% of liver samples had an isotopic fingerprint outside of the ammunition isotopic area, respectively. On the contrary, in Yavarí and Pucacuro River basins only 12.7% and 15.3% of the samples did not match ammunition isotopic area.

Table 3.4 | Ammunition fingerprint in liver samples (n=315).

Study area	Nº of samples into the ammunition area (%)	Isotopic displacement $^{207/206}$Pb	Isotopic displacement $^{208/206}$Pb
Corrientes	21 (38,9) ^{a}	0.0101 ± 0.0098 ^{a}	0.0191 ± 0.0181 ^{a}
Pastaza	67 (67.0) ^{b}	0.0067 ± 0.0109 ^{a}	0.0120 ± 0.0194 ^{b}
Pucacuro	50 (84.7) ^{c}	0.0016 ± 0.0046 ^{b}	0.0026 ± 0.0079 ^{c}
Yavarí-Mirin	89 (87.3) ^{c}	0.0016 ± 0.0039 ^{b}	0.0034 ± 0.0085 ^{c}

Note: Percentage of liver samples inside the ammunition influence fingerprint, with a 99%-interval of confidence, and average distance of the Pb isotopic fingerprint respect to the mean fingerprint of ammunition for the ratios $^{207/206}$Pb and $^{208/206}$Pb, according the study area. Differences among study areas have been determine by Tukey test.

When comparing the distance of Pb isotopic fingerprint in liver samples with the mean fingerprint of ammunition for the ratios $^{207/206}$Pb and $^{208/206}$Pb, separately, livers from areas with oil extraction showed a larger Pb isotopic displacement in both axis from the mean lead-ammunition fingerprint towards the mean lead-oil fingerprint compared to livers collected in areas without oil activities (0.0079 ± 0.0107 vs. 0.0016 ± 0.0042 units $^{207/206}$Pb, $P<0.0001$; and 0.0145 ± 0.0192 vs. 0.0031 ± 0.0082 units $^{208/206}$Pb, $P<0.0001$, respectively, Table 3.3).

Results from three lineal regressions with Pb isotopic fingerprints of (a) soil samples from salt licks, (b) anthropogenic Pb sources (oil and ammunition), and (c) liver collected only in the oil extraction areas show that regressions were parallel, but that of liver samples was much closer to that of anthropogenic Pb sources than to that of soils (Fig. 3.3). The greater isotopic similarity between biological samples and anthropogenic sources suggests Pb from ammunition and oil to have a stronger presence on samples from game species in oil extraction areas than Pb from soils.

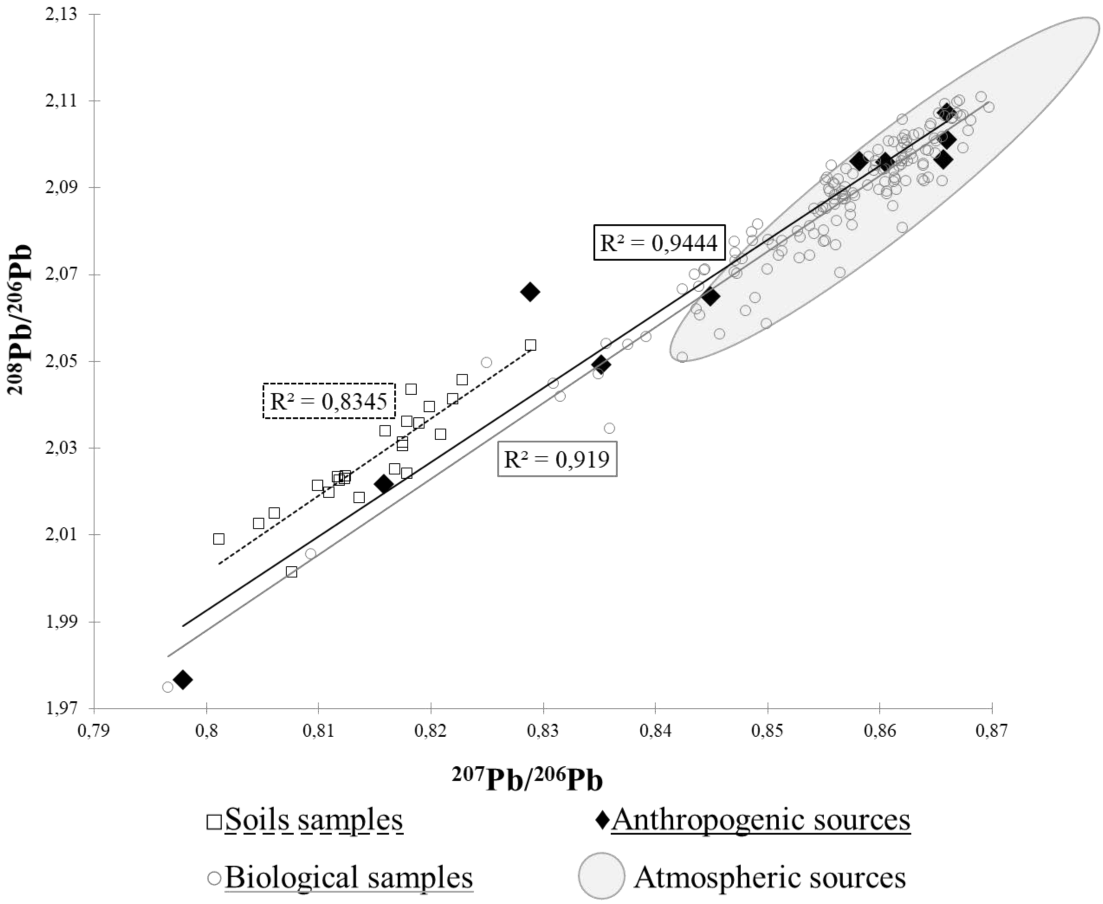


Figure 3.3 | Regression lines of lead sources' fingerprints.
Note: Pb isotopic distribution and regression lines of liver samples, soils from salt licks and Pb anthropogenic sources (ammunition and oil samples). The Pb isotopic composition of aerosols from South America (Brazil, Argentina and Northern Chile) in the period 1994–1999 retrieved from Bollhöfer and Rosman (2000) is shown in grey.

For samples from oil extraction areas, Pb concentrations in wildlife were correlated with the $^{207/206}Pb$ and $^{208/206}Pb$ isotopic ratios. There was a weak, but statistically significant positive correlation between liver Pb concentrations and its isotopic fingerprint: the higher the Pb level, the closer the isotopic ratio to ammunition fingerprint ($^{207/206}Pb$: r=-0.321, P<0.0001; $^{208/206}Pb$: r=-0.327, P<0.0001, Figure 3.4).

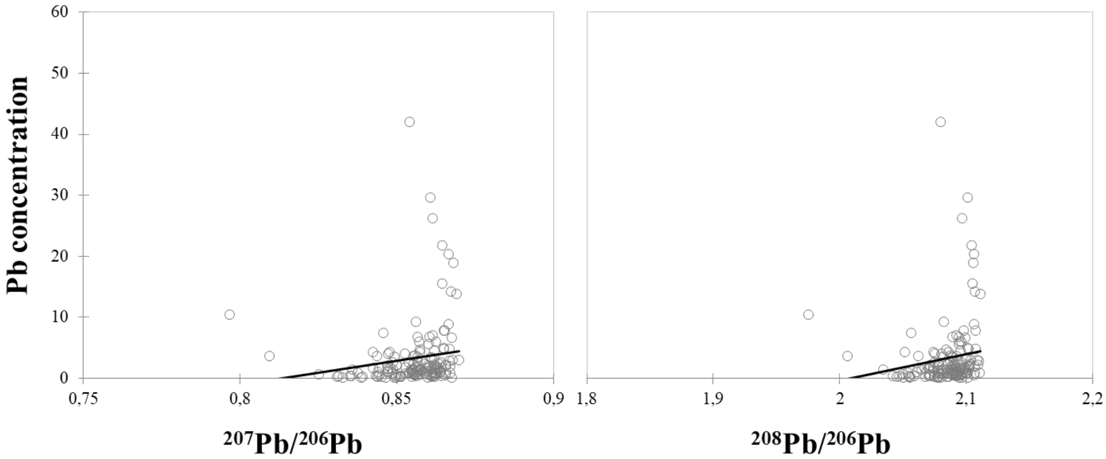


Figure 3.4 | Correlation between lead concentrations and lead isotopic ratios.
Note: Pb concentration in liver samples positively correlate with its isotopic fingerprint.

DISCUSSION

CAVEATS AND LIMITATIONS

One of the main limitations of this subchapter is the lack of soil samples from not-salt lick areas. Since all soil samples have been collected in natural or oil-polluted salt licks, and all these sites are usually visited by hunters, the isotopic fingerprint of geologic Pb cannot be determined, as the absence of lead-based ammunition pollution in the soil samples cannot be guaranteed and the observed Pb fingerprint in soils might be the mixture of both Pb sources: lead-based ammunition and geological Pb.

Another limitation of this subchapter is the reduced oil pollution samples (oil and liquid arising from oil infrastructure samples). Because of the high heterogeneity of Pb isotopic fingerprint found among oil samples, the sample set used in this chapter might be too small for being totally representative. Thus, these results must also be interpreted with caution.

Finally, the methodology used in the collection of animal tissues did not allow the collection of samples not exposed to the deposition of ammunition fragments in animals' tissues during the shot, since all animals where hunted using lead-based ammunition. This limits the capacity of this study to interpret the importance of exposure to Pb sources for alive wildlife (i.e., before the shot). In other words, the "original" Pb fingerprint in alive animals' liver tissues might be masked by the deposition of ammunition fragments during the shot.

LEAD CONCENTRATIONS IN AMAZONIAN WILDLIFE

High Pb concentrations were found in liver samples from wildlife from four remote areas in the Amazon. Comparable results in previous literature refer only to wild game from industrial countries, and mostly relate to wild boar and different deer species (Sevillano, 2013). Results from these studies show that wild boar's and deer's livers had average Pb concentrations of 0.82 µg/g and 0.43 µg/g WW, respectively, ranging from 0.05 to 2.6 µg/g. Pb average concentrations among Amazonian Artiodactyls (mean: 1.46 µg/g WW and median: 0.37 µg/g WW) in this study are similar to values reported for game in industrialized countries. Similarly, liver samples from the studied Amazonian wild tinamou had median Pb concentrations (1.71 µg/g) comparable to values found in liver samples from Tinamidae species in Andean mining areas (~1.34 µg/g) (Garitano-Zavala *et al.*, 2010).

When comparing these data with the maximum safe levels for certain contaminants in foodstuffs for human consumption set by the UE Commission Regulation 1881/2006, results show that about half, 49.8%, of the biological samples studied had Pb concentrations above acceptable limits of offal[15] for human consumption (0.5 µg/g WW), and 90.8% of them exceed the acceptable limits for meat[16] for human consumption (0.1 µg/g WW). In fact, the European Food Safety Authority and the World Health Organisation notice that there are no safe levels of Pb intake for human (EFSA CONTAM, 2010; World Health Organization (WHO), 2017). Nevertheless, all animals whose livers have been analysed in this chapter were consumed by the local population. Moreover, wild game in the area is not only a main source of animal protein, but also an important source of cash, as the meat is also sold in local and urban markets (Bodmer and Lozano, 2001), and for which Pb exposure through wild meat consumption exceeds the scope of subsistence economy reaching urban areas.

[15] The UE Commission Regulation 1881/2006 refers to offal of bovine, ovine, pigs and domestic poultry.
[16] The UE Commission Regulation 1881/2006 refers to bovine, ovine, pigs and domestic poultry meat.

LEAD ISOTOPIC FINGERPRINT IN AMAZONIAN WILDLIFE

The distribution of the biological samples' and putative Pb sources' isotopic fingerprints (Fig.3.2) suggests the existence of diverse sources of Pb exposure.

Overall, data suggest that the role of anthropic Pb sources varies among study areas. On the one side, the elevated match between biological and ammunition samples' isotopic fingerprint, both in the areas with and without oil extraction, suggests that lead-based ammunition is a main source of Pb for wildlife. On the other side, livers from oil extraction areas present a much lower match between biological and ammunition samples' isotopic fingerprint and a larger Pb isotopic displacement from the lead-ammunition fingerprint towards the lead-oil fingerprint compared to livers collected in areas without oil activities, thus suggesting oil-pollution as a major source of Pb in oil extraction areas. Thus, in the Corrientes River basin, 61.1% of liver samples had an isotopic fingerprint outside of the ammunition isotopic area. In fact, 53% of produced water has been discharged in the Corrientes River basin (in contrast, 6% has been discharged into the Pastaza River basin) (Yusta-García *et al.*, 2017). On the contrary, in Yavarí and Pucacuro River basins only 12.7% and 15.3% of the samples did not match ammunition isotopic area. Consequently, both ammunition and oil seem to be important sources of Pb in wild game in the oil extraction areas. These results show that ammunition is the main source of Pb for wildlife in all studied areas, and that oil spills is another important concomitant Pb source in oil extraction areas. Consistently, Pb was found in liquids dropping from oil infrastructure and oil samples from an abandoned oil well and oil spills. Likewise, in oil extraction areas, oil-polluted soils from salt licks had higher Pb concentrations than soils from natural salt licks, also pointing to the importance of oil pollution as a Pb source.

Moreover, the positive correlation observed between Pb concentrations and isotopic fingerprints similar to those of lead-ammunition suggests that ammunition is probably masking the Pb pollution caused by oil spills on wildlife in oil extraction areas.

LEAD-BASED AMMUNITION: EXPOSURE WAYS AND RISKS

In the last decades, research interest on topics related to the health impacts of environmental changes associated to the use of lead-based ammunition has significantly increased, with researchers expressing concerns over the use of Pb in hunting practices (Group of Scientists, 2013, 2014; Arnemo *et al.*, 2016). Pb from ammunition may reach organs of hunted animal through different ways (see Pain, Cromie, & Green, 2014, Fig. S3.1). One route is by direct contact with a Pb shot pellet or bullet. Tissues surrounding the ammunition pathway are the most exposed to Pb. However, disintegration of bullets and gunshots into independently moving parts, sometimes not visible to the naked eye, also occur, and Pb pollution can be found in distant tissues, such as 30 cm away from the bullet pathway (Scheuhammer *et al.*, 1998; Tsuji *et al.*, 1999; Tavecchia *et al.*, 2001; Hunt *et al.*, 2004; Dobrowolska and Melosik, 2008; Pain *et al.*, 2010).

