Universidade de Aveiro Departamento de Biologia
Ano 2016

Anne-Sophie Bertrand

Caracterização e Conservação do Parque Nacional do Iguaçu, Brasil

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia e Ecologia das Alterações Globais, realizada sob a orientação científica do Doutor Amadeu Mortágua Velho da Maia Soares, Professor Catedrático e Diretor do Departamento de Biologia da Universidade de Aveiro e sob a co-orientação científica do Doutor Chris Carbone, pesquisador senior do Instituto de Zoologia da Sociedade Britânica de Londres

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Dedico este trabalho ao Parque Nacional do Iguaçu e à sua Natureza, assim como aos apaixonados pela Conservação na realidade desafiadora do século 21.

o júri

presidente

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palavras-chave

Parque Nacional do Iguaçu, mamíferos, MEA, integridade ecológica, alteração do hábitat,conservação.

resumo

Esta tese é a primeira caracterização da comunidade de mamíferos no Parque Nacional do Iguaçu [PNI] que comemorou 77 anos, e sua zona de amortecimento. Utilizamos armadilhas fotográficas e o Modelo do Encontro Aleatório (MEA) desenvolvido por Rowcliffe et al. (2008), e calculamos estimativas de densidade para 17 espécies dentro e fora dos limites do INP (Capítulo 1). Em seguida, observamos e descrevemos a distribuição dessas espécies ao longo do gradiente de alteração de hábitat oferecido pela região (Capítulo 2). Discutimos também os impactos de uma estrada que cortava o PNI na sua zona intangível (Capítulo 3). Através análises de DNA de fezes de carnívoros, confirmamos a presença oculta de gato-do-mato-grande (*Leopardus geoffroyi*) na região Iguaçu. Esses dados foram reinforçados pela captura de um indivíduo nas redondezas de Foz do Iguaçu (Capítulo 4). Nosso monitoramento também incluiu levantamentos de pegadas, o que permitiu o desenvolvimento de um método para distinguir os pequenos gatos pintados Neotropicais (Capítulo 5). Entre as ~248,000 fotografias que coletamos houve os primeiros registros fotográficos de dois xenartros no PNI, i.e. o tamandua bandeira (*Myrmecophaga tridactyla*) e o tatu-de-rabo-mole-grande (*Cabassous tatouay*; Capítulo 6). Além do mais, avaliamos a integridade ecológica do solo com bait-lamina (Capítulo 7), e o conteúdo bacteriológico das águas do rio São João, um dos maiores rios do PNI (Capítulo 8). Evidentemente, o PNI está sofrendo várias pressões geradas por atividades humanas legais e ilegais. Enquanto o lucro proporcionado pela visitação e fama do PNI representa a maior parte da economia regional, e enquanto este capital é também injetado nas outras Unidades de Conservação Brasileiras menos populares, ao invés de receber a legítima gratidão, o PNI está sendo envenenado com agrotóxicos através sua rede hidrográfica generosa, e está sendo violado por caçadores, pescadores, bandidos e extratores de palmito. Nossos resultados descrevem a tamanha importância ecológica do PNI considerando que ele representa 80% do que sobra de mata Atlântica do interior em todo o estado do Paraná, e porque ainda abriga espécies-chave especialistas deste bioma (e.g. onça pintada, *Panthera onca*). O contexto regional do PNI é onde devemos concentrar esforços conservacionistas, i.e. promovendo conectividade do hábitat e integridade ecológica, e realizando campanhas educacionais para sensibilizar o público quanto ao valor inestimável que o PNI possui, de uma maneira amorosa e colaborativa. Atividades devem promover a reconexão entre os lindeiros e o PNI, de forma que a relação passa de uma perspectiva exploradora a uma perspectiva conservacionista.

keywords

Iguaçu National Park, mammals, REM, ecological integrity, habitat alteration, conservation.

abstract

This thesis is the first characterization of the mammal community of the 77-year old Iguaçu National Park [INP] and region. Using camera-traps and the Random Encounter Model developed by Rowcliffe et al. (2008), we have calculated the first density estimates for 17 species in and out of the INP boundaries (Chapter 1). We then looked at and described the species' distribution along the gradient of habitat alteration offered by the region (Chapter 2). We also discussed the impacts of a road, which used to cut through the INP core area (Chapter 3). Through DNA scat analyses, we confirmed the concealed presence of Geoffroy's cat (*Leopardus geoffroyi*) in the Iguaçu region. These findings were reinforced by the capture of a specimen in the vicinity of Foz do Iguaçu (Chapter 4). Our monitoring also included track surveys, which allowed the development of a method to discriminate small Neotropical spotted felids (Chapter 5). Among the ~248,000 photographs we collected were the first photographs of two xenarthrans for the INP, i.e. the Giant Anteater (*Myrmecophaga tridactyla*) and the Greater Naked-Tailed Armadillo (*Cabassous tatouay*; Chapter 6). Additionally, we assessed the soil's ecological integrity using bait lamina probes (Chapter 7) and the water's bacteriological content in the São João River, one of the main INP rivers (Chapter 8). The INP is definitely under different pressures caused by human legal and illegal activities. While economic profits provided by the INP visitation and fame account for most of the regional economy, and while this capital is also injected into other less popular Brazilian Conservation Units, instead of the legitimate gratefulness, the INP is poisoned with agrochemicals through its generous watercourses network, and is being violated by poachers, fishermen, thieves and palm-tree extractors. Our findings depict the utter ecological importance of the INP given it represents 80% of what is left of inland Atlantic forest in Paraná state and because it still shelters forest specialists keyspecies (e.g. jaguars, *Panthera onca*). The INP regional context is where we should concentrate conservation efforts, i.e. fostering habitat connectivity and ecological integrity, and promoting educational campaigns to raise public awareness about the INP invaluable worth in a loving, collaborative way. Activities should promote reconnection between locals and the INP, so that the relationship switches from an exploitation perspective to a conservation perspective.

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GENERAL INTRODUCTION

In the area of conservation science, a lot of efforts are made in characterizing and monitoring habitat disturbance itself and its impacts on the rest of the ecosystem, may it be water courses, soils, wildlife, plants or any process associated.

The Atlantic forest seems the perfect candidate to look at habitat alteration given its paradoxical situation of being almost as rich as the Amazon biome in terms of fauna and flora, and yet, only 7 to 12% of its original distribution remain (Ribeiro et al., 2011).

Following the same reasoning, the Iguaçu National Park in southwestern Brazil (INP) is also a perfect candidate to focus on for (1) it is the last large inland Atlantic forest continuum under federal protection in southern Brazil, (2) it is the last wildlife natural refuge accounting for 80% of what is left of Atlantic forest in the 199,314.850 km² of the state of Paraná, (3) it is 77-years old and has virtually no historical peer-reviewed data on its wildlife, and last but not least, (4) it is looked at by the rest of Brazil and its 90 national parks as a reference in terms of management and conservation.

The INP, or even the Atlantic forest as a whole, is a fractal of what is happening everywhere human conflict with nature; it is usually more tangible when humans are established close to preservation areas. Typically, these areas were created imposing limits to human villages, sometimes even expropriating families with monetary compensations. Psychologists are rarely involved and locals are rarely heard by local decision-makers, leaving unhealed wounds which do not vanish as time goes. They become instead the weak points through which unethical manipulation can be successful (Mollica, 2008).

When looking at a map, the INP localization is at the junction (or "*foz*" in Portuguese) of two major rivers, i.e. the Iguaçu river, which appears in Curitiba, 700 km from Foz do Iguaçu, and the Paraná River, the second largest river in South America, with 4880 km of extension, which flows from the state of Minas Gerais in central Brazil to the Prata river in southern Argentina. Northward, the municipality of Foz do Iguaçu is delineated by the Itaipu dam and artificial lake. Itaipu Binacional has built the world-largest hydro plant on the Paraná river in the 70's and has therefore physically restricted human's growth and expansion northward, on top of altering the climate regionally (Limberger & Cecchin, 2012). The INP is located south, and extends eastward. Therefore, the city of Foz do Iguaçu can only grow eastward, around the highway BR-277 which connects to the rest of Brazil, and through the INP agriculture-dominated buffer zone. Brazil is likely the South-American country which is thriving the most given its size and its incomparable natural resources. Brazil has been keeping up with unfair competition with industrialized countries by literally raping its land of all its resources in a galloping mode. Technically, it works, but success is short-lived. As expected from Brazilian leaders who look at North America or Europe as role models, the entire Brazilian population puts more value in a noble wood deck in the yard than in an old-growth forest. Soon, without adequate educational programs, it is easy for locals to see Nature as an impediment to prosperity.

As stated above, there is little peer-reviewed literature on the INP, most of what is available consists of punctual undergrad papers (e.g. Guimarães et al., 2003 on mosquitoes; Bortolini et al., 2010 on an algae family, etc.). This thesis is the fourth graduate research that was conducted on mammals and carnivores in the INP. The first PhD thesis, which is referred to several time in this text is Crawshaw's legacy on jaguars and ocelots (Crawshaw, 1995). While Peter's data are 30 years old, Peter's thesis is cited up to these days by carnivore specialists in conservation gatherings as until recently, it was the only scientifically sound information available on jaguars and ocelots for this conservation unit. More recently, Vogliotti (2008) and Silva (2014) wrote on deers and mammals respectively, but these theses do not provide material to implement relevant conservation strategies nor do they provide population estimates for any species.

As common sense suggests, conservation or management require benchmarks to refer to in order to have spatial-temporal perception of current ecological trends (Hunter Jr., 1996), so that adequate strategies can be drawn and undertaken by park managers and local conservationists. The main objective of this research was to provide a first pragmatic characterization of the INP current situation in order to promote its conservation. The INP welcomes annually over 1.5 million visitors from all over the world and one of the most frequent question we hear from visitors is "how many jaguars are left in the park? How many deers? How many tapirs?". While the park will soon turn 80 years old, no one knows.