The intentional or accidental ingestion of Pb ammunition by wild fauna could also be an indirect route for Pb pollution in wild animals. Such ingestion might be due to wild animals mistaking Pb shots by berries, seeds and other food items or grit (Lewis *et al.*, 2001; Kreager *et al.*, 2008), swallowing Pb fragments or Pb dust through soil ingestion (geophagy) (Beyer and Fries, 2002), and/or tasting of Pb salts in the surface of oxidized chunks (Lewis *et al.*, 2001).

Scavengers, predators, including humans, but also the whole ecosystem can be exposed to Pb through the ingestion of polluted animals or by the consumption of lead-polluted soil, water, and vegetation (Pain *et al.*, 1993, 2014; Kendall *et al.*, 1996; Scheuhammer and Norris, 1996; Hunt *et al.*, 2004; Fisher *et al.*, 2006; Mateo *et al.*, 2014). This poisoning might be amplified by the entrance of Pb in the food chain and its bioaccumulation. In that sense, Fustinoni *et al.* (2017) and Knutsen *et al.* (2014) reported a significant association between the ingestion of game meat hunted using lead-based ammunition and increased blood Pb concentrations.

Many European food safety and risk assessment agencies recommend the removal of meat along the bullet channel in a radius of 10-30 cm. However, they also emphasise that removal does not guarantee the expulsion of the Pb content (Knutsen *et al.*, 2014). Nevertheless, ethnographic observations in the area show that the removal of meat along the bullet channel is not at all a habitual practice and all soft tissues are systematically eaten, which increases the risk of Pb exposure for the studied population.

Since 100-300 years are needed to convert the whole metallic Pb bullet into Pb compounds (Jørgensen and Willems, 1987), the ecosystem might have accumulated lead-pellets scattered in the environment, resulting in a progressive increase of Pb concentration in soil, water and vegetation close to salt licks, where hunting is more frequent. This increase of Pb concentration is exacerbated in high hunting pressure areas, as it has been documented in other small areas like shooting ranges (Ma, 1989; Stansley and Roscoe, 1996; Cao *et al.*, 2003), locations of high hunting pressure (Castrale, 1989; Best, Garrison and Schmitt, 1992; Best, Garrison, Schmitt, *et al.*, 1992; Buck, 1998), or metal smelters (Beyer *et al.*, 1985). The same process may be occurring in salt licks frequently used as hunting grounds. Thus, hunting in salt licks increases wildlife and human Pb exposure and can become a public health risk factor.

Although widespread introduction of firearms and Pb shot in the Amazon took place only in the early 20th century, the daily use of shotguns for subsistence hunting was not widespread until the last decade of the 20th century (Monsalve, 2009). Findings presented here suggest that lead-based ammunition has resulted in widespread Pb pollution even in remote areas of the Amazon, becoming -in just few decades- an important threat for Amazonian wildlife and the human populations that rely on subsistence hunting. Fortunately, viable alternatives to lead-based ammunition are already available (Gremse and Rieger, 2014), although vested political and economic interests are important impediments for the phasing out of lead-based ammunition (Arnemo *et al.*, 2016).

LEAD FROM OIL EXTRACTION: OIL INGESTION BY WILDLIFE

In two of the study regions wildlife Pb contamination is compounded by the occurrence of oil extraction activities and the documented widespread spillage for decades of produced waters with elevated Pb concentrations (Yusta-García *et al.*, 2017). The Pastaza and Corrientes River basins overlap with two oil concessions, which were first leased in the late 1960s. Inadequate extraction and remediation practices have led to the regular dumping of produced waters into the local streams and rivers. For instance, in 2008, 939,000 barrels of produced waters were daily released into the environment by the two oil concessions that overlap the Pastaza and Corrientes River basins and with them, 12.88 tons of Pb were annually dumped into the waterways (Yusta-García *et al.*, 2017). Consequently, the spillage of produced waters from oil extraction activities has been a significant anthropogenic source of Pb in the Northern Peruvian Amazon during the 1971–2009 period (Yusta-García *et al.*, 2017). In the study areas, the reinjection of produced waters began in 2009 (Orta-Martínez and Finer, 2010). Analogous operational practices have been common in other oil extraction concessions in the Amazonian rainforests, 733,414 Km^2 of which were covered in 2014 with oil exploration and extraction concessions (Finer *et al.*, 2015).

As mentioned, in the studied oil extraction areas (Pastaza and Corrientes River basins), the large displacement of the Pb isotopic fingerprint in liver samples towards oil fingerprint confirms the concomitant presence of another Pb source related to oil extraction. Consistently, oil polluted salt licks had higher Pb concentrations than natural salt licks in the same regions, suggesting that the Pb increment might be caused by the input of oil spills in the soil. Since geophagy is a common behaviour among Amazon wildlife in salt licks, the Pb isotopic fingerprint analysis suggests that wildlife is ingesting oil products from the soil. In fact, in Chapter II, the ingestion of oil-polluted soils by wildlife was documented. This behaviour could constitute an important risk exposure to other oil-related contaminants, such as many polycyclic aromatic hydrocarbons (PAHs) and the metals Cd, As or hexavalent Cr, that are documented to be carcinogenic, probably carcinogenic, or possible carcinogenic, with some of them also potentially causing multisystem alterations (IARC, 1988, 2012; Mayor *et al.*, 2014; O'Callaghan-Gordo *et al.*, 2016). In other words, the presence of oil-related Pb in wild animals' tissues is worrisome not only for the harmful effects of Pb itself, but also because it suggests the potential presence of other oil-related pollutants.

Moreover, the consumption of oil-polluted wildlife may have alarming health implications for local human populations who depend on subsistence hunting in areas where oil extraction occur. Consistently, in the Corrientes River basin, both DIGESA (2006) and Anticona, Bergdahl, and San Sebastian (2012b, 2012a) found high blood Pb levels in human communities. Despite the fact that they could not determine the Pb source, Anticona *et al.* (2011, 2012a, 2012b) did not found associations between Pb blood concentrations and oil extraction activities (apart from that oil activities could favour the access of human populations to metal Pb from the industry's wastes) and suggested traditional practices such as hunting, fishing or other activities involving the manipulation of Pb as possible risk factors to human populations. Findings presented here suggest that a plausible pathway for the Pb poisoning detected in those human populations could be the ingestion of game meat polluted either through lead-based ammunition and oil pollution, although more research to stablish the association is needed.

LEAD IN SOILS AND LONG-RANGE ATMOSPHERIC LEAD TRANSPORT

This study was conducted in remote areas of the Amazon, where industrial activities, besides oil extraction, are very scarce or inexistent. Nevertheless, the presence of Pb concentrations in natural salt licks was also observed, without differences between areas with or without oil extraction. Pb is a natural component of the Earth's crust (Cheng and Hu, 2010) and long-range atmospheric transport has been proved to increase Pb natural content in soils even in such remote locations as Greenland, the Bolivian Andes, New Zealand and Antarctica (Candelone and Hong, 1995; Correia et al., 2003; Halstead et al., 2000; Ikegawa et al., 1999). In areas without oil extraction, 14% of liver samples (12.7% and 15.3% in Yavarí and Pucacuro, respectively) showed a distinct Pb isotopic fingerprint than that of ammunition, suggesting a minor effect of "naturally" occurring Pb in soils on Pb concentrations in free-ranging wildlife.

Soils incorporate the signal of long-range atmospheric Pb transported and express the mixed geological and atmospheric effect. However, the average Pb isotopic signature described by Bollhöfer and Rosman (2000) from aerosol samples collected in South America was dissimilar to that found in soil samples studied in this chapter (Fig. 3.3). The isotopic fingerprint of the soil samples analyzed seems to reflect the influence of, at least, three endmembers: the Peruvian/Mexican ores, the Mississippi Valley–type Pb used in U.S. gasoline, and a third endmember not identified yet (Bollhöfer and Rosman, 2000; Eichler *et al.*, 2015), reflecting possible atmospheric inputs from the exploitation of polymetallic deposits of the Andean Altiplano and from leaded gasoline. Indeed, the most marked increase in Pb of the past 2000 years took place from the 1960s to 1985, coinciding with the intensification of the use of leaded gasoline in South America (Eichler *et al.*, 2015).

CONCLUSIONS

Many Amazonian indigenous societies depend on subsistence hunting and fishing and the extraction of natural resources for their livelihood. Thus, wild meat is still a key element in Indigenous Peoples' diet and account for a high percentage of daily protein intake (Robinson and Bennett, 2000; Reyes-García *et al.*, 2018). Estimates of annual wild meat harvest report the harvest of 113,000 animals in the Northern Peruvian Amazon (Bodmer and Lozano, 2001) and 89,224 tons in the Brazilian Amazon (Peres, 2000). However, wild meat harvest is not exclusive to the Amazon region; for instance, 23,500 tons of wild meat were consumed in Northwest Borneo (Bennett, 2002), and 1 million–3.4 million tons in Central Africa (Fa *et al.*, 2002).

In recent years, hunting pressure has increased dramatically due to various causes, such as growth of human populations, better access to remaining forests, commercialization of wild meat, increasing use of efficient modern hunting technologies and erosion of traditional hunting institutions due to rapid cultural change. Furthermore, wild meat can also be found in urban markets in forest areas. In the Northern Peruvian Amazon, 7,392 animals (6.5% of total harvest of mammals) were sold in Iquitos during 1996 (Bodmer and Lozano, 2001). Therefore, Pb exposure through the consumption of wild meat exceeds the scope of subsistence economy, and may expose tropical urban populations to Pb contamination.

The effect that the ingestion of lead-polluted soil by Amazonian wildlife may have on wildlife and human health is still understudied. However, it could potentially be hazardous for the conservation of top predators in the tropical rainforests and the health of local human populations, Indigenous and Riverine that rely on subsistence hunting.

This study illustrates how remote natural areas are affected by yet another ubiquitous anthropogenic footprint, Pb exposure. Such footprint might be extended throughout the Amazonian ecosystem illustrating how human impact reaches further than usually envisaged and suggesting the expansion of anthropogenic contaminants from industrial centres to most remote areas. These findings should trigger urgent action focused on diminishing Pb pollution in the Amazon and promoting the health care of the exposed populations. For instance, the removal of lead-based spent shots found in the salt licks soil and the use of other not lead-based ammunition for hunting purposes should be a key step towards the reduction of Pb exposure. Actually, there is a resounding consensus in the scientific community about the importance and need of a strict and wide ban on lead-based ammunition (Tsuji *et al.*, 1999; Dobrowolska and Melosik, 2008; Group of Scientists, 2013, 2014; Knutsen *et al.*, 2014; Arnemo *et al.*, 2016).

SUBCHAPTER B • DRIVERS OF LEAD CONTENT VARIABILITY FOR AMAZONIAN WILDLIFE

DATA ANALYSIS

The aim of subchapter B is to further understand the pathways of wildlife Pb exposure by studying Pb concentrations variability in Amazonian wildlife according to the following potentially confounding variables: oil-activity, basin, species (interspecies differences in metabolism and kinetics), body mass, habitat, and trophic level. To do so, a hypothesis driven analysis was performed.

HYPOTHESES DEFINITION

The main goal of this thesis focus on the study the ingestion of oil-polluted soil and water by Amazonian wildlife. In Chapter II it has been shown that this is a common behaviour among many species (frugivorous and herbivorous mammals and birds), and in the previous subsection of this Chapter III lead-based ammunition and oil have been pointed out as important sources of Pb in wildlife tissues in the study area. Moreover, it was found that ammunition and oil had a different weight as source of Pb for Amazonian game animals sampled in areas with ongoing oil extraction activities and without them. Here, the variability of Pb concentrations in oil extraction and control areas is explored (Hypothesis 1). Moreover, this subchapter also aims to identify the importance of the different Pb exposure pathways for wildlife (i.e., ingestion of Pb versus deposition of Pb fragments during the shot). To evaluate the importance of Pb ingestion as an exposure pathway, Pb biomagnification in higher trophic levels is tested (Hypothesis 2); to study the pollution of animal tissues by lead-based ammunition during the shot, Pb levels in small animals are compared with those in larger ones (Hypothesis 3).

Hypothesis 1: **Samples collected in areas that overlap with oil extraction activities (i.e., the Corrientes and Pastaza River basins) will present higher Pb levels than samples collected in areas without such overlap (i.e., the Yavarí and Pucacuro River basins)**.

Rationale: Shotguns and lead-based ammunition are commonly used for subsistence hunting in the four study areas, but the areas also present important differences that might affect wildlife exposure to oil-related Pb, since the Pastaza and Corrientes Rivers basins have been overlapped by oil concessions for more than 45 years, whereas the Pucacuro and Yavarí River basins have not ever been affected by any industrial activities. As previously mentioned, Pb (among other heavy metals) can be found in oil extraction by-products and wastes. Indeed, produced water dumping from oil extraction activities was a significant anthropogenic source of Pb in the Northern Peruvian Amazon Rivers during 1987–2009 (Yusta-García *et al.,* 2017). Consequently, exposure to oil extraction activities might increase wildlife exposure to Pb. Thus, here it is hypothesized that Pb levels in liver will be higher in samples collected in the study areas overlapped by oil concessions than in control areas. Given the lack on data about other factors that might generate differences on the exposure to lead-based ammunition for wildlife between the study areas (e.g., hunting pressure, human density, or historical use of shotguns), it is assumed that the exposure to lead-based ammunition is similar in the four study sites. To test hypothesis #1, the categorical variables ***basin*** (i.e., Yavarí, Pucacuro, Pastaza, and Corrientes) and ***oil*** (i.e., control areas - Yavarí and Pucacuro- and areas overlapped with the oil concession 192/1AB -Pastaza and Corrientes-) were created.

Hypothesis 2: **Carnivorous species will present higher Pb concentrations than frugivorous and herbivorous species.**

Rationale: Based on the literature, there are two potential routes of exposure to Pb for wildlife: a) Pb ingestion and b) deposition of metal in the carcasses of the hunted animals from bullet/pellets fragmentation during the shot. It is assumed that voluntary or accidental Pb ingestion is a common pathway to lead-exposure among wildlife species and that Pb bioaccumulates in body tissues, travels through the food chain, and biomagnifies in the food web.

Amazonian herbivorous and frugivorous wildlife may ingest spent ammunition and bullet fragments. Birds commonly mistake ammunition for food items or grit (Lewis *et al.*, 2001; Kreager *et al.*, 2008). Wildlife may also be ingesting lead-polluted soil (as shown in Chapter II), water, vegetation, and animals (Pain *et al.*, 1993, 2014; Kendall *et al.*, 1996; Scheuhammer and Norris, 1996; Hunt *et al.*, 2004; Fisher *et al.*, 2006; Mateo *et al.*, 2014). As it has been mentioned before, that process is amplified in high hunting pressure areas, where spent ammunition and ammunition fragments accumulate. Such effect has been found in shooting ranges (Ma, 1989; Stansley and Roscoe, 1996; Cao *et al.*, 2003), wetlands, (Castrale, 1989; Best, Garrison and Schmitt, 1992; Best, Garrison, Schmitt, *et al.*, 1992; Buck, 1998) or around metal smelters (Beyer *et al.*, 1985), and it may be happening the same in Amazonian salt licks.

The ingested Pb may bioaccumulate and travel through the food chain. Despite Pb can be taken up from the intake of air and water, the most usual exposure pathway to Pb for mammals is via the food chain (Ma, 1996). Once Pb is ingested by an animal, the gastrointestinal absorption of Pb takes place in the duodenum and the efficiency in absorption will depend on many factors, including the chemical form and the quantity of the ingested Pb, the animal's age and health and its levels of other dietary constituents, among others (National Research Council, 2005). For mammals, the absorption rate for inorganic Pb through ingestion ranges from 2 to 20% (Ma, 1996). Once absorbed, Pb enters the blood and it is mainly taken up by red blood cells and reaches the soft tissues, where it mostly binds with proteins. Over time, Pb accumulates in the bones, where it replaces calcium during the mineralization processes and it is released to the blood or milk during bone resorption. Resorption is increased during pregnancy, parturition, osteoporosis, prolonged immobility or infection. Pb can also be transferred via placenta to foetus (National Research Council, 2005). The half-life of Pb in different tissues is variable among species. In adult humans, the half-time of Pb in blood and soft tissues is around 1 month (National Research Council, 2005), while in the remaining mammals it has an average half-life of 7.4 days in liver and kidney and 4.6 days in milk (Ma, 1996). Contrastingly, in hard tissues such as bones or teeth, Pb has a half-life in the order of 10 to 20 years (Ma, 1996). Pb may be extracted by both urinary and intestinal excretion routes, although the relative importance of this routes varies among species (National Research Council, 2005).

The second exposure route, the deposition of ammunition fragments in animal tissues during the shot, has been broadly documented in radiographs studies for diverse types of ammunition including shot pellets, several types of bullets, and shotgun slugs

(Scheuhammer *et al.*, 1998; Tsuji *et al.*, 1999; Tavecchia *et al.*, 2001; Hunt *et al.*, 2004, 2006, 2009; Dobrowolska and Melosik, 2008; Grund *et al.*, 2010; Pain *et al.*, 2010). For instance, Hunt et al., (2006) found ammunition fragments in both carcasses and offal piles of hunted animals, and Dobrowolska & Melosik (2008) found that ammunition fragments might penetrate deep into the animal bodies, up to 30 cm away from the bullet pathway. Based on the broad evidences provided by the scientific community, many European food safety and risk assessment agencies recommend the removal of meat for consumption along the bullet channel in a radius of 10-30cm. However, they emphasise that this removal do not always guarantee the expulsion of all the Pb content (Knutsen *et al.*, 2014). In this kind of animals' "post-mortem" exposure, human consumers are the most affected by Pb poisoning, while animals from higher trophic levels are expected to be less exposed to Pb pollution through this second exposure pathway. Therefore, the biomagnification (increment of Pb levels from one link in a food chain to another) of Pb in animals from higher trophic levels due to this second exposure route is also expected to be much lower or even negligible in comparison with the biomagnification related with the first mentioned exposure route. However, predators and scavengers can be exposed to this pollution through the ingestion of polluted animals' viscera discarded by hunters in the forest or through the ingestion of animals that have been injured but survived (Stauber *et al.*, 2010; Pain *et al.*, 2014).

Most previous research has highlighted Pb bioaccumulation in body tissues (showing that Pb concentrations in the tissues are higher than those in the environment). However, Pb biomagnification is a matter of debate and some authors claim that it does not take place in aquatic environments (Demayo *et al.*, 1982; Sydeman and Jarman, 1998; Chen and Folt, 2000; Barwick and Maher, 2003; National Research Council, 2005; Agengy for Toxic Substances and Disease Registry, 2007; Cui *et al.*, 2011; Cardwell *et al.*, 2013), although recent research suggested Pb biomagnification among aquatic invertebrate predators (Rubio-Franchini *et al.*, 2008; Rubio-Franchini and Rico-Martínez, 2011). Another recent study found higher Pb levels in bald eagles than in other birds at lower trophic levels (Burger and Gochfeld, 2009).

In this subchapter, to test the hypothesis 2, samples were classified in three groups according to the ***feeding pattern*** of the sampled species: Carnivorous, frugivorous, and herbivorous (Table 3.5).

Hypothesis 3: **Pb levels in liver samples from species with a small body mass will be larger that Pb levels in liver samples from species with a larger body mass**.

Rationale: Dobrowolska and Melosik (2008) analysed Pb concentrations in muscle tissues of wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) harvested by hunters in Poland. The authors concluded that muscle tissue closer to wounds had higher Pb concentrations. Other authors found similar results (Scheuhammer *et al.*, 1998; Tsuji *et al.*, 1999; Tavecchia *et al.*, 2001; Hunt *et al.*, 2004; Dobrowolska and Melosik, 2008; Pain *et al.*, 2010), implying that the closer the liver to the shoot, the higher the Pb concentration found in the liver. Therefore, the smaller the animal is, the larger the Pb levels in its liver, as the distance to the shoot will be always smaller than in larger animals. In other words, the probabilities of deposition of ammunition fragments in the liver tissue will be larger in small than in big animals. It is important to keep in mind that in the study area all hunters used the same ammunition (with the same fragmentation patterns) to harvest all the species. To test this hypothesis, the categorical explanatory ***body mass***[17] was created and species sampled were classified into three groups: low (weight < 5 Kg), medium (5 Kg ≤ weight ≤ 10 Kg) and high body mass (weight > 10 Kg).

Additional analysis:

To better interpret the results for the aforementioned hypothesis, the relationship between Pb levels and other factors that might influence the liver Pb content (i.e., the taxonomical order and the species of the sample and its habitat) has also been tested.

The species' habitat might influence the Pb levels found in liver samples, as Pb concentrations might be different in different habitats. Thus, the exposure route to this heavy metal might be different among terrestrial, arboreal and aquatic species. To test the association between species habitat and Pb levels, the sample was divided into three categories for the variables ***habitat*** according to whether species were arboreal, aquatic and terrestrial (Table 3.5).

The association between Pb content and the taxonomical order and the species to which the samples belong was also tested. Species differences not reflected in the habitat, feeding patterns, and body mass (e.g., the ingestion of grit, differences in metabolism and biokinetics) might determine the Pb content found in the animals' tissues. For this reason

[17] Body mass data has been obtained from literature (Aquino *et al.*, 2001; Escobedo and Rios, 2004; Mayor *et al.*, 2015).

it is expected to detect important variations among samples belonging to different species and orders. Samples were divided into 11 categories for the explanatory variable ***order*** and 18 categories for the explanatory ***species*** (Table 3.5).

Despite in this subchapter it would have been interesting to test whether geophagy is an exposure pathway to oil-related Pb and lead-based ammunition, this analysis could not be performed because all the frugivorous and herbivorous collected species (or species from the same family) have been reported visiting salt licks (Izawa, 1993; Montenegro, 2004; Blake *et al.*, 2011). A more detailed analysis examining the association of amount of soil ingested (ingesta) and Pb concentrations could neither be carried out because of the scarcity of data on the available literature.

Table 3.5 | Order, body mass, feeding pattern and habitat of the studied species.

	Body mass Kg	Body mass Category	Feeding	Habitat
Anura				
Leptodactylus pentadactylus	1	Low	Aquatic	Carnivorous
Artyodactyla				
Mazama americana	33	High	Terrestrial	Herbivorous
Tayassu pecari	33	High	Terrestrial	Frugivorous
Tayassu tajacu	25	High	Terrestrial	Frugivorous
Carnivora				
Nasua nasua	5	Medium	Arboreal	Carnivorous
Potos flavus	3	Low	Arboreal	Carnivorous
Crocodilia				
Caiman crocodilus	12.5	High	Aquatic	Carnivorous
Galliformes				
Penelope jaqcuaqu	2	Low	Terrestrial	Frugivorous
Gruiformes				
Psophia leucoptera	1.3	Low	Terrestrial	Frugivorous
Perissodactyla				
Tapirus terrestres	160	High	Terrestrial	Frugivorous
Primatia				
Alouatta seniculus	6.5	Medium	Arboreal	Frugivorous
Cebus albifrons	3	Low	Arboreal	Frugivorous
Cebus apela	3.5	Low	Arboreal	Frugivorous
Lagothrix sp	8.71	Medium	Arboreal	Frugivorous
Psittaciformes				
Ara sp.	1	Low	Arboreal	Frugivorous
Rodentia				
Cuniculus paca	9	Medium	Terrestrial	Herbivorous
Dasyprocta fuliginosa	5	Medium	Terrestrial	Herbivorous
Tinamiformes				
Tinamous major	1.17	Low	Terrestrial	Frugivorous

Notes: Own elaboration based on (Emmons and Feer, 1997; Aquino *et al.*, 2001; Escobedo and Rios, 2004; Mayor *et al.*, 2015).

DATA ANALYSIS

Log transformation of Pb concentrations (*LnPbDW*) in liver samples was performed to approach the assumptions of normality and homoscedasticity. A bivariable analysis was performed using one-way ANOVA and Tukey test to study the independent relation of each of the aforementioned variables with the log-transformed Pb concentration

(*LnPbDW*) found in liver samples. A detailed analysis for the variates *basin* and *oil* was also performed for the species *Cuniculus paca,* the species with most balanced data availability among the four study sites. Furthermore, a second bivariate analysis for the variable *feeding* has been performed, grouping the categories herbivorous and frugivorous.

A multivariate analysis including different combinations of explanatory variables was performed to determine which of the variables had the largest impact on Pb concentration. Ten different Linear Models (LMs) were created by combining the different variables and their interactions and testing their association with Pb concentration (Table 3.5). Following Lehikoinen *et al.* (2016), the candidate models were fitted to Linear Models (LMs) using the function "lmer" from the "lme4" R package and were controlled for the mixed model fitting using the "lmerControl" function with optimizer BOBYQA. Kenward–Roger approximation was used to obtain the parameter specific p-values. The Akaike information criterion (AIC) values were used as an estimator of the relative quality of the different statistical models. These values were calculated following Burnham and Anderson (2002, in Lehikoinen *et al.*, 2016). The model with the lowest AIC value and the models with a similar AIC (AIC differences <2.00) are considered as the models with higher relative quality. The Akaike information criterion considers both the goodness of fit of the model and the simplicity of the model, by penalizing overfitting.Following Burnham and Anderson (2002, in Lehikoinen et al., 2016), a model validation has been performed for the top ranking models by analyzing the distribution of the residuals.

RESULTS

Animal liver samples collected in the four study areas had a *PbDW* median (IQR) of 2.00 µg/g (2.94 µg/g), ranging from 0.07 µg/g to 375.00 µg/g. 29 outliers, or samples with a *PbDW* > 8.38 (Table 3.3), have been observed, mostly including samples from Yavarí (n=13) and Pastaza (n=8), but also some from Pucacuro (n=4) and Corrientes (n=4). Outliers belong to the Artyodactyla (n=6), Rodentia (n=6), Galliformes (n=6), Tinamiformes (n=4), Carnivora (n=3), Gruiformes (n=2), Primatia (=1) and Crocodilia orders (n=1).

BIVARIATE ANALYSIS

Oil – *LnPbDW* mean concentration was significantly higher in samples collected in areas without oil extraction activities than in areas located inside the oil concession (ANOVA ($F(2,312) = 21.058$, $p < 0.001$, Table 3.6). Similarly, the bivariate analysis for *Cuniculus paca* showed a *LnPbDW* mean concentration significantly higher in *C. paca* samples collected in areas without oil extraction activities than in areas located inside the oil concession (ANOVA ($F(1,76) = 12.221$, $p < 0.001$, Table 3.7)

Table 3.6 | Pb concentration (DW) in samples collected in areas overlapping and not overlapping with oil extraction activities.

Oil	N	Min.	Max.	1st Q.	Median	3rd Q.	Tukey
No overlap with oil extraction	161	0.15	375.00	1.68	2.40	4.51	a
Overlap with oil extraction	154	0.07	42.00	0.74	1.50	3.41	b

Note: Categories sharing the same letter in the "Tukey" column do not differed significantly at $p < 0.05$ regarding *LbPbDW* mean values.

Table 3.7 | Pb concentration (DW) in *Cuniculus paca* samples collected in areas overlapping and not overlapping with oil extraction activities.

Oil	N	Min.	Max.	1st Q.	Median	3rd Q.	Tukey
No overlap with oil extraction	40	0.46	7.67	1.79	2.14	3.08	a
Overlap with oil extraction	38	0.07	13.85	0.41	1.22	2.28	b

Note: Categories sharing the same letter in the "Tukey" column did not differ significantly at $p < 0.05$ regarding *LbPbDW* mean values.

Basin - There was a statistically significant difference between basins' *LnPbDW* mean values as determined by one-way ANOVA ($F(3,311) = 8.405$, $p < 0.001$). Tukey test showed that samples collected in the Pucacuro and Yavarí basins presented significantly higher *LnPbDW* mean concentrations that those collected the Corrientes basin. The mean *LnPbDW* for samples collected in the Pastaza basin was only significantly different than that of Yavarí. In the analysis carried out only with *Cuniculus paca* samples, there was also a statistically significant difference in the *LnPbDW* mean values of samples collected from different basins', as determined by one-way ANOVA ($F(3,74) = 10.224$, $p < 0.001$).

Tukey test showed that *C. paca* samples collected in the Pucacuro, Yavarí and Pastaza River basins presented significantly higher *LnPbDW* mean concentrations that samples collected the Corrientes basin.

Table 3.8 | Pb concentration (DW) in samples according to collection *basin*.

Basin	N	Min.	Max.	1st Q.	Median	3rd Q.	Tukey
Pucacuro	59	0.15	46.00	1.82	2.78	4.64	a,b
Yavari	102	0.46	375.00	1.62	2.21	4.08	a
Pastaza	100	0.07	26.21	0.83	1.67	3.88	b,c
Corrientes	54	0.10	42.00	0.43	1.35	2.19	c

Note: Categories sharing the same letter in the "Tukey" column did not differ significantly at $p < 0.05$ regarding *LbPbDW* mean values. Note: Tukey test has been conducted for mean log-transformed Pb concentrations (*LnPbDW*), but this table show median values for not log-transformed Pb concentrations (DW).