Numbers. Humans need numbers to visualize things through reference systems. Knowing the number of individuals per species is indeed a very useful tool for conservation and management (Martins et al., 2007). Colleagues from the Institute of Zoology have been working on developing such a tool – the Random Encounter Model (REM) - which derives density estimates for species using the theory of gas molecule collision (Rowcliffe et al., 2008). They have tested it on captive ungulates and verified its robustness. The REM was also used and further tested with pine martens in Italy (Manzo et al., 2012) and more recently, in Africa with lions (Cuzack et al., 2015). Chapter 1 is the first use of the REM technique on a whole community of mammals, and on the American mainland. It provides robust density estimates for seventeen mammal species in the INP and in its buffer zone. Prey and generalist species were found in high numbers while other forest specialists were rarer. Hypotheses to explain our findings are discussed at the light of the most up-to-date knowledge at the regional level or in similar ecological contexts.

Once one knows the number of individuals there are per species, one needs to look at the species' distribution across the landscape. The INP region could be pictured as a large continuous forest remnant surrounded by a sea of soy-corn-wheat crops mixed with pastures, farms, plantations and disturbed secondary vegetation thickets. The Iguaçu region and other deforested areas elsewhere are very much alike. How do animal respond to that gradient of habitat alteration? How do they react to foreign environments or to forest edges? What about habitat connectivity and the need for the animals to wander in other areas to ensure genetic flux? In Chapter 2, we used multivariate analyses to look at habitat alteration and we observed how these environmental gradients could explain species distribution. Species' sensitivity and representativeness were looked at individually and discussed. While certain species showed zero tolerance to poor habitat (e.g. giant anteater (*Myrmecophaga tridactyla*) or jaguar (*Panthera onca*), some highly adaptive species such as coatis (*Nasua nasua*) or tayras (*Eira barbara*), appear to be thriving in man-made disturbed landscapes.

Ecology and our understanding of the infinitely complex, correlated relationships occurring within ecosystems have rapidly evolved (Kay, 2000). After using surrogates in the 90's to assess the ecological integrity of a given ecosystem (e.g. Fleishman et al., 2000), we have reached a point in our understanding where it becomes necessary to look at ecosystems considering the human communities living within them, some refer to this as "eco-social systems" (Waltner-Toews & Kay, 2005). Chapter 3 deals with a social-historical dimension of the INP that has had tremendous ecological repercussions over the past three decades. It tackles an everlasting wound hidden in the INP, i.e. a dirt road that was located in its core area. It was called Settler's Road due to its primary role in the late 50's, when colonizers used it to reach the Iguaçu River. It has been a headache all along because locals and INP managers could not come to an agreement, which depicts a communication crisis. Between 1986 and now, there were many frictions between locals and local authorities, and the police was forced to keep the road closed and under permanent surveillance. While this is a historical and social wound, it is also fertile ground for greedy politics to gather a few votes. The insidious part of the story is that a deputy used this regional wound to write a bill where, not only was he asking to re-open that road but he was also suggesting creating another category of Conservation Units (i.e. "parkways") in the Brazilian federal legislation (for more details, see the Brazilian National System of Conservation Units law n°. 9.985/2000). In truth, this bill came about at the time where Brazil was daily red-flagged with countless cases of environmental backtracking, and "parkways" were just another strategy to legally get authorization to open roads in conservation units, weakening and fragmenting even more the remaining Nature preserves (Baptiston & Garcia, 2014).

The carnivore feces encountered were georeferenced, silica-dried and sent to Dr. Salisa Rabinowitz from the Global Felid Genetics Program at the American Museum of Natural History in New York for species identification analysis. When the results came in, we were surprising as we had collected 10 feces samples of Geoffroy's cat (*Oncifelis geoffroyi*), which was not known to occur in the region. DNA analyses were run twice and the same results were confirmed. Such striking news coincided with the capture of a specimen in the outskirts of Foz do Iguaçu. Chapter 4 discusses the implication of such findings for the Iguaçu region and was successfully published in the IUCN Cat Specialist Group journal called Cat News in 2014.

It is also relevant to stress that in order to provide robust results from inventories, species identification is key and is entirely dependent on the observer's skills. It has been noted a lot of confusion especially among the little spotted cats inhabiting the tropics (Haemig, 2001; Wang, 2002; Oliveira et al., 2010). They are elusive, most are nocturnal, some are arboreal, and all often display niche overlap when sympatric (Wang, 2002). Chapter 5 is an attempt to rid the subjectivity of observer's identification by a series of measurements on tracks. We used multivariate analysis on footprints measurements from known captive and free-ranging wild individuals and successfully identified which track outline measurements were useful for species discrimination. The objective was to provide a robust technique to reduce the amount of mistakes produced among the scientific community, and sometimes in educational material. This chapter was published in the Brazilian Journal of Nature Conservation in 2012.

Our data collection on mammals mainly consisted of a 24-month continuous camera-trap monitoring, along with track surveys and feces collection. We obtained an enormous amount of information, among which were first photographic records for the INP of some species, such as the giant anteater and the greater naked-tailed armadillo (*Cabassous tatouay*). Chapter 6 presents in details these historic findings and compare with what is known in the literature about these species' ecology and conservation situation. In addition to these two species, we present occurrence information and relative abundance for the nine-banded armadillo (*Dasypus novemcinctus*), the six-banded armadillo (*Euphractus sexcinctus*), and the lesser anteater (*Tamandua tetradactyla*) in the INP and in its buffer zone. The IUCN xenarthran specialist commission informed us such data were cruelly needed for this part of their range and the manuscript is currently being reviewed.

Globally, it is safe to affirm that humans are increasingly aware of their contribution to worldwide alterations. As a matter of fact, landscape changes are increasingly fast and impact everything living in the system, including ourselves. While charismatic wildlife receives lots of attention and research efforts, the less charismatic soil is however where it all begins. Only recently have we started to look at soil as a living organism. Clearly, we have not started to treat it with the respect it requires to thrive. As Primavesi (2006) relevantly stressed, deforestation replaces forest cover with sugar cane or soy crops, causing a lot of water evaporation as wind freely brooms over the naked fields. Moreover, exposed soils are compacted with the use of heavy machinery and are hotter than soils that benefit from the shade and cool air of the forest. A living soil creates a thriving ecosystem. Soil organisms' activity has extensively been used as a shortcut to assess ecological integrity in a wide range of environmental issues. How do soil's microorganisms respond to habitat changes? In order to look at this research question, we applied a method that was poorly used in Brazil, i.e. the bait lamina probes (Von Törne, 1990). Bait-lamina are perforated plastic sticks in which minuscule apertures are filled with a mix (i.e., bait) usually composed of cereal bran, activated charcoal and cellulose. This technique is based on the assumption that apertures will be emptied out as a result of soil organisms' feeding activity. Bait lamina probes are inserted vertically into top soil for a determined period of time. It has shown to be robust in various scenarios, and has gained popularity when compared to other more energy-consuming methods such as litterbags, mini-containers, or wheat-straw degradation (Kula & Römbke, 1998). In Chapter 7, we compared the feeding activity in three zones, i.e. within the INP boundaries, on the INP transition zone and in the INP buffer zone. We discuss the ecological implications of our findings and assess the technique itself.

In addition to wildlife and soil, landscape changes also affect waters. Watersheds are heavily impacted by soil use. *Y'guaçu* in Guaraní, the indigenous name means "Big Waters". The INP buffer zone has a very rich network of water courses. Water is the most important element to all living forms

(Karr & Chu, 2000). Exception made of the Floriano River, which is the only river to appear and disappear within the INP boundaries, all rivers that run through the INP have their head waters located in the buffer zone, in the midst of cereal crops and pastures. They can easily be located because they are usually surrounded by the remainder of forest vegetation as this is a legal requirement from the Brazilian environmental legislation to preserve riparian forests. Empirical data have already shown that the ecological integrity of our rivers is threatened in many ways, e.g. agrochemicals, solid waste, erosion, drainage, artificial lakes, etc. (Bertrand & Garcia, 2010). This study consisted of a series of ecological assessments to verify how the INP handled all the pressures from anthropization.

Ecological services for instance, are described as natural vital processes that guarantee the integrity and life of organisms living in a given area (Tonhasca, 2004). Water purification is one of the ecological services a preserved forest usually does, along with other services. According to Parkes & Horwitz (2009), water could be used as a “surrogate for the distribution of all natural resources”. In Chapter 8, we conducted bacteriological analyses on the São João River, one of the main INP rivers, from its source to where it merges into the Iguaçu River. We described what we found in details and discuss the implications for public health and for the INP conservation situation.

Summarizing, the objective of this thesis is to bring to the scientific and academic community the first characterization of the INP conservation conundrum. Efforts were concentrated to gather as much information as possible on mammals using camera-trapping, feces analysis and track surveys. Furthermore, the Settler’s Road story is a scar, which should be used to orient and define human-oriented strategies to INP preservation. And last but not least, soil and water analyses provide important broader insights on the general ecological condition at the regional scale. In a nutshell, this thesis intends to become a reference document readily usable by the park managers to develop sound applied conservation and management strategies.

CHAPTER 1

CHARACTERIZATION OF THE MAMMAL COMMUNITY USING THE REM METHOD IN THE IGUAÇU REGION

ABSTRACT

Pioneer 24-month monitoring using camera-traps was conducted between September 2012 and October 2014 in the region of the Iguaçu National Park in southwestern Brazil. We used the Random Encounter Model (REM) proposed by Rowcliffe et al. (2008) to calculate density estimates for 17 mammal species for the park and its buffer zone. Our results are the first estimates for this 77-year old Conservation Unit, which annually welcomes 1.5 million visitors. We compared our results with other research conducted in the same eco-region or in similar ecological contexts. Environmental factors such as the disappearance of top predators, intense poaching pressure, and recent bamboo seed production are potential causes for prey species increase, as observed for white-collared peccaries, red brockets, lowland tapirs and rodents. We also observed predominance of ubiquitous generalist, alteration-tolerant coatis in and out the park. Domestic dogs were present in the same proportions in and out the park, almost outnumbering ocelots. Conservation implications of our findings are discussed. This work is the first REM application (1) at the community level, and (2) on the American continent.