Table 3.9 | Pb concentration (DW) in *Cuniculus paca* samples according to collection *basin*.

Basin	N	Min.	Max.	1st Q.	Median	3rd Q.	Tukey
Pucacuro	20	1.76	6.93	2.17	2.86	5.01	a
Yavari	20	0.46	7.67	1.50	1.80	2.03	a
Pastaza	22	0.07	13.85	0.87	1.55	4.42	a
Corrientes	16	0.11	6.75	0.30	0.44	1.20	b

Note: Categories sharing the same letter in the "Tukey" column did not differ significantly at $p < 0.05$ regarding *LbPbDW* mean values. Note: Tukey test has been conducted for mean log-transformed Pb concentrations (*LnPbDW*), but this table show median values for not log-transformed Pb concentrations (DW).

Feeding - The highest median Pb concentrations were found in samples from carnivorous species, but the difference in *LnPbDW* means among carnivorous, herbivorous and frugivorous species were not statistically significant, as determined by one-way ANOVA ($F(2,312) = 0.805$, $p=0.448$). The difference in *LnPbDW* means among the two categories -carnivorous on one side and herbivorous and frugivorous on the other- was also not statistically significant ($F(1,313) = 0.365$, $p=0.365$).

Table 3.10 | Pb concentration (DW) in biological samples according to *feeding pattern*.

Feeding	N	Min.	Max.	1st Q.	Median	3rd Q.
Carnivorous	30	0.11	29.58	1.61	3.30	4.11
Herbivorous	124	0.07	375.00	1.00	1.97	3.50
Frugivorous	161	0.15	89.73	1.05	1.85	4.10

Body mass – There was a statistically significant difference between body mass groups' mean *LnPbDW* as determined by one-way ANOVA (F(2,312) = 6.380, p = 0.002). A post hoc Tukey test showed that species with a body mass lower than 5 Kg presented significantly higher mean *LnPbDW* values than species with a body weight higher than 10 Kg. The mean *LnPbDW* in the group of species with a medium body weight was not significantly different from the mean of the other two groups.

Table 3.11 | Pb concentration (DW) in biological samples according to *body mass*.

Body mass	N	Min.	Max.	1st Q.	Median	3rd Q.	Tukey
Medium: 5 Kg ≤ Weight ≤ 10 Kg	139	0.07	375.00	1.40	2.17	4.17	a,b
Low: Weight < 5 Kg	74	0.26	89.73	1.40	2.11	5.96	a
High: Weight > 10 Kg	102	0.08	199.45	0.75	1.55	3.10	b

Note: Categories sharing the same letter in the "Tukey" column did not differ significantly at $p < 0.05$ regarding *LbPbDW* mean values. Note: Tukey test has been conducted for mean log-transformed Pb concentrations (*LnPbDW*), but this table show median values for not log-transformed Pb concentrations (DW).

Habitat – There was no statistically significant differences between the Pb concentration found in samples taken from species living in different habitats, as determined by one-way ANOVA (F(2,312) = 1.173, p = 0.311).

Table 3.12 | Pb concentration (DW) in biological samples according to *habitat*.

Habitat	N	Min.	Max.	1st Q.	Median	3rd Q.
Arboreal	65	0.43	29.58	1.60	2.40	4.17
Aquatic	17	0.11	21.85	0.98	2.39	3.80
Terrestrial	233	0.07	375.00	0.93	1.85	3.70

Order - There was a statistically significant difference between orders' *LnPbDW* mean values as determined by one-way ANOVA ($F(10,304) = 2.626$, $p = 0.004$). Samples from the birds' orders Galliformes and Gruiformes and the Carnivora order showed the highest Pb median concentrations. Contrastingly, samples from Artyodactyla, Perissodactlya and Tinamiformes showed the lowest Pb median concentrations. The post-hoc Tukey test showed that the orders Artyodactyla, Perissodactyla, Rodentia and Crocodilia presented significantly lower *LnPbDW* values than the order Galliformes at $p<0.05$. The remaining orders did not present statistically significant differences among them nor with the aforementioned orders.

Table 3.13 | Pb concentration (DW) in samples according to the species' *order*.

Order	N	Min.	Max.	1st Q.	Median	3rd Q.	Tukey
Galliformes	13	0.64	89.73	2.04	5.65	20.66	a
Gruiformes	7	1.11	26.21	1.66	4.37	13.25	a,b
Carnivora	13	0.62	29.58	2.70	3.40	5.90	a,b
Anura	4	0.74	6.06	0.92	2.55	4.60	a,b
Crocodilia	13	0.11	21.85	1.60	2.39	3.61	b
Psittaciformes	3	1.30	4.51	1.70	2.11	3.31	a,b
Primatia	49	0.43	13.18	1.60	2.06	4.10	a,b
Rodentia	100	0.07	375.00	1.19	2.03	4.04	b
Tinamiformes	24	0.26	42.00	0.95	1.71	3.11	a,b
Perissodactyla	1	1.50	1.50	1.50	1.50	1.50	b
Artyodactyla	88	0.08	199.45	0.73	1.21	2.93	b

Note: Categories sharing the same letter in the "Tukey" column did not differsignificantly at $p < 0.05$ regarding *LbPbDW* mean values. Note: Tukey test has been conducted for mean log-transformed Pb concentrations (*LnPbDW*), but this table show median values for not log-transformed Pb concentrations (DW).

Species - Samples from the bird species *Penelope jaqcuaqu* and *Psophia leucoptera*, and the primate species *Alouata seniculus* showed the highest Pb median concentrations. On the contrary, the large ungulates *Tayassu tajacu*, *Mazama americana* and *Tapirus terrestris* showed the lowest Pb median concentrations. There was a statistically significant difference between species' *LnPbDW* mean values, as determined by one-way ANOVA ($F(17,297) = 2.671$, $p < 0.001$). The Tukey test showed that *LnPbDW* mean values of the species *Penelope jaqcuacu* were significantly higher than that of *Cuniculus paca, Tayassu pecari, Tapirus terrestris, Cebus apela, Mazama americana* and *Tayassu tajacu* at $p<0.05$. No statistically significant difference was found for the other species.

Table 3.14 | Pb concentration (DW) in biological samples according to the *species*.

Species	N	Min.	Max.	1st Q.	Median	3rd Q.	Tukey
Penelope jacquacu	13	0.64	89.73	2.04	5.65	20.66	a
Allouatta seniculus	2	2.22	6.79	3.36	4.50	5.65	a,b
Psophia leucoptera	7	1.11	26.21	1.66	4.37	13.25	a,b
Potos flavus	5	2.01	9.23	3.53	4.11	8.90	a,b
Cebus albifrons	7	1.60	13.18	2.40	4.10	6.02	a,b
Dasiprocta fuliginosa	22	0.39	375.00	2.23	3.55	6.98	a,b
Nasua nasua	8	0.62	29.58	2.40	3.10	4.03	a,b
Leptodactylus pentadactylus	4	0.74	6.06	0.92	2.55	4.60	a,b
Caiman crocodilus	13	0.11	21.85	1.60	2.39	3.61	a,b
Ara sp.	3	1.30	4.51	1.70	2.11	3.31	a,b
Lagothrix sp	29	0.43	7.38	1.72	2.08	4.17	a,b
Cuniculus paca	78	0.07	13.85	0.97	1.85	2.94	b
Tinamous major	24	0.26	42.00	0.95	1.71	3.11	a,b
Tayassu pecari	46	0.15	46.00	0.65	1.69	3.62	b
Tapirus terrestris	1	1.50	1.50	1.50	1.50	1.50	b
Cebus apella	11	0.54	2.79	0.96	1.50	1.90	b
Mazama americana	24	0.08	199.45	0.82	1.25	2.81	b
Pecari tajacu	18	0.22	28.18	0.84	1.09	1.67	b

Note: Categories sharing the same letter in the "Tukey" column do not differed significantly at $p < 0.05$ regarding *LbPbDW* mean values. Note: Tukey test has been conducted for mean log-transformed Pb concentrations (*LnPbDW*), but this table show median values for not log-transformed Pb concentrations (DW).

In sum, results from bivariate analysis suggest that there are statistically significant differences on *LnPbDW* mean values from biological samples with different body mass (P=0.002) and from areas with different exposure to oil extraction activities (P<0.001), but not according to species feeding patterns (p=0.311). Moreover, statistically significant differences according to the sampling basin (P<0.001), the species (P<0.001), and the order (P=0.004) have also been found, but not according to the species' habitat (p=0.448).

MULTIVARIATE ANALYSIS

Ten models have been created by combining the *basin*, *body mass*, *feeding* and *habitat* variables and their interactions (Table 3.15). The variables *order* and *species* were not included in the models since the sample was unbalanced regarding the number of observations from individuals in the different categories in these variables. *Oil* was not included either, since this variable is redundant with the variable *basin*.

Models 8 and 10 presented the lowest AICs values, for this reason they are the most accurate according to the AIC estimator for relative quality of models. Both models (8 and 10) indicate that the explanatory variables *basin, body mass* and *feeding* are the variables having a significant association with *LnPbDW*. Results from these models show that (1) samples from the Corrientes basin have significant lower Pb concentration than samples from the Pastaza, Pucacuro and Yavarí basins, being the basins that do not overlap with extractive activities (i.e., Pucacuro and Yavarí) those with higher Pb concentration values; (2) smaller animals (*body mass* < 5Kg) have significant higher Pb concentrations than larger animals (*body mass* > 10 Kg), although there is no clear pattern for medium-body mass animals; and (3) carnivorous species present higher Pb concentrations than herbivorous and frugivorous species, although the statistical significance of this finding is weak.

Table 3.15 | Results of the ten models explaining fluctuations in Pb concentrations in liver samples evaluated based on their AICc values.

Model	Description	dAIC	W
1	lnPbDW ~ basin + feeding + body mass + habitat	5.32	0.03
2	lnPbDW ~ basin + feeding * body mass + habitat	4.27	0.05
3	lnPbDW ~ basin + feeding * habitat + body mass	5.32	0.03
4	lnPbDW ~ basin + feeding + body mass * habitat	3.88	0.06
5	lnPbDW ~ basin + feeding * body mass * habitat	4.27	0.05
6	lnPbDW ~ basin + body mass + habitat	4.11	0.06
7	lnPbDW ~ basin + feeding + hábitat	21.31	0.00
8	**lnPbDW ~ basin + body mass + feeding**	**1.70**	**0.18**
9	lnPbDW ~ basin + body mass	2.78	0.11
10	**lnPbDW ~ basin + body mass + feeding^**	**0.00**	**0.43**

Note: *dAIC* is the AIC differences compared to the model with the lowest AIC value, and *W* are the AIC weights. Bold models are those with a higher relative quality, according to the AIC estimator. The symbol ^ in the variable *feeding* (i.e., Feeding^) indicates that herbivorous and frugivorous have been grouped into the same category for the analysis.

Table 3.16 | Coefficients and test values of the linear model 8 explaining changes in Pb concentrations in livers.

	Estimate	SE	t value	Pr(>|t|)	
(Intercept)	0.926	0.278	3.334	0.001	***
Basin - Pastaza	0.433	0.196	2.206	0.028	*
Basin - Pucacuro	1.150	0.232	4.963	0.000	***
Basin - Yavari	0.953	0.195	4.891	0.000	***
Body mass - Medium	-0.299	0.210	-1.422	0.156	
Body mass - High	-0.839	0.194	-4.325	0.000	***
Feeding - Frugivorous	-0.460	0.235	-1.956	0.051	
Feeding - Herbivorous	-0.554	0.255	-2.175	0.030	*

	Df	Df Sum Sq	Mean Sq	F value	Pr(>F)	
Basin	3	35.620	11.873	8.973	0.000	***
Body mass	2	26.540	13.268	10.028	0.000	***
Feeding	2	6.590	3.296	2.491	0.084	
Residuals	307	406.210	1.323			

Note: R^2 = 0.145. *Basin*=Corrientes, *Body mass*=Low and *Feeding*=carnivorous was defined as the intercept in the model. SE stands for Standard Error. Df stands for Degrees of freedom. Significance codes are the following: P < 0.001 (***), 0.001 ≤ P < 0.01 (**), 0.01 ≤ P ≤ 0.05 (*).

Table 3.17 Coefficients and test values of the linear model 10 explaining changes in Pb concentrations in livers.

	Estimate	SE	t value	Pr(>|t|)	
(Intercept)	0.467	0.200	2.335	0.020	*
Basin - Pastaza	0.424	0.195	2.171	0.031	*
Basin - Pucacuro	1.152	0.231	4.979	0.000	***
Basin - Yavari	0.950	0.195	4.883	0.000	***
Body mass - Medium	-0.366	0.168	-2.175	0.030	*
Body mass - High	-0.863	0.188	-4.588	0.000	***
Feeding^ - Carnivorous	0.492	0.227	2.169	0.031	*

	Df	Df Sum Sq	Mean Sq	F value	Pr(>F)	
Basin	3	35.620	11.873	8.994	0.000	***
Body mass	2	26.540	13.268	10.051	0.000	***
Feeding^	1	6.210	6.211	4.705	0.031	*
Residuals	308	406.590	1.320			

Note: R^2 = 0.144. *Basin* = Corrientes and *Body mass* = Low was defined as the intercept in the model. SE stands for Standard Error. Df stands for Degrees of freedom. Significance codes are the following: P < 0.001 (***), 0.001 ≤ P < 0.01 (**), 0.01 ≤ P ≤ 0.05 (*). The symbol ^ in the variable *feeding* (i.e., Feeding^) indicates that herbivorous and frugivorous have been grouped into the same category for the analysis.

HYPOTHESIS VALIDATION

The results obtained in bivariate and multivariate analyses support the rejection of the hypothesis 1: "**Samples collected in areas that overlap with oil extraction activities (i.e., the Corrientes and Pastaza River basins) will present higher Pb levels than samples collected in areas without such overlap (i.e., the Yavarí and Pucacuro River basins)**". The bivariate analyses for the variable *oil* (both when using the sample including all species and when using the sample for *Cuniculus paca*) suggested that there are statistically significant differences in *LnPbDW* mean values between areas overlapping and areas not overlapping with oil extraction activities, with higher Pb concentrations in areas not overlapping with oil extraction activities. The bivariate analysis for the variable *basin* (including all the observations in the sample) also showed significant differences in *LnPbDW* mean values between Yavarí and Pucacuro areas and Corrientes, while Pastaza only showed significant differences with samples from Yavarí. Considering only *Cuniculus paca* observations, the bivariate analysis for the variable *basin* showed significant differences in *LnPbDW* mean values between Corrientes and Yavarí, Pucacuro and Pastaza, being the Corrientes River basin the one showing lower *LnPbDW* mean values. In the same line, in the multivariate analysis selected by the AIC methodology, the variable *basin* displayed a statistically significant difference in the same direction: Pb concentrations are significantly higher in basins that do not overlap with oil extraction activities (Yavarí and Pucacuro) than in the Corrientes River basin, which actually do overlap with oil extraction activities. However, samples from Pastaza also show significantly higher *LnPnDW* mean concentrations than samples from Corrientes.

Results obtained in the analysis presented here support hypothesis 2: "**Carnivorous species will present higher Pb concentrations than frugivorous and herbivorous species**". As hypothesized, the highest median Pb concentrations were found in samples from carnivorous species. Results from bivariate analysis suggest no significant difference in the *LnPbDW* mean among different feeding categories. However, for the most significant multivariate models (models 8 and 10, Table 3.16 and Table 3.17), the variable *feeding* is associated in a statistically significant way to Pb concentrations, being *LnPbDW* mean among carnivorous species higher than among frugivorous and herbivorous.

Results obtained in bivariate and multivariate analyses also support hypothesis 3: "**Pb levels in liver samples from small animals will be larger in species with a larger body mass**". The bivariate analysis showed that low-weighted species had significant higher *LnPbDW* mean values than high-weighted species, low-weighted species. In the same vein, the two most significant multivariate models (models 8 and 10, Table 3.16 and Table 3.17) showed that *body mass* bears a strong statistical significance with Pb concentrations: samples from low body mass species presented significantly higher Pb concentrations than samples from high body mass species.

DISCUSSION

CAVEATS AND LIMITATIONS

A major limitation of this subchapter is that some variables that may affect liver Pb levels in wildlife (e.g., sex, age and weight of the animal) were not collected. For instance, since Pb is a cumulative heavy metal, old animals are expected to show higher Pb concentrations than young ones. Actually old animals tend to show higher Pb levels than younger animals (National Research Council, 2005; Agengy for Toxic Substances and Disease Registry, 2007). The association, however, is not direct since a mother with high Pb levels can also transfer Pb to the developing foetus transplacentally and during lactation (Pb in milk is very bioavailable, National Research Council, 2005). Moreover Pb is more efficiently absorbed by young than by older animals (National Research Council, 2005). In any case, since information regarding the age of the sampled individual was not collected, there is no possibility to test any of these associations.

In this research, the average species' body mass was used as a proxy of the weight of the sampled hunted animals (which was not collected for limitations of the sampling method[18]). In turn, the weight of the sampled hunted animal was a proxy for the distance between the ammunition placement and the liver. As data on the animal's weight or the exact spot where the ammunition impacted the animals' was not collected, results presented here should be considered with caution.

[18] Although the protocol created for the participative collection of biological samples included the weight out and annotation of the weight of the animal and the weight of the liver by local hunters, these data were scarcely or inaccurately collected.

Another limitation of this work relates to the unbalanced sample size. The sampling method used does not target particular species and, therefore, the final sample provided a heterogeneous representativeness in relation to the variables *order* and *species* (Table 3.2). To better understand the variations of Pb concentrations, a bigger and more balanced sample size would have been necessary, since some orders and species show a very small sample size and/or have not been collected in all the sampling areas. This introduces a considerable noise in the variance of the dataset in relation to the variables *species* and *order*. For this reason, results considering these two variables should also be interpreted with caution. To test the reliability of the findings obtained, some additional analysis using only the sample *Cuniculus paca* were conducted*,* and while results found in this analysis resemble results from the full sample, a note of caution is still in order.

The low representativeness of the sample for carnivorous species is also a limitation of this study. Actually, the biomagnification of Pb (Hypothesis 2) has been tested by comparing Pb concentration between species belonging to different trophic levels, but not belonging to the same food web. In other words, the carnivorous species collected do not commonly ingest any of the frugivorous and herbivorous species analysed: both *Caiman crocodilus* and *Leptodactylus pentadactylus* are aquatic carnivorous while the frugivorous and herbivorous species collected are terrestrial or arboreal; the arboreal carnivorous *Potos flavus* (Carnivora) mostly eat fruit, flowers and leaves (Charles-Dominique *et al.*, 1981; Bisbal, 1986; Julien-Laferriere, 1999; Kays, 1999); and the also arboreal carnivorous *Nasua nasua* (Carnivora) predominantly eats invertebrates and fruits, and its consumption of vertebrates is uncommon (Kaufmann, 1961; Russell, 1982; Schaller, 1983; Bisbal, 1986; Gompper, 1996; Gompper and Decker, 1998; Beisiegel and Mantovani, 2006). Notwithstanding, in examining the variations of metals along trophic levels, other authors have also compared different trophic levels (even inside members of the same group, like seabirds), instead of measuring the metal concentrations in each link of a food chain (e.g., plankton, invertebrates, fish, fish-eating seabird) (Burger and Gochfeld, 2009). So these results are comparable to those previous studies.

Finally, as it has been mentioned, many frugivorous and herbivorous Amazonian wildlife –many of them game species- ingest soil deliberately in salt licks or incidentally while feeding. Data on ingestion rates (which can be up to 30% of the intake, Beyer et al., 1994; Hui, 2004) may be key to understand bioaccumulation of environmental contaminants, including Pb. However, these data are very scarce, hindering the interpretation of the findings.

RESULTS INTERPRETATION

Results from this study show that the two explanatory variables *basin* and *body weight* were associated to Pb concentration in wildlife species' liver samples. However, the best of the multivariate models explained just 14.5% and 14.4% of the variance observed, probably because of the aforementioned limitations of this study, including omitted variable bias.

Overlap with oil extraction areas

Contrary to what it was predicted in hypothesis 1, higher Pb levels were found in samples from Pucacuro and Yavarí, the two basins that do not overlap with oil extraction activities, than in samples from the basin which overlap with oil extraction. Some possible explanations of these results are suggested here, although further studies are needed to further explore any of them:

1. *Hunting pressure varies across areas, and especially in salt licks.* Differences on human density in the study areas might affect hunting pressure. A higher hunting pressure would be then translated into a higher Pb environmental pollution from spent ammunition and, consequently, a higher risk for wildlife to ingest Pb. However, this explanation does not seem very plausible as, currently, the two basins where animals show higher Pb concentrations (Yavarí and Pucacuro) are less populated than Corrientes and Pastaza, contradicting this suggested explanation. When sharing these findings with local people, they suggested that the findings could be explained because hunting activities are more common in salt licks in the Yavarí region than in salt licks in the Corrientes and Pastaza basins. As they explained, hunters in Yavarí typically hided in the salt licks to wait for preys, while hunters from the Corrientes and Pastaza also hunt while walking through the hunting paths (*caminos de mitayo*), and therefore use less intensively salt licks. Additionally, in the Corrientes and Pastaza basins there is a primary road system opened by the oil industry that allows motor-based circulation. Roads are frequently used by local hunters when looking for their

preys, a behaviour that de facto increases the hunting area and the number of salt licks that hunters visit at the same time that reduces spent ammunition and ammunition fragment density in any specific salt lick. In other words, differences on hunting practices across areas might result in higher density of Pb spent shots in some salt licks versus others. While this explanation was suggested by local people, data collected in this work do not seem to support it as the analysis presented in the previous subchapter showed no differences on Pb concentrations in soils of natural mineral licks for areas overlapping and not overlapping with oil extraction activities.

2. *Hunters' practices vary from one area to another.* It might be the case that hunters in the different study areas might target different kill points when hunting (e.g., head, heart, lungs, etc.). The closer the target kill point from the liver, the higher the expected liver Pb concentrations. Unfortunately, this information has not been collected so far.

3. *The historical use of shotguns differs across the studied sites*, some areas having been exposed to lead-ammunition for longer time than other. If this was the case, the amount of Pb spent ammunition dumped in the environment will also be different across sites. Again, this information has also not been collected.

4. *The geological Pb content is a very important Pb exposure source for wildlife and it is different in the study areas*. Results presented in the previous subchapter suggested that geological Pb is a minor source of Pb exposure for wildlife, compared to lead-based ammunition and oil-related Pb. However, because the fingerprint of the geological soil might have not been fully identified since all soil samples belonged to hunting areas –salt licks-, this study might have underestimated the importance of geological Pb as an exposure source. If so, differences on Pb concentrations between the study areas (unknown in this study) might explain the observed differences on liver Pb levels.

5. Finally, it is also possible that the observed results do not reflect the reality since it is too much affected by the noise in the sample set, maybe related to the age, sex, order and species of the animals from which the tissue samples were

collected. The analysis performed using only the observations of the species *Cuniculus paca*, which eliminated variability associated to order and species, showed similar results than when using the full sample, for which it can be argued that results might not be largely affected by these variables. However, animal's age and sex could still be important factors in explaining liver Pb levels.

So, definitively, more research is needed to understand the difference on Pb concentrations found in wildlife tissues from samples from the different studied basins.

Feeding patterns

The results presented here show significant –although weak- differences in Pb concentration among species with different feeding patterns. As hypothesized, higher Pb median levels were found among carnivorous species. However, the statistical significance of these differences is weak. The low statistical significance can be due to the fact that this study lacks enough data on carnivorous species occupying the highest trophic level in the terrestrial ecosystem (e.g., top predators like felines) or to the fact that trophic levels belonging to different food webs are grouped in this study.

Nevertheless, the findings presented here are in line with new evidences that suggest the possibility of Pb biomagnification from one trophic level to another (Burger and Gochfeld, 2009; Rubio-Franchini and Rico-Martínez, 2011). It should be noticed that this literature differs from most of the previous literature –mostly in aquatic environments- suggesting that Pb biomagnification does not to typically occur (Demayo *et al.*, 1982; Sydeman and Jarman, 1998; Chen and Folt, 2000; Barwick and Maher, 2003; National Research Council, 2005; Agengy for Toxic Substances and Disease Registry, 2007; Cui *et al.*, 2011; Cardwell *et al.*, 2013). For example, Ma (1996) argued that large mammalian predators (Carnivora) are not expected to show high Pb levels since muscle is a poor Pb accumulator. Moreover, Pb mostly bioaccumulates in bones, while most predators especially ingest soft tissues for which they might be less exposed to Pb ingestion. It is also been argued that the percentage of Pb dose absorbed through gastrointestinal absorption decreases as the dose increases (National Research Council, 2005), counteracting biomagnification. Contrary to this literature, results presented here – documenting an increase of Pb levels from one trophic level to another - suggest both Pb bioaccumulation and biomagnification in food webs. Since Pb biomagnification is expected to occur, especially when Pb ingestion is a pathway of Pb exposure for animals (vs. deposition of ammunition fragments in animal tissues during the shot), results presented here point at the importance of Pb ingestion as an exposure way, as it has been also suggested in previous literature (Pain *et al.*, 1993, 2014; Kendall *et al.*, 1996; Scheuhammer and Norris, 1996; Lewis *et al.*, 2001; Beyer and Fries, 2002; Hunt *et al.*, 2004; Fisher *et al.*, 2006; Kreager *et al.*, 2008; Mateo *et al.*, 2014).

As a note of caution, it should be keep in mind that Pb bioaccumulation in animal tissues is influenced by many physical and biological factors, such as the amount and bioavailability of Pb exposure, the metallothionein level -a protein family that can bind Pb and prevent toxicity-, or the individual's sex, age, dietary composition, body condition, or metabolism (Ma, 1996; Cui *et al.*, 2011). For example, soluble forms of Pb are absorbed more efficiently than insoluble forms; young animals absorb Pb more efficiently than older animals; lactation, pregnancy or deficiencies in calcium or iron also increase the Pb absorption efficiency (National Research Council, 2005). In addition, all collected samples might be affected by Pb pollution through the deposition of ammunition fragments during the shot. So, although data presented here suggest the veracity of hypothesis 1, the aforementioned limitations should be overcome for a firmer test of this hypothesis.

Body mass

Results presented here confirm the hypothesis that Pb level concentration is higher in liver samples from small body mass species than for large-size species. These results resemble results presented by Pain *et al.* (2010), who also suggested that Pb levels tend to be higher in small species, such as gamebirds, than in larger species, like ungulates. This finding supports the argument that the probabilities of deposition of ammunition fragments in the liver tissue are higher in smaller animals, although data to test this argument has not been obtained in the framework of this thesis. Confirming this hypothesis might highlight the importance of the deposition of ammunition fragments in animal tissues during the shot as a Pb exposure pathway for wildlife.

Orders and species

The results presented in this subchapter show that *Penelope jaqcuaqu* (Galliformes) and *Psophia leucoptera* (Gruiformes) are the species with higher liver Pb concentrations, while *Pecari tajacu* and *Mamaza americana* (Artyodactyla) are the species with the lowest Pb average levels. Some possible explanations for the obtained results are suggested in the following lines:

Both *Penelope jaqcuaqu* (Galliformes) and *Psophia leucoptera* (Gruiformes), the two species with the highest levels of Pb, in addition to ingesting soil in salt licks, they also deliberately ingest grit to facilitate the fragmentation of food. Individuals from these species might also ingest food items (such as seeds) with a similar aspect to lead spent ammunition. Thus, these species may mistakenly ingest more ammunition than other species not displaying these feedings behaviours, being therefore especially susceptible to Pb exposure (Pain *et al.*, 2009). Moreover, the abrasion of the Pb in the gizzard accelerates the dissolving of lead-based ammunition fragments (Pain *et al.*, 2009), and Pb fragments can remain long time in the gizzard acid environment, increasing its toxicity (Ma, 1996). Indeed, Pb poisoning of species from these two groups has been widely reported trough European and North American countries (Pain *et al.*, 2009). Despite samples from *Ara sp.* (Psittaciformes), a bird species which does not ingest grit, showed a lower (although not significantly different) Pb concentration average value, samples from the bird species *Tinamus major* (Tinamiforme), a species that does ingest grit (Erard *et al.*, 1991), showed the lowest Pb average levels among birds species in the study sites, contradicting the aforementioned explanation, and highlighting the need for further research.