KEYWORDS

Mammals, Density estimates, REM, Iguaçu National Park, Conservation, Brazil.

CAPÍTULO 1

CARACTERIZAÇÃO DA COMUNIDADE DE MAMÍFEROS NA REGIÃO IGUAÇU UTILIZANDO O MÉTODO MEA

RESUMO

Um monitoramento pioneiro de 24 meses com o auxílio de armadilhas fotográficas foi realizado de setembro de 2012 até outubro de 2014 na região do Parque Nacional do Iguaçu no sudoeste do Brasil. Recorremos ao Modelo de Encontro Aleatório (MEA) proposto pelos Rowcliffe et al. (2008) para calcular estimativas de densidade para 17 espécies de mamíferos para o parque e sua zona de amortecimento. Nossos resultados fornecem as primeiras estimativas para uma unidade de conservação de 77 anos de idade, que recebe anualmente 1.5 milhão de visitantes. Comparamos nossos resultados com outras pesquisas realizadas na mesma eco-região ou em contextos ecológicos semelhantes. Fatores ambientais como a disparição de predadores de topo de cadeia, pressão de caça furtiva intensa, e a recém produção de sementes dos bambus no PNI são causas potenciais para explicar o aumento drástico de espécies de presas, como foi observado com os catetos, os veados mateiro, as antas e os roedores. Também observamos predominância de uma espécies generalista, ubíqua, e resistente à alteração do hábitat: o coati, tanto dentro como fora do parque. Cachorros domésticos foram presentes nas mesmas proporções fora e dentro do parque, mais numerosos do que as jaguatiricas. As implicações conservacionistas de nossos resultados são discutidas. Este trabalho é a primeira aplicação do MEA (1) ao nível da comunidade, e (2) no continente Americano.

PALAVRAS-CHAVES

Mamíferos, estimativas de densidade, MEA, Parque Nacional do Iguaçu, Conservação, Brasil.

INTRODUCTION

Obtain the number of individuals present in an area is very helpful when it comes to propose adequate conservation and management strategies (Rowcliffe et al., 2008; Trolle et al., 2008; Nuñes-Peres, 2011). In actuality, this is the objective of any researcher working on mammal species, especially on elusive carnivores (Sollmann et al., 2011; Chandler & Royle, 2013; Cusack et al., 2015a). However, most often than not, logistics, equipment or fund restrictions resulted in capture frequency indexes instead densities estimates (Kelly, 2003; Moraes-Junior et al., 2003; Trolle, 2003; Di Bitetti et al., 2008). The problem with such indices is that they do not provide individual recognition and therefore can over or underestimate populations. Camera-traps started being used in the early 2000's in the Neotropics (Maffei et al., 2002; Noss et al., 2003) and are now the most commonly chosen method to tackle conservation or ecology studies (O'Connell et al., 2011). Karanth's (1995) work with tigers in India inspired the world to use capture-recapture (C-R) models using camera-traps to extract density estimates for marked species, typically, spotted or striped felids. He proved it was possible to estimate densities using a grid system and a buffer calculation initially based on half the size of the home range of a female of the species of interest. Since then, most researchers followed his path and used the C-R models on different species (e.g., Karanth & Nichols, 1998; Trolle et al., 2008; Paviolo et al., 2009; Foster & Harmsen, 2012; Tobler et al., 2013) but it remained essentially restricted to individually identifiable species (Rowcliffe et al., 2008).

As the C-R method was being used and assessed worldwide, several critics appeared, suggesting optimizing camera placements, reducing camera spacing and increasing the recapture window to up to 60 days (Noss et al., 2003; Foster & Harmsen, 2012). The calculation of the Mean Maximum Distance Moved (MMDM), also referred to as 'buffer', also raised many questions (Noss et al., 2003; Dillon & Kelly, 2008; Maffei & Noss, 2008). Some authors suggested increasing MMDM to at least four average home ranges of the target species (Maffei et al., 2011). Density estimates have proved to be extremely sensitive to the calculation of the effective survey area, which depends on the buffer surrounding traps (Maffei et al., 2011; Torres et al., 2012).

More recently, spatially explicit capture-recapture (SECR) modeling appeared as a potential solution for the effective trapping area problem (Foster & Harmsen, 2012; Chandler & Royle, 2013). SECR have proved to perform better than C-R models, provided the home ranges considered are not highly asymmetric or elongated. SECR do provide unbiased density estimates for individually identifiable species, like spotted or striped felids (e.g. Sollmann et al., 2011). While this represents great progress for the C-R modeling, it does not solve the problem for species for which individuals cannot be identified by specific marks, which represent the majority of species in camera-trap censuses (Tobler et al., 2008; Cusack et al., 2015a).

Foster & Harmsen (2012) analyzed 47 camera-trap studies and the flaws they have found in too many studies clearly emphasize that the assumptions to C-R and even SECR are constraining and hardly respected in living ecosystems.

In 2001, Carbone et al. suggested that camera-trapping results and home range sizes could likely be used to derive density estimates for species for which individual recognition was not possible. They also suggested photographic rates were correlated to species' absolute abundance. The Random Encounter Model (REM), which provides density estimates without the need for individual recognition, was born a few years later (Rowcliffe et al., 2008). The approach used the gas molecule theory and derived density estimates from contact rates between moving animal and static camera detection zone (Cusack et al., 2015a). Unlike C-R models, the REM does not require the use of a systematic grid (e.g. Karanth et al. 2006). Nor does it require to find the best trap sites or to avoid "holes", i.e. areas where the elusive species has a 0 capture probability (Karanth & Nichols, 2000). However, the REM key assumption is random camera placement in relation to animal movement (Rowcliffe et al., 2008) as this could create biased results for encounter rates (Cusack et al., 2015a). While it has been stressed that the gas molecule scenario might difficultly apply to animal movements and camera detection zone (Rovero & Marshall, 2009), the technique has proven robust in estimating density estimates for ungulates (Rowcliffe et al., 2008), pine martens (Manzo et al., 2012), and lions (Cusack et al., 2015a), despite potential conflicts with its underlying assumptions.

The present study was conducted in one of the largest fragment of charismatic yet highly threatened Atlantic forest. With over 20,000 plant species, over 650 vertebrate species, the Atlantic forest is one of the most valuable biodiversity hotspots (Myers et al., 2000; Mittermeier et al., 2005; Tabarelli et al., 2010) and yet, it is the most threatened and decimated biome throughout Brazil (Mittermeier et al., 1982; Ranta et al., 1998). Since the first European settlements in the 16th century, this biome suffered from timber exploitation, soil use conversion to agriculture, industry and urbanization (Keuroghlian et al., 2004). While the Atlantic forest is home to thousands endemic species - most of which still need scientific studies - and may shelter between 1 and 8% of the world's total species (Silva & Casteleti, 2003), less than 12% of its original distribution remains (Ribeiro et al., 2009). The Iguaçu National Park (INP) is comprised in this number. It is a 77-year old conservation unit, the second national park to be created in Brazil. It is also the most visited park in Brazil, annually welcoming over 1.5 million visitors from all over the world. It became a World Heritage Site in 1986 given its scenic beauty. Despite being such a national and international reference, the INP has not received the scientific attention its outstanding ecological value deserves, and there are no population estimates available for its fauna up to this day, except for jaguars and ocelots based on one doctoral telemetry study conducted between 1985 and 1995 (Crawshaw, 1995).

The objectives of the present study were (1) to characterize the mammal community occurring in the region of the INP and (2) to provide population estimates for as many mammal species as possible using the REM method. Last, we compare and discuss our findings and existing peer-reviewed data from the closest contexts for species which are known to play important ecological roles to the balance of the ecosystem. To our knowledge, this work is the first application of the REM on wildlife on the American mainland.

MATERIAL AND METHODS

Study Area

The Iguaçu National Park (INP; 25°05'S - 25°41'S, 53°40'W - 54°38'W) accounts for 1,850 km^2 of continuous subtropical forest in south-western Brazil. It was created on January 10, 1939 through federal decree nº. 1.035 (Andrade, 2003; Baptiston & Garcia, 2014). The INP is located in the Upper Paraná Atlantic Forest ecoregion (Di Bitetti et al., 2003). Native vegetation is predominantly semi-deciduous moist broad leaf forest, Araucaria (Brazilian pine) forests are however well represented in the INP's northeastern tip, where altitude reaches ≥600 meters (MMA/IBAMA, 1999). Topography consists of hills of inland Atlantic forest and iron-rich red latossol and nitossol soil types (Embrapa, 2013). The INP is the most important conservation unit of south and southwestern Brazil. It is the one and only public continuous fragment of inland Atlantic forest under federal protection (MMA/IBAMA, 2006).

Camera-trapping & the REM method

Sixteen Reconyx P900 camera units were randomly installed at approximately 20 cm from the ground, locally electing most strategic locations to ensure capture success, i.e. cross roads of game paths or poaching trails, brook edges, marshlands, etc. (Trolle, 2003; Negrões et al., 2010). While this may somewhat seem conflicting with the REM randomness assumption (Rowcliffe et al., 2008), we believe it may interfere only minimally with inferences at community level as (1) overall camera display was totally random and as (2) sampling effort was >1400 camera trap nights (Cusack et al., 2015b). Camera trap spacing varied anywhere between 60 m and 1 km. These cameras do not produce sound or flash, reducing the odds of being stolen and/or destroyed by infringers in the INP. Cameras were set on fire shooting mode, taking 15-frame sequences for as long as movement was detected. We started installing cameras in the INP and its buffer zone (up to 5 km from the INP edge) near São Miguel do Iguaçu (Paraná, Brazil; Fig. 1) and progressively went westward, changing camera locations approximately every 30 days, for 25 consecutive months, until we reached the INP visitation area. All microhabitat types were covered through our monitoring to ensure representativeness (Cusack et al., 2015a).