It might also be argued that the species with a higher mobility might show lower Pb concentration in tissues than species with lower mobility, since the firsts might access foodstuff and water from a wider territorial range, for which the effect of local contamination might be less strong on them. However, results presented here do not show a clear relation between Pb concentrations and home ranges (HR). Although some species like *Tayassu pecari* (HR: 130 Km^2, Kiltie & Terborgh, 1983), *Pecari tajacu* (HR: 6.85 – 11.70 Km^2, Taber et al., 1994; Vieira-Fragoso, 1999) and *Tapirus terrestris* (HR: 0.50 – 5.78 Km^2, Noss et al., 2003) show large home ranges and low median Pb concentrations (under 2.00 µg/g DW), other species with also large home ranges show high median Pb concentrations. For example, *Nasua nasua* (HR: 4.45 – 5.54 Km^2, Beisiegel & Mantovani, 2006) or *Lagothrix sp*. (HR: 7.60 Km^2 for *Lagothrix lagothricha*, Defler, 1996). Similarly, although some species with small home ranges present high median Pb concentrations (over 2.00 µg/g DW), like *Allouatta seniculus* (HR: 0.07 Km^2, Mitani & Rodman, 1979), *Potos flavus* (HR: 0.53 – 0.82 Km^2, Kays & Gittleman, 1995) and

Caiman crocodilus (HR: 0.12 – 0.36 Km^2, Ouboter & Nanhoe, 1988), some species with small home range show median Pb concentrations under 2.00 µg/g (DW), like *Mazama americana* (HR: 0.52 Km^2, Maffei & Taber, 2003) and *Cebus apella* (HR 0.77 – 1.61 Km^2, Di Bitetti, 2001).

Habitat

Surprisingly, no statistically significant differences in Pb concentrations were found for species living in different habitats. Since the exposure routes to Pb for the aquatic species largely differ from those of terrestrial and arboreal species, it was expected to detect important differences on Pb concentrations. Neither the ingestion of soil in salt licks nor the ingestion of spent ammunition are supposed to be an important pathway for the two aquatic species for which data were collected (*Caiman crocodilus* and *Leptodactylus pentadactylus*). Furthermore, individuals from these species are mostly not hunted with shotguns, so the deposition of ammunition fragments in the carcasses might not be a main source of exposure for the studied aquatic species. Here it is argued that the lack of differences across habitats might be explained by the fact that fresh water might also be highly polluted with Pb. Indeed, many rivers from the Corrientes and Pastaza River basins have been exposed to the daily dumping of aprox. 1,000,000 barrels of produced water for almost 30 years (Orta-Martínez and Finer, 2010) and, as mentioned before, Pb is usually found in this by-product of oil activities (Fakhru'l-Razi *et al.*, 2009). However, fresh water from control areas (i.e., Pucacuro and Yavarí River basins) it is a priori not expected to be polluted with Pb. Unfortunately, data on fresh water and sediments was not collected in this work, therefore a broader analysis should be done to obtain a comprehensive picture of the exposure pathways for species living in different habitats.

Pb concentrations

As mentioned, average Pb concentrations found in the studied liver samples are comparable to average levels found in wild game from industrial countries. However, the liver Pb concentrations of the 29 outliers (PbDW: 8.45 µg/g – 375 µg/g) resembles, and in some cases are higher, than the concentrations found in animal samples collected close to smelters (17 µg/g – 110 µg/g DW in carcasses samples) (Beyer 1985), and firearms shooting ranges (0.9 µg/g – 34.5 µg/g DW in liver samples,6.2 µg/g – 90.0 µg/g WW in liver samples) (Ma 1989, Lewis 2001).

According to Franson (1996), Pb levels in livers (WW) in Galliformes are considered subclinical (asymptomatic) when ranging between 2-6 µg/g, toxic when they exceed the 6 µg/g, and deathly when exceeding 15 µg/g. In this chapter, 13 liver samples belonging to *Penelope jaqcuaqu* (Galliforme) were analysed, from which two showed subclinical Pb levels and three presented toxic Pb levels. One of the samples showed a Pb concentration (WW) of 24.88 µg/g, a concentration that is lethal for Galliformes according to Franson (1996). Overall, a worrying 38.50% of *Psophia leucoptera* (Gruiformes) samples collected for feeding purposes showed Pb levels over the subclinical threshold. In mammals, Pb toxicity is expected for liver Pb levels above 5 µg/g DW and clinical signs of Pb poisoning are associated with liver concentrations over 30 µg/g DW (Ma, 1996). Among the 251 samples belonging to mammal species, only eight samples (3.19%) showed liver Pb levels over 5 µg/g DW and two of them presented levels over 30 µg/g DW. So, 0.80% of mammal samples (one *Mazama americana* and one *Dasyprocta fuliginosa*) might have shown clinical signs of Pb poisoning. However, as stated before, Pb health risk for mammals depend on a multitude of confounding variables, including dietary composition, nutritional status, age or metabolism, among others (Ma, 1996). Moreover, subclinical, clinical and mortal Pb exposure levels show higher variation in mammals than in birds (Lewis *et al.*, 2001). And finally, it is important to keep in mind that in this study it is not possible to separate Pb from the deposition of ammunition fragments during the shot and Pb ingested. Therefore, it is not possible to surely conclude whether animals showing high liver Pb levels were affected by this heavy metal during their life or whether the exposition to Pb only occurred at the moment of their death. For all the aforementioned reasons, these results might be interpreted with caution.

The species *Tapirus terrestres, Tayassu pecari, Alouatta seniculus,* and *Lagothrix sp* deserve an especial attention since they are considered endangered or vulnerable in the IUCN red list of threatened species (International Union for Conservation of Nature and Natural Resources, 2016). Altough *Tapirus terrestris* and *Tayassu pecari* showed relatively low median lead liver levels, the primates *Lagothrix sp* and *Alouatta seniculus* presented higher concentrations, especially the species *Alouatta seniculus*, which showed the second highest median lead level (4.50 µg/g DW) among all the studied species. Considering that these species are already strongly menaced by mupliple threats, lead exposure might further exacerbate their worrying conservation status.

Finally, tt is also important to keep in mind that Pb in liver tissues does not have a very long half-life. As mentioned before, the average half-life of Pb in mammal liver tissues is 7.4 days, and around 1 month for adult humans. (Ma, 1996; National Research Council, 2005). The Pb levels observed in this study represent the recent exposition to Pb, not the long-term exposition. Therefore, those animals with low Pb levels might just have had higher (and harmful) Pb levels some weeks before they were hunted.

CONCLUSIONS

Although only 14% of the variance has been explained with the selected models, findings from this section point at the same direction that findings in the previous subchapter A, as both highlight the importance of ammunition as the main driver of wild species Pb contamination. The deposition of lead-based ammunition fragments in the liver tissue during the shot arises as an important pathway of Pb exposure, an interpretation derived from the findings that (a) there is higher Pb levels in smaller species than in larger species and (b) the most frequent visitors to salt licks (i.e., Perissodactyla and Artyodactyla as reported in Chapter II) showed the lower Pb levels, indicating that geophagy might be a less prominent exposure pathway than the deposition of ammunition fragments in the animal tissues during the shot. Pb ingestion is also suggested as another route of exposure: the higher Pb concentrations in species located in upper levels of the trophic chain suggest that biomagnification of Pb is occurring, a process that would not take place if the deposition of ammunition during the shot was the only pathway of exposure to Pb.

Interestingly, higher liver Pb concentrations were found in samples from the Pastaza River basin than in samples from the Corrientes River basin. In the previous subchapter, liver samples from the Corrientes River basin showed an isotopic fingerprint closer to that of oil, in comparison with samples from the Pastaza River basins. These two findings could suggest that the fingerprint of oil-related Pb is more visible in samples from the Corrientes River basin because it is less masked by the fingerprint of other Pb sources – probably lead-based ammunition-. Contrarily, since Pb pollution is higher in the Pastaza River basin, the fingerprint of oil-related Pb is more strongly masked in this study area.

Finally, although there is no doubt that oil extraction activities have strongly affected Corrientes and Pastaza River basins (Yusta-García *et al.*, 2017; Rosell-Melé *et al.*, 2018), the higher Pb concentrations in the areas that do not overlap with oil extraction activities (i.e., Yavarí and Pucacuro) suggest the existence of other factors strongly influencing the Pb content in livers. Although some explanations have been outlined above, this research is not conclusive on the identification of these factors, thus, further research should focus on the avoidance of the limitations reported in this study. Moreover, future research might consider the analysis of Pb concentrations in bones, as these data might provide very useful information for the interpretation of results, since Pb in bones would reflect the cumulative Pb exposure for wildlife and not only the exposure of the last days or weeks before the sample was collected (Ma, 1996).

CHAPTER IV
CONCLUSIONS

Picture in the previous page: *The Pastaza River after the storm*.
Source: Mar Cartró-Sabaté

CHAPTER IV • CONCLUSIONS

In this last chapter, the general conclusions from this thesis are presented. The chapter is organized by differentiating between the major theoretical and methodological contributions, and the policy implications of this research. It ends pointing at some methodological caveats of this work and providing some suggestions for future research.

THEORETICAL CONTRIBUTIONS

The results from the work presented here provide four major theoretical contributions to diverse fields within environmental sciences and ecology, namely conservation biology, tropical ecology, ecological epidemiology, and environmental pollution.

First, Amazonian wildlife ingestion of oil-polluted soil and water was new-to-science until local people reported this behaviour to the scientific team involved in the overall research project in which this thesis is framed. The team published first evidences of this behaviour after a pilot experiment consisting on a one-week camera trap survey in two potentially oil-polluted sites (Mayor et al., 2014). In this thesis, this behaviour has been confirmed and its characteristics and drivers are broadly studied. Results show that geophagy redirected to oil-polluted sites is a widespread behaviour amongst several mammal species. At least eight species belonging to three different orders (i.e., Rodentia, Artiodactyla and Perissodactyla) of the class Mammalia and one order of the class Aves (i.e., Psittaciformes) have been recorded ingesting oil-polluted waters and/or soils. Results also show that this behaviour is not uncommon, as it has been observed among numerous species in all four studied oil-polluted sites and over an extended period of time (12 months). Therefore, results presented here suggest that wildlife ingestion of oil-polluted soils and/or waters is an extended behaviour in taxonomical, geographical, and temporal terms. Indeed, results suggest that wildlife seems to be redirecting geophagy from natural salt licks, a well-documented behaviour, to oil-polluted sites, a new-to-science behaviour. Moreover, this redirected behaviour can be a route of exposure to petrogenic contamination for Amazonian wildlife and, subsequently, for human populations living in the vicinity of oil extraction areas and relying on subsistence hunting for consumption.

The second important theoretical finding of this thesis is that Pb, a toxic metal that may cause deleterious effects over most body systems (Komárek *et al.*, 2008; WHO, 2010), bioaccumulates up to high concentrations in liver tissues from wild and free-ranging Amazonian species. The high concentrations of Pb found in the tissues of animals analysed are comparable to those found in wildlife from industrial countries and mining areas. Specifically, 49.8% of biological samples studied had Pb concentrations above UE acceptable limits of offal for human consumption (0.5 µg/g WW), and 90.8% exceed the EU acceptable limits for meat of human consumption (0.1 µg/g WW). The finding highlights Pb as an important threat for the health of the studied species, a finding with important conservation implications. Moreover, results presented here also have implications for the whole ecosystem, since Pb can travel through the food chain impacting species beyond the ones actually analysed, including top predators and human populations that rely on subsistence hunting for their livelihoods, such as Indigenous and Riverine communities. Although data collected for this dissertation do not allow to prove a causal relationship, it is possible that the high concentrations of Pb found in liver tissues from wild and free-ranging Amazonian species may partly explain the worrisome Pb blood levels detected in local human populations of the study area (DIGESA, 2006; Anticona *et al.*, 2011; Anticona, Ingvar A. Bergdahl, *et al.*, 2012).

A third important theoretical finding of this thesis relates to the high prevalence of Pb in wildlife tissues from remote areas of the Amazon. This thesis has identified two important sources of Pb exposure for Amazonian wildlife. On the one side, oil-pollution from oil extraction activities represents an important source of Pb in the areas where oil-activities are taking place. Considering the large expansion of the oil extractive frontier in the Amazon rainforests and beyond (Orta-Martínez, Rosell-Melé, *et al.*, 2018), oil extraction activities may be threatening vast areas of rainforests worldwide. On the other side, results from this thesis also suggest that an important source of Pb exposure for Amazonian wildlife is lead-based ammunition. Although the health and environmental risks of lead-based ammunition are well known in western countries, they had never been described before for Indigenous Peoples living in tropical forests. Findings of this thesis suggest that, in just some decades, lead-based ammunition may have raised up as an important threat for both wildlife and human communities in remote areas, potentially posing a widespread environmental and health risk all over the Amazon and beyond.

Overall, results from this work illustrate the existence of an important knowledge gap regarding the diversity of negative impacts that the expansion of anthropogenic contaminants from industrial centres is having in the most remote areas of the earth. This thesis thus highlights the ubiquitous anthropogenic footprint in so-called wilderness areas. The findings from this work might also apply to other parts of the Amazonian basin, illustrating how human impact reaches much further than usually envisaged (Mittermeier *et al.*, 2003).

METHODOLOGICAL CONTRIBUTIONS

This thesis also brings three important methodological contributions. First, a transversal characteristic of the methodological approach used through this work is that it has relied on citizens' participation at almost all the stages of the research: from the definition of the research question (i.e., the Achuar people wanted to know whether Amazonian game species were ingesting oil-polluted soil and water), to data collection (e.g., camera trapping survey supported by the monitors of the Indigenous Federations or animal samples collected by Indigenous hunters), data analysis (e.g., local hunters helped on the identification of the species recorded in the videos), and the interpretation of results (e.g., local people suggested explanations for the results obtained in chapters II and III in communal meetings hold to present them some first results). The collaborative and active attitude of local people through the project has been crucial to collect and interpret data used to elaborate this thesis. Probably the high levels of local participation in this project relate to the fact that this research project was born from the own interest of local people, a critical factor that has strengthened engagement and blurred many barriers to participation. Indeed, since 2005, local people have been carrying out a community-based monitoring programme in collaboration with one of my supervisors (Dr. Orta-Martínez) to identify, map, and monitor oil extraction impacts in their territories (Orta-Martínez, 2010). The bond of trust between local people and the research team created during that previous project strongly facilitated local people's close collaboration that made this thesis possible. This thesis is an example of how combining different stakeholders' strengths in documenting and investigating oil impacts creates synergies that help reach a better understanding of oil extraction activities impacts and to overcome knowledge gaps.

Second, in expanding even more this project participation palette by including volunteers from all over the world, the work conducted during this thesis will also contribute to explore the benefits and limitations of online volunteered information. In order to analyze the large amount of data collected by this project (8,200 videos representing about 50,000 survey hours), a digital web platform has been created (*Amazo'N'Oil,* https://www.zooniverse.org/projects/marcartro/amazonoil). The platform, active since early April 2018, seeks the participation of worldwide volunteers in the analysis of videos recorded during the camera trap survey. This participation will accelerate the analysis of the big amount of videos collected. At the same time, the platform is a powerful educational and dissemination tool for public awareness, a side benefit that might contribute to enhance extractive industries' accountability. Only during April 2018, 1,300 volunteers have collaborated to the project through the platform and more than 30,000 video classifications have been obtained. The potential of collaborative science to produce crowdsourced data is clear. The comparison of the data produced by the online volunteers with the data produced by scientific experts (presented in Chapter I) will allow to assess the quality of identifications performed by non-expert online volunteers. For calendar limitations, this thesis does not present the data collected through the platform, neither the quality assessment of massive crowdsourced data, a work that I plan to undertake in the near future.

Finally, this thesis is a text-book example of interdisciplinarity. This thesis was developed within the framework of a PhD in Environmental Science and Technology. By definition, environmental sciences are interdisciplinary, and so is this thesis. A myriad of tools and methods from different disciplines have been used to study the ingestion of oil-polluted soil by wildlife: from camera trapping to metal and hydrocarbon content laboratory analysis, and from GIS spatial analysis to Community-Based Monitoring or Participatory Action Research. The combination of very diverse domains of knowledge has allowed to obtain a holistic view of wildlife ingestion of oil-polluted soils and its potential impacts. Pb concentrations and isotopic analysis techniques have been used to study the bioaccumulation and the sources of Pb in wildlife tissues. Keystone concepts from ecology have been considered, combined with the application of techniques from analytical and environmental chemistry, environmental forensics, exposure routes evaluation, and environmental impact assessment. In fact, the diverse fields of expertise of my supervisors and other colleagues involved in the research presented here (i.e., veterinary, chemistry, biology, anthropology, political ecology and epidemiology) have contributed to make of this thesis a truly interdisciplinary project. Although the dialogue among disciplines is -in itself- a great challenge, the discoveries that have been presented in this PhD thesis encourage the scientific community to further embrace interdisciplinarity to deal with the serious socio-environmental problems that society is currently facing.

POLICY IMPLICATIONS

The policy implications that derive from this work expand from the global to the local levels. At the global level, the findings of this thesis illustrate the serious threat that oil extraction activities represent for the tropical ecosystem, including wildlife and human populations, as well as the existing knowledge gaps on the impacts of oil-extraction activities. Considering the magnitude of the potential impacts of oil extraction activities and the vulnerability of the exposed ecosystems and human populations, the study of the impacts associated to extractive activities in tropical rainforests should be given importance and urgency and that projects aiming to study the topic should be entrusted with the necessary investment (budget and human resources).

In the same vein, the findings presented here make an urgent call upon governmental institutions and oil companies to promptly and efficiently review the operational standards and remediation practices of oil companies operating in tropical areas. Oil spills and deficient reinjection of produced water in the study area generate every year new oil-polluted sites (Hill, 2017; La República, 2018) that could potentially turn into oil-polluted salt licks. Moreover, after more than 45 years being operational, the lifetime of the pipelines in the study area is overdue, as reflected in the numerous oil spills occurring in the last years along the North-Peruvian Pipeline (PUINAMUDT, 208AD; El Comercio, 2016; Staff Reuters, 2017). Some of the study sites had been affected by recurring oil spills, some of them occurred more than 30 years ago and -at the time of writing, - have not yet been remediated. Agencies enforcing remediation to oil-spills should also guarantee a proper reinjection of produced waters, as salty springs have been reported in the area since reinjection started in 2009 (Orta-Martínez, personal communication).

Guaranteeing the conservation of the rainforest ecosystems and the human rights of Indigenous Peoples (i.e., right of life and security of the person, Article 3 of the Universal Declaration of Human Rights, United Nations, to begin with) should be considered fundamental and be a governmental and company's priority and never be questioned nor postponed.

The documented impacts of oil activities should be broadly disseminated to gain the attention not only of responsible governmental institutions and extractive companies, but also of the scientific community and the general population. It is more than likely that the impacts reported here are also occurring in many other rainforest areas where oil activities are being conducted. The recently launched platform *Amazo'N'Oil* aims to contribute to the dissemination of information of oil-extractive activities' impacts, complementing efforts to reach a broader audience done through the publication of research articles in scientific journals and other dissemination articles.

One of the important findings of this thesis relates to contamination through the use of lead-based ammunition. There is an overwhelming evidence of the toxic effects of Pb, and multiple studies around the world point out the discharge of lead-based ammunition into the environment as an important threat to humans and wildlife (Arnemo et al., 2016). Pb replacement with non-toxic alternatives is urgent, and indeed there is an international scientific consensus on the need to phase out and eventually eliminate the use of lead-based ammunition (Arnemo et al., 2016). In 2013, a consensus statement was signed by many experts with a particular focus on the impacts of lead-based ammunition on the USA (Group of Scientists, 2013). One year later, a similar scientific consensus statement with the focus on Europe was signed (Group of Scientists, 2014). Despite these efforts, progress in Pb regulations has been very slow. The first international agreement to ban the use of Pb was the African-Eurasian Migratory Waterbirds Agreement (AEWA). The signatory parties (22 countries in 1995 and 75 in 2015) agreed to phase out Pb ammunition in wetlands. Although the Agreement was signed in 1995, only 50% of the initial signatory countries and 27% of all the signatory countries had fully eliminated the use of lead-based ammunition in wetlands in 2015 (Stroud, 2014). Few countries, i.e., Denmark, Norway or the Netherlands, decided to extend the ban to all game species (Mateo, 2009). In 2014, the call of the Convention on the Conservation of Migratory Species of Wild Animals (CMS) was a big step forward, prompting its 126 parties (including Peru, Fig. S4.1) to expand the phase-out of the use of lead-based ammunition to all habitats -wetland

and terrestrial- (Stroud, 2014). Despite these efforts, and although it is widely known that wild meat consumption is key for the livelihood of many human populations living in rainforests, scarce attention has been drawn to the use of lead-based ammunitions in these regions. The alarming findings of this thesis generates further evidence for the argument that the replacement of lead-based ammunition with non-toxic alternatives should be a top priority for worldwide governments, especially in regions where human populations rely on wild meat for their livelihood. Fortunately, Pb is not indispensable and non-toxic alternatives to lead-based ammunition are widely available, apparently perform well, and some of them (e.g., steel shots) have similar prices to lead-based ammunition (Thomas, 2014).

Finally, several examples from previous work had shown how the recognition of Indigenous and local knowledge systems and the cross-fertilization between them and natural and social sciences can lead to new findings and to improve the interpretation of data, resulting in more socially-meaningful science (see examples in Tengö, Brondizio, Elmqvist, Malmer, & Spierenburg, 2014). In regions like the Amazon, local people own an incommensurable amount of knowledge about their environment that exceed the frontiers of academic scientific knowledge, as it has been shown in this research. In this line, diverse global assessment programs and science-policy platforms (e.g., the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) or the Scientific Advisory Board (SAB) of the Secretary-General of United Nations) highlight the importance of recognizing, protecting, and promoting Indigenous and local knowledge systems. They also see the diversity of knowledge as vital for solving contemporary problems (Tengö *et al.*, 2014; United Nations Secretary, 2016). Results from this thesis enhance the value of legitimizing Indigenous and local knowledge systems and creating synergies among knowledge systems to explore their complementarities. The aforementioned theoretical and methodological contributions of this thesis are the result of these synergies. Findings from this work highlight the crucial importance of really recognizing and taking into account local knowledge systems in research, as well as of developing transversally participative research projects, to face the many complex problems that this planet and its inhabitants are facing. There has been a recent increase of the so-called "digital Citizen Science" projects, in which a crowdsourced and volunteered data collection through worldwide non-expert volunteers allows for the massive collection or analysis of data (the platform *Amazo'N'oil* is an example of these types of projects). However, there is still a long way to go on in terms of citizens' participation and engagement in knowledge production in all the phases of a research project (Turreira-García *et al.*, 2018). In the same vein, public policies should also embrace and integrate the variety of knowledge systems and promote a transversal participation of local people in the design of public policies. The present thesis is a fine example of how transversal participation can not only foster science beyond the limits of academia by integrating local knowledge but also produce social-environmental and political meaningful information which, in turn, is crucial to create contextualized and efficient public policies. This thesis reinforces the idea that the implementation and respect of the Indigenous Peoples' FPIC right by developing proper participation and consultation processes about extractive activities in their territories is an essential requirement for sustainable, respectful, and suitable political decisions.

CAVEATS AND LIMITATIONS

Although detailed description of caveats and limitations are provided in each chapter, in this concluding section the most relevant limitations of this thesis are exposed. The first caveat of the work presented here relates to the external validity of its findings largely because the small sample size. For example, the results obtained through statistical tests in Chapter II have to be considered with caution, since the number of camera sites included in the analysis is too low to obtain concluding findings. Similarly, in Chapter III, the sample size of oil samples might be too small for being totally representative, particularly due to the high heterogeneity of Pb isotopic fingerprint among oil pollution samples (oil and liquid arising from oil infrastructure samples).

Second, only soil samples from salt licks, natural and oil-polluted, have been analysed. The lack of data on the soil composition in other areas (i.e., not salt lick areas) limits the interpretation of findings in Chapters II and III. In Chapter II, salt licks' mineral composition cannot be compared with the composition of adjacent soils. Thus, the hypothesis that animals ingest soil and water in oil-polluted salt licks seeking mineral supplementation cannot be tested. Moreover, oil-related metal concentrations in oil-polluted sites cannot be compared with those in adjacent soils, but only with those of natural salt licks. This lack of comparative information on soil composition makes difficult the interpretation of results. Similarly, in Chapter III, the isotopic fingerprint of geologic Pb cannot be determined because all soil samples collected might be exposed to lead-based ammunition pollution, since all salt licks are used by hunters.

Finally, the methodology used in the collection of animal tissues had multiple limitations. Thus, this methodology did not allow the collection of a very balanced sample set in terms of species and trophic level. Since samples have been collected for food by local hunters, the sample set is more representative of the human game meat ingestion than of the rainforest ecosystem. Additionally, the sample did not provide important information of the subjects captured (e.g., age, sex and weight), characteristics that might have explained part of the variability observed in liver Pb concentrations. Finally, the sample did not permit a separated analysis of the ingestion of Pb as an independent exposure pathway (i.e., independent of the exposure to lead-based ammunition during the shot) since all animals collected have been hunted using lead-based ammunition. Therefore, all tissues samples might have been polluted by ammunition fragments during the shot. Unfortunately, information on the location of the wounds created by the ammunition in the hunted animal was not collected. Thus, the findings obtained in Chapter III-B cannot be considered as concluding.

FUTURE RESEARCH

To face the urgency of identifying and facing oil and Pb pollution impacts in tropical rainforests, several questions that deserve further research are exposed in the following paragraphs.

First, this study shows that oil pollution is one important source of the Pb content found in wild Amazonian animals' livers, raising questions about the impact and the bioaccumulation of other toxic petrogenic compounds and other pollutants related to oil extraction. Potential examples of such pollutants include many heavy metals such as cadmium (Cd), mercury (Hg), chromium (Cr), or arsenic (As) as well as polycyclic aromatic hydrocarbons (PAHs). Further research in this direction is urgently needed to get a comprehensive knowledge of the impacts of oil pollution in the ecosystem, including wildlife and human populations. Moreover, results from such research are essential before we can plan proper actions to remediate impacts of oil-extraction activities. In fact, the health effect that the ingestion of oil-polluted soil by Amazonian frugivorous and herbivores may have on wildlife and on human population has not been studied yet. An epidemiological study is urgently needed to establish the relations between oil pollution detected in surface water, sediments, soils and, now, in game species, and, the presence of these substances in humans, and the possible long-term effects on the health of the Indigenous Peoples living in the vicinity of oil extraction areas. Without such a study, any action plan to address health impacts of oil-extraction activities will fall short.

Second, since oil pollution and oil-related spills are unfortunately a common impact of oil extraction activities around the world and since geophagy is a widespread animal behaviour in other ecosystems beyond the global rainforests, the ingestion of oil-polluted soil and water by wildlife might also be occurring in other oil extraction areas of the world. For this reason, the prompt study of this behaviour in other ecosystems is encouraged.

Third, further research is needed to answer some questions opened in this thesis, and that have very important policy implications. These questions include 1) "why are there higher Pb concentrations in wildlife in the Pucacuro National Park and in the Yavarí River basin than in the two study areas which overlap with oil extraction activities: Corrientes and Pastaza?" and 2) "how Pb from lead-based ammunition reaches wildlife liver tissues: through deposition of ammunition fragments in the carcasses of the hunted animals or through the accidental ingestion of Pb spent ammunition and ammunition fragments deposited in the salt licks?" To answer these important questions, future research should use a different protocol for sample collection than the one used in this thesis (see the aforementioned limitations).

Fourth, the findings of this thesis suggest that Pb pollution may be alarmingly widespread in remote tropical rainforest worldwide where lead-based ammunition is used for subsistence hunting. To confirm that the Peruvian Amazon is not an exceptional severely polluted area by lead-based ammunition, research in other territories should be carried out. To do so, it might be of great interest to analyze other variables that might affect Pb pollution, such as the hunting pressure or the historical use of shotguns.

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APPENDICES

APPENDIX I

Some results of this thesis have been published in the following peer-reviewed articles, although none of the published articles correspond to the main body of any of the chapters presented here:

- Chapter II – Petrogenic hydrocarbon analysis in soils

Rosell-Melé, A., Moraleda-Cibrián, N., Cartró-Sabaté, M., Colomer-Ventura, F., Mayor, P., & Orta-Martínez, M. (2018). Oil pollution in soils and sediments from the Northern Peruvian Amazon. *Science of the Total Environment*, *610*, 1010-1019.

- Chapter III – Video analysis (partly) and soil analysis for the oil-polluted sites 4 and 5

Orta-Martínez, M., Rosell-Melé, A., Cartró-Sabaté, M., O'Callaghan-Gordo, C., Moraleda-Cibrián, N., & Mayor, P. (2018). First evidences of Amazonian wildlife feeding on petroleum-contaminated soils: A new exposure route to petrogenic compounds? *Environmental research, 160*, 514-517.