We defined one 'event' by a sequence of images of the same animal. Each wildlife photograph was individually georeferenced and ecology notes were added when judged necessary (i.e. interspecific relationships, presence of juvenile, perception of camera by animal, couple, group, etc.). Density estimate (D) is provided by the REM according to the following equation (Rowcliffe et al., 2008):

$$D = \frac{y}{t}\frac{\pi}{vr(2+\theta)}$$

where *y*/*t* is the trapping rate, *v* is the animal speed of movement through the detection zone, whose dimensions are described by π (angle) and θ (depth).

A grid was built using *in loco* distance measurements to measure all distances corresponding to any trajectory moved within the detection zone. If the animal would go in a zigzag mode, every angle/distance of such broken-line path would be measured. Angles were measured *ex situ* using

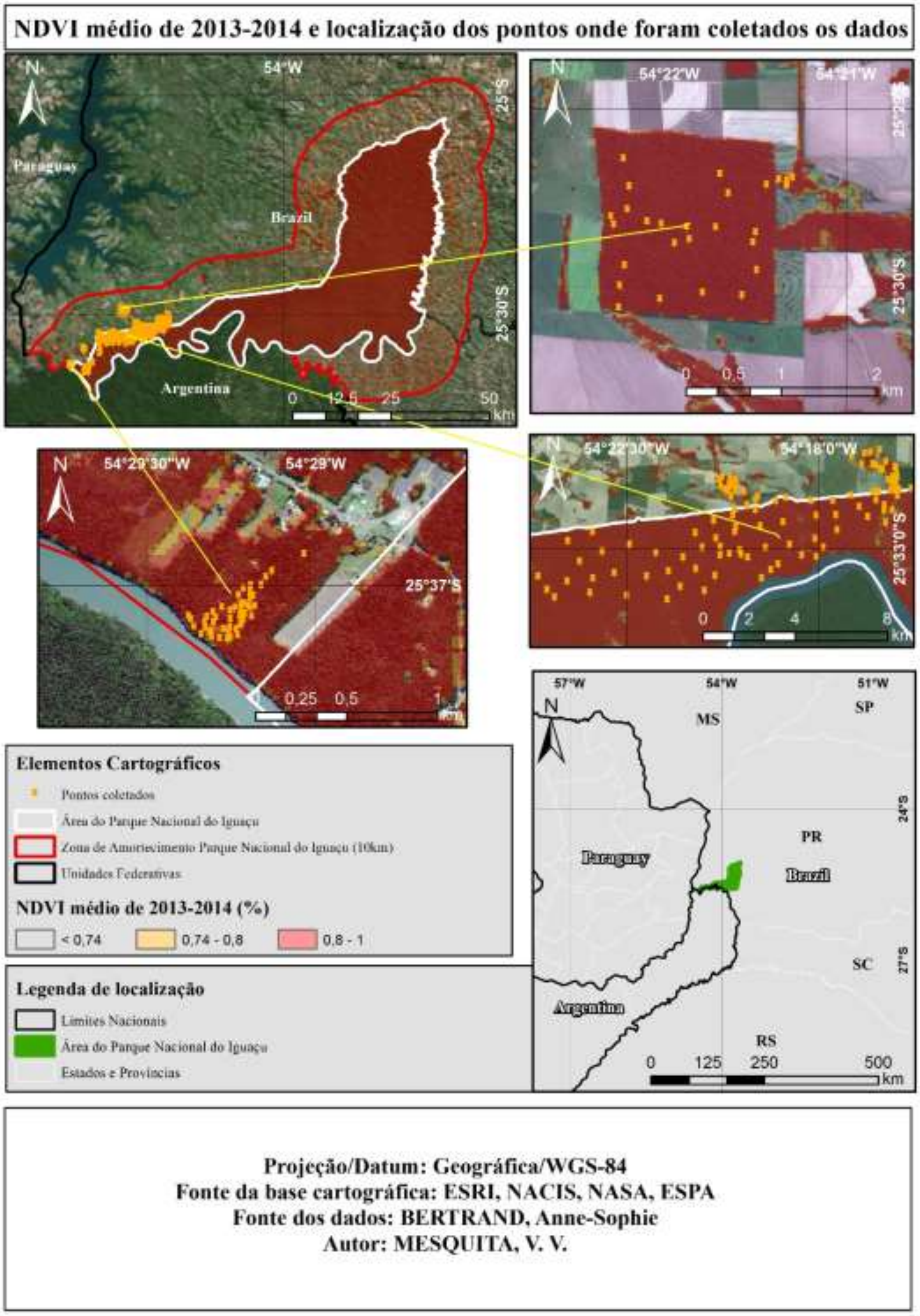

Figure 1. Iguaçu National Park location in south western Brazil and sampling effort (yellow dots are sampling stations).

the x-position of the mouse on each photograph using PixelRuler™ and later converted into radians. We used the center of the animal body to standardize both angle and distance measurements. Camera detection zone was also measured *ex situ*, using a measuring tape to calculate the width and depth of the detection zone. Detection zone models used animal body mass, activity levels, and location (i.e. INP or buffer zone). Data distribution and variable transformation analysis and model selection were assessed using the Akaike Information Criterion (Akaike, 1974; Aho et al., 2014). REM calculation was done using the R ‘activity’ package (Rowcliffe, 2014).

RESULTS

We monitored 130 km^2 of the INP and its buffer zone in 755 days. Sampling effort was 6,190 trapping days. We collected 247,693 photographs, which corresponded to 7,340 individual record events, which corresponded to an encounter rate of 118.58/100 cam-days. Data collection was successful in 193 of 207 established sampling stations. Camera malfunctions in the remainder prevented collecting photographs. Detection rates were higher in the INP than in its buffer zone, i.e. 59.22% vs. 40.78% respectively, while sampling effort was lower in the INP (84 stations) than in the buffer zone (109 stations).

Relative abundance was obtained for 30 taxa in and out of the INP (Table 1 and 2). Most represented species in the INP were red brockets, agoutis, white-collared peccaries, and coatis. Forest specialists like tapirs were also very well represented, along with opossums and armadillos. Outside the park boundaries, agoutis were the most frequent animal, followed by coatis, armadillos, opossums and, to a lesser extent, red brockets. Some forest dwellers were restricted to the INP habitats, i.e. jaguar, puma, skunk, hedgehog, giant anteater and greater naked-tailed armadillo (see Chapter 2).

Density estimates were calculated for species with over 8 occurrences. Prey density estimates were successfully obtained for ten species within the INP and 11 in its buffer zone (Table 3 and 4). The INP highest densities were obtained for rodents, agoutis, collared peccaries, coatis, and opossums. In the buffer zone, coatis, agoutis and capuchin monkeys were the species with the highest densities.

Carnivore density estimates could successfully be obtained for six species in the INP and seven in its buffer zone (Table 5 and 6). Coati density was much higher than the remainder of carnivore species. There were almost as many hunting/domestic dogs as ocelots within the boundaries of this conservation unit.

Activity patterns were plotted for 15 species (Fig. 2). Diurnal species were agoutis, coatis, capuchin monkeys, capybaras, tayras, peccaries and dogs. Nocturnal species were nine-banded armadillos, pacas, rabbits, possums and rodents. Ocelots and raccoon showed cathemeral patterns. Tapirs, agoutis and red brockets showed bimodal activity patterns, with peaks in the first hours of the day and in the first hours of the night.

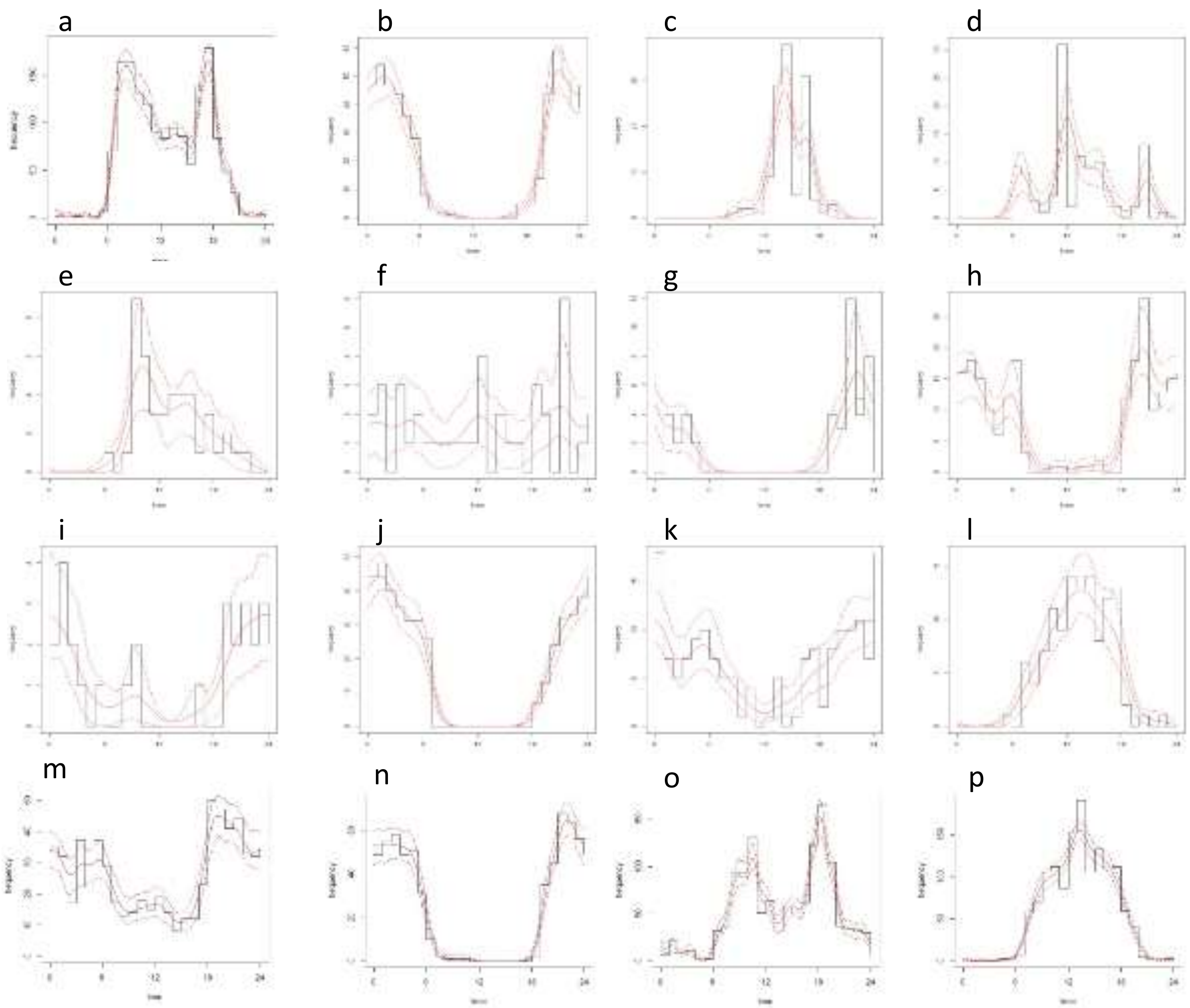

Figure 2. Activity patterns for 15 most common mammal species in the Iguaçu National Park region (2012-2014). a: Agouti (*Dasyprocta azarae, n* = 1565); b: Nine-banded Armadillo (*Dasypus novemcinctus, n* = 485) ; c: Black-capuchin Monkey (*Cebus apella, n* = 126); d: Capybara (*Hydrochaerus hydrochaeris, n* = 111); e: Domestic dog (*Canis lupus, n* = 44); f: ocelot (*Leopardus pardalis, n* =37); g: Paca (*Agouti paca, n* = 43); h: Brazilian rabbit (*Sylvilagus brasiliensis, n* = 200); i:Raccoon (*Procyon cancrivorus, n* = 27); j: Rodents (*n* = 402); k: Tapir (*Tapirus terrestris, n* = 148); l: Tayra (*Eira barbara, n* = 116); m: red brocket (*Mazama americana*, *n* = 621); n: opossum (*Didelphis* spp.; *n* = 578); o: White-Collared Peccary (*Tayassu tajacu*, *n* = 1272); p: Quati (*Nasua nasua*, *n* = 1357).

Table 1. Naive occupancy (i.e. observed proportions of occupied sites) of carnivore species in the Iguaçu National Park (INP) and its buffer zone (BZO; 2012-2014).

Species	PNI (%)	BZO (%)
Nasua nasua	61.90	56.88
Eira barbara	19.05	22.94
Leopardus pardalis	13.10	10.09
Leopardus wiedii	13.10	2.75
Canis lupus	11.90	13.76
Leopardus tigrinus	9.52	3.67
Puma concolor	8.33	0.00
Herpailurus yagouaroundi	4.76	7.34
Panthera onca	3.57	0.00
Cerdocyon thous	2.38	7.34
Galictis cuja	1.19	0.00
Procyon cancrivorus	0.00	9.17

Table 2. Naive occupancy (i.e. observed proportions of occupied sites) of prey species in the Iguaçu National Park (INP) and its buffer zone (BZO; 2012-2014).

Taxa	INP (%)	BZO (%)
Mazama americana	84.52	42.20
Dasyprocta azarae	80.95	65.14
Pecari tajacu	67.86	16.51
*Didelphis**	55.95	50.46
Dasypus novemcinctus	54.76	51.38
*Roedentia***	50.00	32.11
Tapirus terrestris	47.62	2.75
Sylvilagus brasiliensis	16.67	23.85
Caniculus paca	8.33	5.50
Myrmecophaga tridactyla	3.57	0.00
Hydrochoerus hydrochaeris	2.38	5.50
Sciurus aestuans	2.38	0.92
Tamandua tetradactyla	2.38	3.67
Cabassous tatouay	1.19	0.00
Cebus apella	1.19	18.35
Coendou prehensilis	1.19	0.00
Euphractus sexcinctus	1.19	0.92
Lontra longicaudis	0.00	0.92

* Genus ** Order

Table 3. Prey population estimates for the Iguaçu National Park (2012-2014).

Taxa	Density (ind./km^2)	Estimate SE*	Record nb.	Estimated nb. indiv.
Rodentia**	50.00	13.48	289	92515.86
Dasyprocta azarae	46.30	9.11	958	85659.62
Pecari tajacu	43.22	9.31	1207	79950.37
Nasua nasua	21.21	3.68	510	39251.48
Didelphis***	19.22	3.96	353	35555.18
Mazama americana	8.69	1.55	442	16077.15
Dasypus novemcinctus	4.99	0.98	125	9225.38
Sylvilagus brasiliensis	2.63	0.94	36	4872.68
Tapirus terrestris	1.70	0.40	144	3142.73
Caniculus paca	1.26	0.74	18	2336.00

* Standard Error **Order ***Genus

Table 4. Prey population estimates for the INP's buffer zone (2012-2014).

Taxa	Density (ind./km^2)	Estimate SE*	Record nb.	Estimated nb. indiv.
Nasua nasua	29.10	5.42	847	68389.36
Dasyprocta azarae	24.22	24.22	607	56921.64
Cebus apella	20.57	8.30	119	48333.39
Rodentia**	16.11	3.69	113	37867.19
Dasypus novemcinctus	11.86	3.92	360	27872.09
Didelphis***	10.11	1.99	225	23755.07
Sylvilagus brasiliensis	9.90	3.78	164	23265.32
Mazama americana	2.91	0.62	179	6838.64
Pecari tajacu	1.92	0.62	65	4521.35
Hydrochaerus hydrochaeris	1.63	1.17	109	3828.55
Caniculus paca	1.45	0.78	25	3404.82

* Standard Error **Order ***Genus

Table 5. Carnivore population estimates for the Iguaçu National Park (2012-2014).

Species	Density (ind./km^2)	Estimate SE*	Record nb.	Estimated nb. indiv.
Nasua nasua	21.22	3.68	510	39251.48
Eira barbara	1.94	1.03	65	3596.43
Leopardus wiedii	0.56	0.24	13	1034.26
Leopardus pardalis	0.39	0.17	16	723.23
Canis lupus	0.38	0.17	18	714.01
Leopardus tigrinus	0.25	0.19	12	459.73

Obs. Species with 8 or less records are not listed

Table 6. Carnivore population estimates for the INP's buffer zone (2012-2014).

Species	Density (ind./km^2)	Estimate SE*	Record nb.	Estimated nb. indiv.
Nasua nasua	29.10	5.42	847	68389.36
Eira barbara	1.16	0.49	51	2732.98
Canis lupus	0.46	0.19	26	1082.79
Procyon cancrivorus	0.43	0.19	27	1012.60
Leopardus pardalis	0.42	0.18	21	996.26
Cerdocyon thous	0.35	0.19	20	837.29
Herpailurus yaguarondi	0.07	0.03	10	157.56

Obs. Species with 8 or less records are not listed

DISCUSSION

This study represents a pioneer attempt to characterize and quantify the mammal community in the 77-year old INP. Due to the lack of historical density estimates for the INP, efforts were made to compare our results to studies with the most similarities, i.e. regional studies, same biome studies and/or similar animal assemblages. Findings and conservation implications are discussed. The use of the REM is also discussed.

Carnivores

Ocelots

Ocelots were more abundant and more widely distributed than any other felid in the census, which corroborates recent presence and occupancy studies conducted in both sides of the INP (Di Bitetti et al., 2010; Silva, 2014). They were found in both the Iguaçu National Park and in its buffer zone, being even more frequently recorded outside of the park then within its boundaries (Table 5 and 6). It has indeed been stated elsewhere that ocelot density does not appeared to be correlated with habitat type (Maffei et al., 2005), being found in any cover type, from pristine to highly disturbed sides (Oliveira et al., 2009). According to the review made by the authors, ocelots are more abundant than smaller cats, with margays ranking second, regardless of habitat type. As for their activity pattern, ocelots are found to be active both during day and night time (Di Bitetti et al., 2006). Crawshaw (1995) obtained 41% of his telemetry activity locations at night, and 34% during day time, in the INP, which is consistent with our findings (n = 37, Fig. 2). Also, they have shown that prey biomass and body mass, which are acknowledged as influencing factors to carnivore density and space use (Litvaitis et al., 1986; Carbone & Gittleman, 2002), are not correlated to ocelot density.