Besides the aforementioned articles, during my PhD I also participated as a co-author in the following scientific publications:

Benyei, P., Turreira-Garcia, N., Orta-Martínez, M., & Cartró-Sabaté, M. (2017). Globalized Conflicts, Globalized Responses. Changing Manners of Contestation Among Indigenous Communities. In *Hunter-gatherers in a Changing World* (pp. 233-250). Springer, Cham.

Mayor, P., Rosell, A., Cartró-Sabaté, M., & Orta-Martínez, M. (2014). Actividades petroleras en la Amazonía:¿Nueva amenaza para las poblaciones de tapir? *Tapir conservation*, 23(32), 26-29.

Moreover, I also participated in the publication of some results of the thesis in the following dissemination magazine article:

Fernández-Llamazares, A., Cartró-Sabaté, M. & Reyes-Garcia, V. (2015). Els pobles indígenes amazònics davant del Canvi Global. *Revista Papers - Publicació de la Lliga dels Drets dels Pobles, 60*, 6-9. October 2015

Additionally, the results of this dissertation were presented in the following international congresses (* speaker):

Cartró-Sabaté, M.*, Mayor, P., Rosell-Melé, A., Reyes-García, V. & Orta-Martínez, M. (2015). *Oil spills ingestion by game species in the Peruvian Amazon.* 28th Annual Conference of the Society for Tropical Ecology - Gesellschaft für Tropenökologie (gtö), Zurich, Switzerland. 7-10 April

Cartró-Sabaté, M.*, Mayor, P., Rosell-Melé, A., & Orta-Martínez, M. (2016). *Identifying sources of lead in Amazonian wildlife by lead isotope analysis.* 53rd Annual Meeting of the Association for Tropical Biology and Conservation. Montpellier, France. June 19-23. ● Winner of the Alwyn-Gentry award for the best oral presentation among PhD students ●

Cartró-Sabaté, M., Mayor, P., Orta-Martínez, M. & Rosell-Melé*, A. (2016) Lead isotopes track exposure of Amazonian wildlife to oil spills. 26th Goldschmidt Conference. Yokohama, Japan. 26 June – 1 July

APPENDIX II

Appendix II.a. – Survey days carried out obtained in each study site

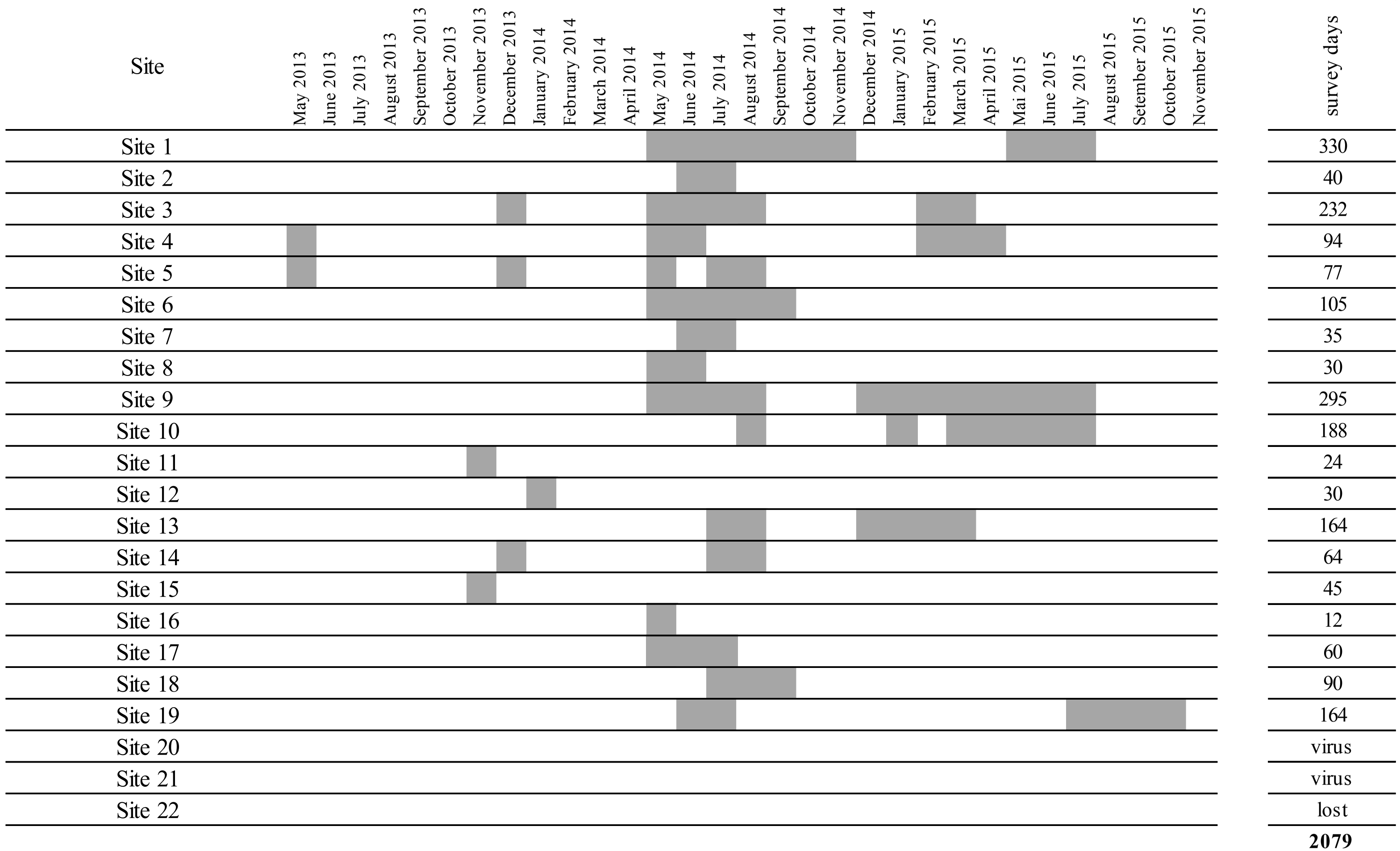

Figure S2.1 | Survey days carried out in each study site.

Appendix II.b. – Maximum Permissible Levels of heavy metals in soils

Table S2.1 I Peruvian Soil Quality Guidelines: Maximum Permissible Levels of heavy metals in soils

Metal		Peruvian Soil Quality Guidelines *Concentration (mg/kg dry weight)*		
		Agricultural	Residential / Parkland	Commercial/ Industrial/ Extractive
As	Arsenic	50	50	140
Ba	Barium	750	500	2000
Cd	Cadmium	1.4	10	22
Pb	Lead	70	140	1200
Hg	Mercury	6.6	6.6	24

Note: Own elaboration based on MINAM, (2013).

Table S2.2 I Canadian Soil Quality Guidelines: Maximum Permissible Levels of heavy metals in soils

Metal		Canadian Soil Quality Guidelines *Concentration (mg/kg dry weight)*			
		Agricultural	Residential / Parkland	Commercial	Industrial
Be	Beryllium	4	4	8	8
Cr	Chromium total	64	64	87	87
Ni	Nickel	45	45	89	89
As	Arsenic	12	12	12	12
Ba	Barium	750	500	2000	2000
Cd	Cadmium	1.4	10	22	22
Cu	Copper	63	63	91	91
Pb	Lead	70	140	260	600
Hg	Mercury	6.6	6.6	24	50
V	Vanadium	130	130	130	130
Zn	Zinc	200	200	360	360

Note: Own elaboration based on CCME, (1999).

APPENDIX III

Appendix III.a. – Exposure routes and impacts on wildlife from lead-based ammunition

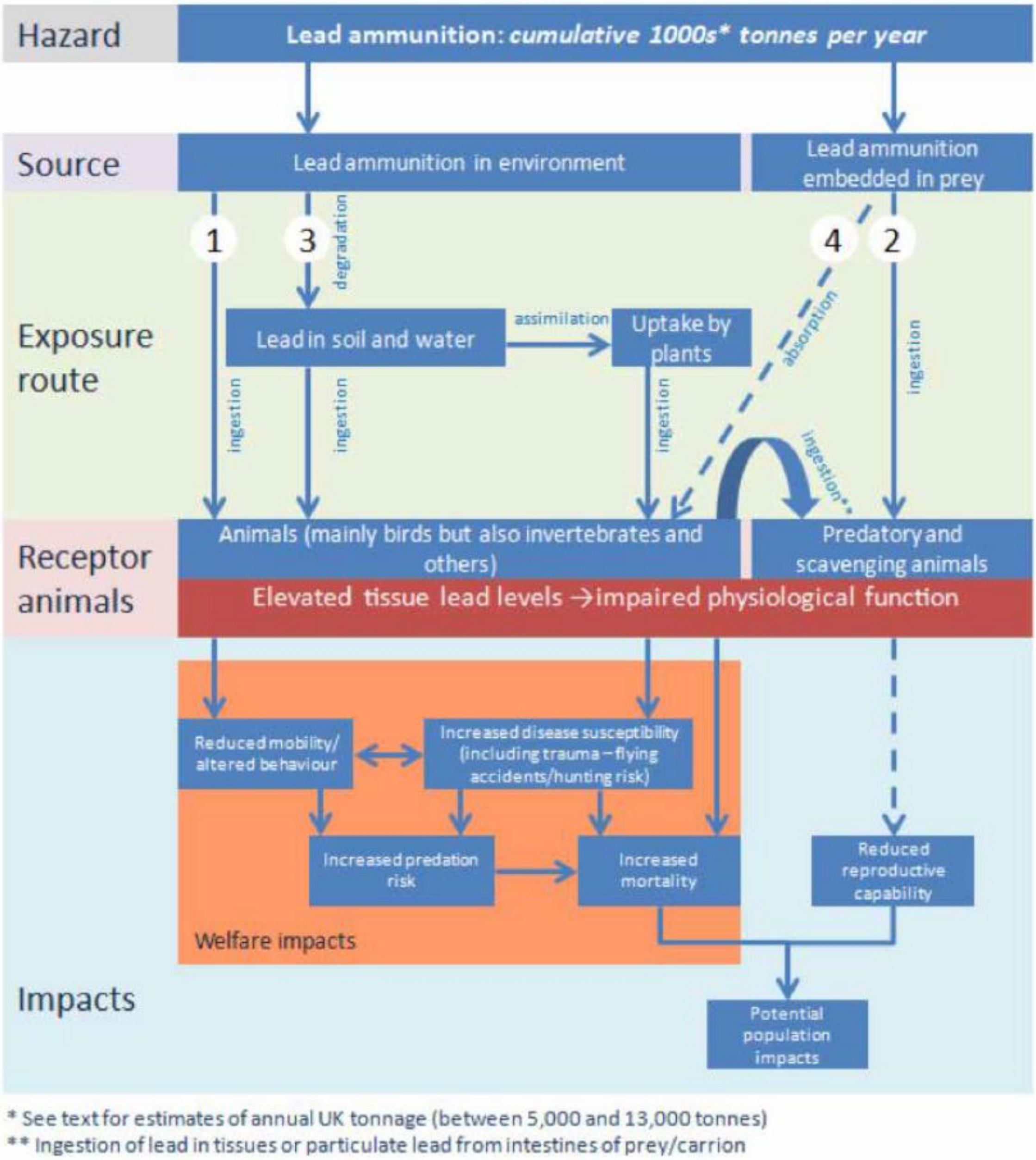

Figure S3.1 | Exposure routes and impacts on wildlife from lead-based ammunition
Note: Schematic illustrating and summarising the 4 exposure routes and range of impacts on wildlife of poisoning from lead ammunition sources (Paint et. al. 2014).

APPENDIX IV

Appendix IV.a. – Convention on Migratory Species (CMS) Parties

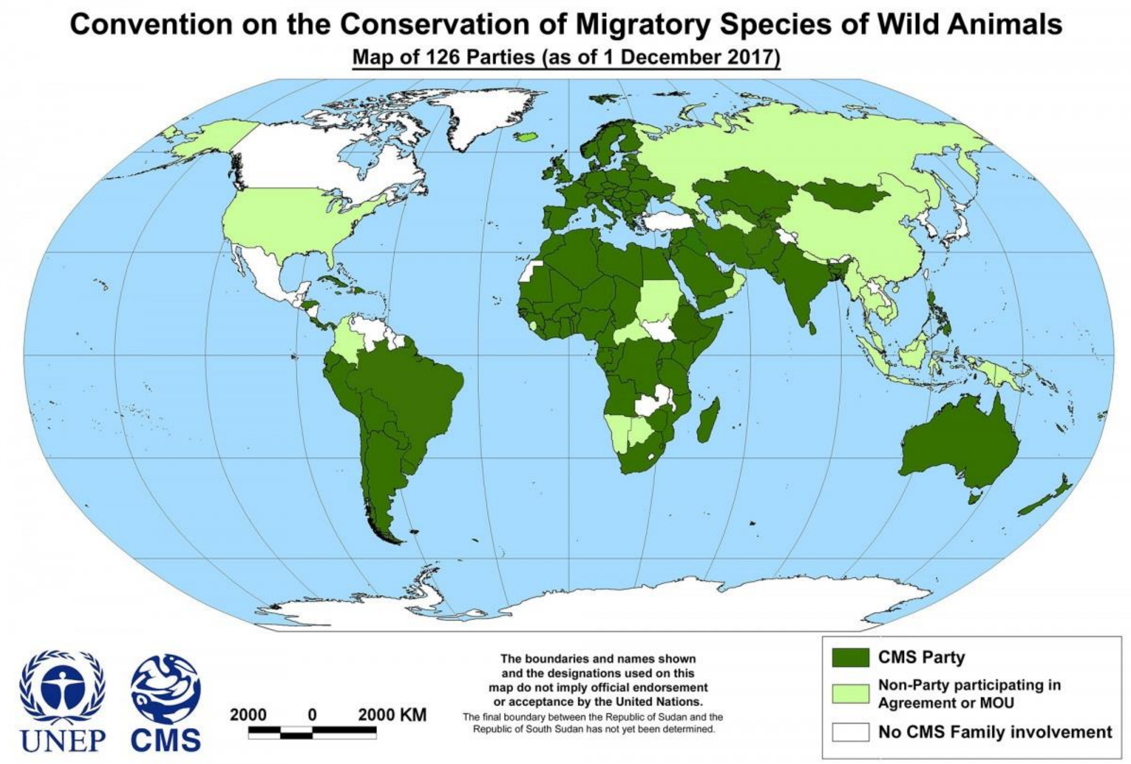

Figure S4.1 | Map of the Convention on Migratory Species (CMS) Parties in 2017
Note: Obtained from (CMS, 2017)