Small cats have larger home range size and population densities 3.5 times lower than expected; while ocelots have densities 1.3 times lower than expected. While ocelots are sympatric to both large and smaller felids, smaller felids seem to reach high densities only when the ocelot is rare or absent. Last, according to Donadio and Buskirk (2006), intraguild competition through killing peaks when the larger species is 2 to 5.4 larger than the smaller species, and ocelots are thought to create a competitive release over the three smaller species, i.e. margays, oncillas and jaguarundis (Di Bitetti et al., 2010). The ocelot is indeed 2.2-2.4 times larger than the three other smaller felids, and dominance is correlated with body mass (Fuller & Sievert, 2001). Finally, Oliveira et al. (2009) described the “ocelot effect” or “pardalis effect” as the negative pressure ocelots exerted onto smaller sympatric felids because of their high adaptability (Goulart et al., 2009), generalist behavior, tolerance to habitat alterations (Jacob, 2002) and dominance over smaller felids (Oliveira et al., 2008). Likewise, smaller felid numbers would increase as the ocelot number decreases. Ocelot densities were found to vary greatly from 0.08 to 1.00 individual/km^2, averaging 0.31 ± 0.22 ind/km^2 (n = 22 studies, Oliveira et al., 2009). Results from a 39-camera-trap study in the neighboring Iguazú National Park in Argentina revealed densities estimates ranging from 0.13 ± 0.03 to 0.19 ± 0.04 ind./km^2 (Di Bitetti et al., 2006) and the only historical data we have for the study area is Crawshaw’s

PhD telemetry PhD study between 1985 and 1995, where he obtained 0.14 ± 0.02 ind./km^2 based on 21 monitored individuals. Our density estimates (Table 5) are likely higher than before given the rarefaction of jaguars (Moreno et al., 2006), the profusion of prey availability (i.e. rodent populations outbreaks from bamboo seed production in the INP in 2006 and 2009; Jaksic & Lima, 2003; Sage et al., 2007) and the tolerance to habitat disturbance exhibited by this species (Oliveira et al., 2009 and present study).

Small felids

Small felids are elusive, poorly known (Di Bitetti et al., 2010) and confoundable, especially spotted ones (see Chapter 5). First attempts to locally measure presence and detectability are recent (Di Bitetti et al., 2010; Silva, 2014). As observed by Oliveira et al. (2015), the margays ranked second, soon after the ocelots. They were present in the same number of stations than ocelots within the INP boundaries, while rarer outside the park (Table 1). Small felids are described by Oliveira et al. (2008) as “widespread but everywhere in small population sizes”. They are more associated to forest habitats than ocelots (Goulart et al., 2009) and our findings confirm this statement, with almost twice as many margays and oncillas in the INP as compared to outside of the park. As found by Di Bitetti et al. (2006), margays were also strictly nocturnal, jaguarundis were exclusively diurnal while oncillas were cathemeral being out in day light in 50 % of the records. This could be a coping strategy to intra-guild competition, just like pumas tend to become more nocturnal when jaguars are rare (Paviolo et al., 2009). Also, oncillas were well represented outside the park (i.e. 44% of records) while only 31% of margay records were collected in the same area, suggesting the latter are more closely associated with dense forest cover than oncillas (Oliveira, 1998). No less than 74% of jaguarundi records were collected in the INP buffer zone, corroborating what is known about the species’ ecology (Oliveira, 1998a; 1998b; Oliveira et al., 2009). Oncillas are likely the most adaptive of all three small felids (Oliveira-Santos et al., 2012), for being active at any time of the day, for being disturbance-tolerant, venturing more than margays out of the INP. Its dietary niche extensively overlaps with that of ocelot (Rosa-Mandes et al., 2010) which exerts a negative pressure onto all subordinated guild species. That is why oncillas tend to become more abundant where ocelots are rare or absent (Oliveira et al., 2009).

Dogs

Domestic dogs are looked at by wildlife conservationists as the major wildlife predators in protected areas worldwide (Galetti & Sazima, 2006). These dogs are stray dogs or farm owned, which usually unrestrictedly move about, eventually exploring preserved areas and interacting with wildlife (Paschoal et al., 2012). The problem has progressively increased and has caught the attention of scientists for ten years. In our study, domestic and/or hunting dogs were incredibly common in and out of the INP, almost in the same proportions (Table 5 and 6). Dogs were more abundant than ocelots (Fig. 3), ranking in 12th position in terms of individual records, being found in 13% of all sampling stations. This is not an isolated phenomenon (Bertrand, 2009b; Espartosa, 2009; Torres & Prado, 2010; Paschoal et al., 2012). All four referred studies were conducted in the Atlantic forest in Brazil

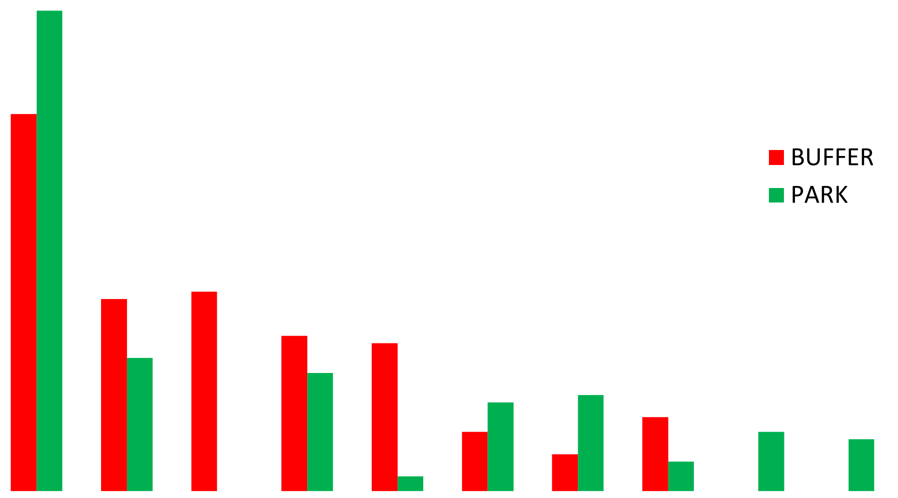

Figure 3. Number of individual records for all recorded carnivores species (but coatis) in the Iguaçu National Park (PARK) and its buffer zone (BUFFER).

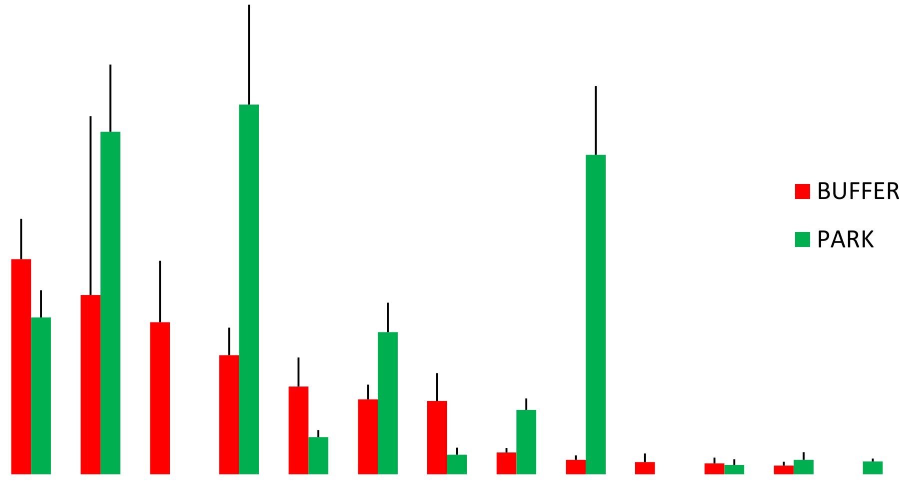

Figure 4. Density estimates with standard errors for main prey species in the study area.

and dogs ranked in top positions in all four studies. Dogs are a negative byproduct of the anthropogenic changes over forested ecosystems (Paschoal et al., 2012; Hughes & McDonald, 2013). Dogs' presence is not correlated to distance from the forest edge or to the number of mammal species found in the location (Paschoal et al., 2012). In their analysis, Ribeiro et al. (2009) found that over 73% of Atlantic forest remnants are located less than 250m from villages or farms, and a little less than 8% is located >1 km away from human settlements. Dogs have travelled longer distances than this (e.g. up to 5.5 km; Paschoal et al., 2012). On the other hand, dogs' presence is highly associated with the presence of humans, who - in our 400-km track survey in the INP - were found to be the most abundant mammal in the INP, being twice as common as the second most common animal (i.e. brocket deer; Bertrand, 2009a). Poaching is widely spread in preserved areas in Brazil and the lack of law enforcement has generated pitiful crime impunity (Peres, 2000; Hanazaki et al., 2009; Fragoso et al., 2011). Logically, the presence of dogs is related to the presence of villages and farms, but also to the reserve perimeter size and the existence of poaching and game trails. In a severely fragmented Atlantic forest, dog density reached 6.2 ind./km^2 (Torres & Prado, 2010). It is acknowledged that rural dogs are used by their owners for services, and also for hunting (Paschoal et al., 2012). We encountered and registered hound packs within the INP in sites with visible poaching indices. Dogs' intra-guild pressure on carnivores is now being discussed among the scientific community (Silva-Rodríguez & Sieving, 2011), and their impact can very well be compared to the "ocelot effect", as their feeding choices overlap other wild carnivores' ones. Reports of dogs predating medium-sized wild mammals have increased (Galetti & Sazima, 2006; Oliveira et al., 2008; Srbek-Araujo & Chiarello, 2008). They are likely competing with ocelots and its wild cousin, the crab-eating fox (Paschoal et al., 2012). Last, while dogs may not be the most efficient predators (Oliveira & Cavalcanti, 2002), they impact negatively forested ecosystems by bringing domestic-borne diseases into wildlife (Butler et al., 2004; Fèvre et al., 2006), killing medium-sized mammals (Silva-Rodríguez & Sieving 2011), displacing other species and competing with wildlife for resources (Paschoal et al, 2012). Attention and efforts should be dedicated to sensitize dog owners to become more responsible with their animals.

Foxes

Foxes were not found in sufficient numbers in the INP to derive population estimates. However, it was found in the INP buffer zone with 0.35 ± 0.19 ind./km^2 (Table 6). In a study conducted in the Itapuã state park, an Atlantic forest remnant in the state of Rio Grande do Sul in southern Brazil, it showed great tolerance to habitat alteration, even occupying open lands, with a density of 0.78 ind./km^2.

Quatis

Coatis are diurnal social species (Beisiegel & Mantovani, 2006; Fig. 2). We found that like everywhere else in Central and South America, coatis were the most abundant of all carnivores in the animal assemblage (Table 5 and 6), being 54 times more abundant than ocelots. Despite being so abundant, there were only few density studies conducted on the species. Density estimates vary according to the biome and depending on whether conditions are favourable or not (e.g. food, mates, etc.). Where food abounds or where pressures are limited, coati group sizes increase (Beisiegel & Mantovani, 2006). And so do densities, such as in the Chamala-Cuixmala Biosphere Reserve in Mexico where Valenzuela & MacDonald (2002) estimated 55.6 ind./km^2 or, in Barro Colorado Island, Panama, where Gompper (1997) estimated 51.5 ind./km^2. In Pantanal, densities varied from 2.14 ind./km^2 (Schaller, 1983) to 16.5 ind./ km^2 (Desbiez & Borges, 2010). Costa et al. (2009) found 33.71 ind./ km^2 in a 1,335 km^2 park in the *Cerrado*. In a secondary forest in the Brazilian Amazonia, Parry (2004) estimated 15.4 ind./km^2. In five Atlantic forest fragments ranging from 1700 to 35,000 ha, Cullen et al. (2001) found densities ranging between 3.11 and 5.20 ind./km^2. In our scenario, buffer zone density was slightly higher than INP density, suggesting great adaptability habitat alteration, as stated elsewhere (Costa et al., 2009). Coatis are able to cope with many habitat contexts and environmental pressures, adopting adequate group structures (Gompper, 1997), food choices (Beisiegel, 2001) or inter or intraspecific competition (Beisiegel & Mantovani, 2006).

Prey

Tapir

Lowland tapirs presented a clear bimodal activity pattern, with activity peaks early in the morning and night, which corroborates what exists in the literature (Lizcano & Cavelier, 2000). Lowland tapirs have shown activity pattern differences based on locations (e.g. trail vs. salt licks, see Lizcano & Cavelier, 2000) but overall, it however displays the same bimodal activity pattern. As for density estimates, first estimates using camera-trapping appeared in the early 2000's (Noss et al., 2003). The numbers existing in the literature show great variability depending on the habitat type and sampling method used (Medici et al., 2012). For instance, in the Amazon region, values range between 0.11 ind./km^2 (diurnal line transects; Peres, 2000) to 3.7 ind./km^2 (diurnal and nocturnal line-transects; Mendes-Pontes, 2004). As far as the Atlantic forest biome is concerned, first density estimates were provided by Cullen (1997), who determined 0.30 ind./km^2 using line transects in the Morro do Diabo State Park in São Paulo. Later on, Cullen et al. (2000) found 0.47 ind./km^2 for the same area. Most recent estimates are provided by Medici (2010), who estimated the lowland tapir population to 130 individuals for this 350 km^2 Atlantic forest remnant, with density estimates ranging from 0.35 to 1.35 ind./km^2. In the Green corridor of the province of Missiones, Cruz (2012) observed a drastic distinction between areas of distinct level of protection with 0.32 ± 0.18 ind./km^2 in the relatively preserved Argentinean Iguazú National Park, and 0.07 ± 0.05 ind./km^2 in the Urugua-í reserve where infraction rate is overwhelming. In the state of Paraná, Rocha (2001) estimated a high 2.2-2.5 ind./km^2 using direct observations in a 7 km^2 Atlantic forest remnant, but we question replicability and representativeness of this number given the method and the study area chosen. Compared to other reliable estimates for the Atlantic forest biome in Brazil, the density estimates found in this study (Table 3) is relatively high. When extrapolated to the whole INP, it would represent 3,150 individuals, of which 1,500 would be adult tapirs (Medici, 2010). The main ecological explanation for this high population density is the disappearance of jaguars (Paviolo et al., 2008), which releases the typical prey population control by its main predator. Of course, other pressures exist and contribute to lowland tapir populations decline. In the Atlantic forest, lowland tapir populations are mainly threatened by poaching, habitat loss, fragmentation and alteration (Medici et al., 2007). Tapir is the largest neotropical herbivore which has arrived during Pleistocene (Thoisy et al., 2010) and also the last representative of that Paleolithic megafauna in South America (Galetti et al., 2001). Often referred to as 'gardeners' or 'architects', they play a key role in the forest as seed predators and dispersers (Fragoso, 2000). Paradoxically, they might not be the most efficient 'garderners' for (1) they digest many seeds and defecate in unfavorable areas for seedlings (Salas & Fuller, 1996); (2) they defecate huge amounts of seeds, creating important competition among them (Galetti et al., 2001), and (3) facilitate location for seed predators (Fragoso, 1997). However, it does not decrease the ecological importance of the long-distance seed dispersal, especially the large-seeded species (>25mm), of this unique largest frugivore in the Neotropics (Galetti et al., 2001). In the Atlantic forest, tapir disappearance would have deleterious effects for at least 50 of its 1380 plant species (Siqueira, 1994; Galetti et al., 2001).

White-Collared Peccary

This species was among the most represented of the large mammal community (Table 2, Fig. 4). The activity patterns observed is similar to what has been observed by others (Carrillo et al., 2002), with two activity peaks around 7:00 and 17:00 (Fig.2). Existing density estimates for continuous tropical forest remnants vary from 2 ind./km^2 to 12 ind./km^2 depending on the hunting pressure (Wright et al., 1994; Peres, 1995; Cullen et al., 2001). Outside of the INP, we found 1.92 ± 0.61 ind./km^2 where the anthropogenic pressures are the most intense (Table 4). Inside the INP, we obtain a striking 43.22 ± 9.31 ind./km^2 (Table 3; Fig. 3), which is the highest density estimate ever recorded for the species. Several reasons to this, starting with its strong alteration tolerance documented elsewhere (Peres, 1995; Keuroghlian et al., 2004; Altrichter & Boaglio, 2004). Collared peccaries can tolerate high degree of hunting pressures, habitat fragmentation or degradation. They require 5 to 10 times less space than white-lipped peccaries (Fragoso, 1999), and form small herds. Their home ranges and seasonal ranges vary minimally (Keuroghlian et al., 2004). Furthermore, the two species that exert population control over this species, either by competition or predation, i.e. the white-lipped peccaries and the jaguars, are extinct and almost extinct, respectively, leaving vacant niches and releasing trophic pressure, causing the population to unrestrictedly thrive.

The INP used to shelter both the white-lipped peccary (*Tayassu pecari*) and the collared peccary but no record of the former species has been made in the last two decades (MMA/IBAMA, 1999). In a monitoring of these two species, Peres (1995) clearly observed a high sensitivity to disturbances by the white-lips as opposed to a high tolerance to disturbances by collared peccaries. Moreover, despite their apparent similarity, these two species differ in morphology, ecology and herd structure. The white-lips form the largest herds and foraging biomass of all the Neotropical ungulates, with usually around 200 individuals, as opposed to a 9 or 10, average herd size, for collared peccaries (Keuroghlian et al., 2004). Sadly, there is no peer-reviewed information on the INP white-lipped peccaries' history but this species has been widely studied elsewhere, enabling us to draw conclusions regarding its disappearance (e.g. Peres, 1995; Carrillo et al., 2002; Keuroghlian et al., 2004). The white-lips requirement of preserved continuous forest is the largest of all Neotropical mammals, with values ranging between 6,000 and 20,000 ha (Kiltie & Terborgh, 1983), which is inevitably associated with undisturbed forests (Keuroghlian et al., 2004). The scientific community is still shared regarding the herd movements; some argue that the species is migratory (Bodmer, 1990; Peres, 1995), while others suggest they are nomadic within a large home range (Kiltie & Terborgh, 1983). The consensus is that they required large continuous forested areas to survive, and that is probably why it has vanished from most of its original distribution, as the forest was destroyed or removed.

Brocket Deer

Red brockets are the most widely distributed South American deer (Emmons & Feer, 1997). It is also one of the hunters' favourite game animals (Hurtado-Gonzales & Bodmer, 2004; Di Bitetti et al., 2008) and therefore is harvested all over its range (Ojasti, 1993). Activity pattern is typically bimodal with activity peaks between 18:00 and 22:00 and between 01:00 and 05:00, as found by our neighbours in the Argentinean Iguazú National Park (Fig. 3; Di Bitetti et al., 2008). Rivero et al. (2005) in Bolivia also observed most activity between sunset and sunrise. Different methods have been used to try to quantify brockets in forested areas, mostly line-transects (Chiarello, 1999; Hurtado-Gonzales & Bodmer, 2004), one short telemetry study (Maffei & Taber, 2003) and one dung count study (Rivero et al., 2004). Telemetry study provided 1.92 ind./km^2. Line-transect density estimates found for this species ranged between 0.20 ± 0.07 ind./km^2 to 1.16 ± 0.29 ind./km^2 (Hurtado-Gonzales & Bodmer, 2004; Naranjo & Bodmer, 2007), most strikingly, densities in slightly hunted and non-hunted sites were lower than in persistently hunted or heavily hunted. With dung counts in Bolivian lowlands, densities were higher, ranging between 3.52 ± 4.6 to 6.98 ± 7.2 ind./km^2 (Rivero et al., 2004). While the density we found in the INP buffer zone is a little under what was obtained with dung counts, it was much higher than existing values. In the INP, density was much higher than what exists in the literature as a probable response to a diminution in predator numbers. While Di Bitetti et al. (2008) had a larger sampling effort using camera-traps, they provided frequency counts but no density estimates. During their 2004 cam-trap monitoring in the Argentinean Iguazú Park, they reported the presence of red brockets in 92% of sampling sites. In this study, the red brocket was present in almost 85% of sampling stations. It was indeed the species with the widest distribution. As our Argentinean colleagues, we observed that red brockets were more abundant in less-hunted areas although they are more tolerant to hunting than other mammal species (Bodmer et al., 1997). However, as they relevantly stressed, there is no place in the park that is hunters-free but law enforcement is being restructured so that crime rates should be now start decreasing in this conservation unit (I. Baptiston, *pers. comm.*).

Prey – Predator

As Carbone & Gittleman (2002) showed, carnivore density is correlated to the density of their prey. According to the authors, no less than 10.000 kg of prey is required to maintain 90 kg of predator. Large cats in the INP are known to feed preferentially on tapirs, brocket deers, peccaries, agoutis and capybaras. According to Peres (1999), the Atlantic forest offers 891 kg/km^2. Extracting average prey weight from our dataset, the INP offers 2,114 kg/km^2 of tapir, brocket deer, peccary, paca, agouti and coati. At the time he monitored jaguars in the INP, Crawshaw (1995) had estimated seventy individuals in the park. Based on scat prey item analyses and considering that a jaguar eats 5% of its body weight daily (Emmons, 1987), such population would annually require 102,200 kg of prey food, of which 81,000 kg would come from peccaries. According to Crawshaw et al. (2004), one adult jaguar would annually require 38 peccaries, nine deers, six coatis, ten armadillos, five agoutis and ten opossums to fulfill its dietary needs. Based on our analyses, if one jaguar requires 1,460 prey kg/year (Emmons, 1987), there is food for 2,687 jaguars, if we only consider tapirs, peccaries, deers, coatis, opossums, agoutis and armadillos. While there is no previous database to compare with our data, the rarefaction of the major predator seems to impact the trophic guild in many ways, making larger prey available for other carnivores as Moreno et al. (2006) observed for ocelots and pumas in Panama, and allowing prey populations to explode (Terborgh et al., 2001). Density estimates for rodents, agoutis, white-collared peccaries, coatis and opossums we obtain were extremely high (Table 3; Fig. 4), which are likely the reflection of the disappearance - or at least diminution - of top predators and by the enormous amount of food resources available with recent bamboo seed production throughout the INP (Sage et al., 2007).

REM ASSESSMENT

Based on existing peer-reviewed literature, the REM applied to our dataset provided sound density estimates for ten mammal species, including six carnivore species for which we had no prior information in this part of their range. Generally, it performed well for species with over 40 records, and detection distance increased with body mass. While speed estimates accuracy is crucial to obtain reliable density estimates, it is rarely available. While some may use day range to access this parameter (e.g. Manzo et al., 2012), we did not have this problem for everything was measured. While the task may be time-consuming depending on the size of the dataset, it solves the major hurdle to the use of the REM. Another observation was regarding gregarious species, which were treated like solitary ones. In other words, each animal encounter was treated individually, but it was acknowledged by others as plausible, especially when group counts are not easily obtained (Thomas et al., 2010). Furthermore, animals showed curiosity and sometimes ‘played’ with the cameras. Plastic-covered wires were used to hold cameras locked, as agoutis, capuchin monkeys and even peccaries had the ability to open them. Rowcliffe et al. (2008) acknowledged that the REM was robust to behavior patterns which likely violate underlying model assumptions. Also, wet days resulted in smaller detection zones but REM estimates showed little sensitivity to it, as found elsewhere (Cusack et al., 2015a). It showed great sensitivity to long sequences of inactive animals, causing density estimates to be a hundred times higher than the actual estimates obtained without these sequences. Such phenomenon was also observed by other REM users (Cusack et al., 2015a).

LIMITATIONS

Camera technical failure occurred in 8.8 % of cases, invalidating analyses in 19 sampling locations. A majority of our cameras presented temporal dysfunctions, e.g. infra-red lightening not turning on; camera taking pictures without stopping; camera stopping for some time and getting back to normal after some time, etc). Such failures impacted data collection to a lesser degree, causing partial data loss in the remainder of the sampling stations. In their one-year camera trap monitoring study in Panama, Kays et al. (2011) observed that only 30% of their cameras never failed. Furthermore, camera batteries had very variable lifespan given unknown shelf-life, alkaline or lithium type, brand, etc., causing them to sometimes discharge in a few days like others experimented (Lizcano & Cavelier, 2000).

Underestimation of large cats was a trade-off that was required to undertake this research project. Illegal activities were so common in the INP and surrounding areas that the major challenge of this project was to maintain cameras safe and running. We opted for the no-glow technology to minimize camera loss to infringers. Pumas and jaguars are known to prefer trails over forest (Paviolo, 2010; Cusack et al., 2015a). In 2006, a pilot camera-trapping monitoring consisted of two recreational trails in the intensive use INP area. Pumas were the second most photographed species (19.8% of all 1311 records; Bertrand, 2006). This research project rather focused on the overall mammal assemblage and was run upon license, in parallel with the Carnivores Project within the INP, whose main objective was to provide density estimates for jaguars and pumas.

In terrestrial carnivore species, home ranges and densities were found to be negatively correlated (Karanth & Chundawat, 2002). They are also correlated to the prey densities and carnivore metabolic needs (Carbone & Gittleman, 2002) or to prey's home range size (Herfindal et al., 2005). Density estimates vary greatly, even within each eco-region (see for instance, Trolle & Kerry, 2005). Indeed, studies based in few individuals, typically 2 to 11, provide highly variable results and may likely distort findings (Maffei & Noss, 2008; Oliveira et al., 2009).

CONCLUSION

Statistics and projected estimates are a useful yet dangerous tool for they are blind to real-world fluctuations of the numerous parameters influencing species' distribution. Things become even more delicate when it comes to elusive rare species such as the jaguar. Small survey areas (Maffei & Noss, 2008), inadequate trap spacing (Dillon & Kelly, 2007), and the blind yet generalized use of half MMDM collected from camera-traps (Soisalo & Cavalcanti, 2006) often provide overestimated density estimates for all these parameters because of effective sampled size underestimation (Negrões et al., 2010).

If taken at face value, a population viability analysis provides challenging values in terms of individual numbers and time frames. It was said that only population with over 85 individuals are viable for over 200 years (Oliveira et al., 2009). Reed et al. (2003) stated that only populations with over 5.000 individuals are safe from extinction for the next four decades. Throughout Brazil, only the Amazon populations fulfilled all the requirements for long-term conservation (Sollmann et al., 2008). Also, population estimates for the entire country like the jaguar estimates suggested in Morato et al. (2013) are likely off. The Frankham (2009) correction applied to obtain an effective population estimate is a mere 0.1 factor. The literature presents highly variable density estimates for the species, considering the habitat type, prey abundance, habitat connectivity and quality, surveyed area size, number of individuals monitored, used method and poaching intensity (Paviolo et al., 2008; Torres et al., 2012; Morato et al., 2013; Galetti et al., 2013). Dispersal behavior is also poorly known, and is likely to be pondered by environmental pressures, making it hard to estimate.

While so much time, money and efforts are put into trying to obtain estimates, which are most of the time, worryingly low or totally off (Nuñes-Peres, 2011), time has come for resource focus to prioritize working on prevention and rehabilitation solutions and conservation strategies instead.

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CHAPTER 2

THE IGUAÇU NATIONAL PARK AND ITS BUFFER ZONE: MAMMALS' RESPONSE TO HABITAT ALTERATION

ABSTRACT

Habitat selection by species is complex to fully understand because it relies on several multi-scale environmental elements that are sometimes hard to measure or even determine. In addition, anthropogenic habitat alterations force species to alter their own ecological requirements as a coping strategy, generating highly variable responses throughout the literature. This study was the first camera-trapping mammal monitoring ever conducted in the region of the 77-year old Iguaçu National Park (INP) in southwestern Brazil. It allowed identifying most important environmental parameters associated with the presence of mammal species. We used constrained multivariate analyses to observe the relationship between the distribution of species and environmental variables associated with the habitat gradient offered by the Iguaçu region. Forest dwellers such as *Panthera onca*, *Puma concolor* and *Tapirus terrestris* were strongly associated with the highest level of protection and forest cover. Other species showed great tolerance to habitat alteration and the presence of humans. Among them, carnivores *Cerdocyon thous*, *Eira barbara*, *Leopardus tigrinus*, *Herpailurus yagouaroundi*, *Nasua nasua* but also *Cebus apella*, *Hydrochaerus hydrochaeris*, *Cuniculus paca* and *Dasypus novemcinctus*. *Leopardus pardalis* showed strong association with the presence of water, and *L. wiedii* showed strong association with the distance from roads and therefore, from humans. Habitat alteration-sensitive species play important ecological roles within the ecosystem and their disappearance would add up to the already disturbed context. As shown here, such forest dwellers are exclusively limited to the most preserved forests of the INP. Ecological integrity and habitat connectivity should be conservation priorities to promote genetic flux and enhance population health in the long term.

KEYWORDS

Iguaçu National Park, mammals, buffer zone, habitat alteration, CCA.

CAPÍTULO 2

O PARQUE NACIONAL DO IGUAÇU E SUA ZONA DE AMORTECIMENTO: RESPOSTA DOS MAMÍFEROS À ALTERAÇÃO DO HÁBITAT

RESUMO

A seleção de hábitat por espécie é um processo complexo de se entender pois depende de vários elementos ambientais multi-escala, que são as vezes complicados de mensurar ou até definir. Além do mais, as alterações antropogénicas do hábitat obrigam as espécies à mudar seus próprios requerimentos ecológicos como estrategia de sobrevivência, gerando respostas variáveis na literatura. Este estudo é o primeiro monitoramento com armadilhas fotográficas jamais realizado no Parque Nacional do Iguaçu que já tem 76 anos. Permitiu identificar os parâmetros ambientais os mais importantes para explicar a presença de espécies de mamíferos. Utilizamos análises multivariáveis para observar a relação entre a distribuição das espécies e as variáveis ambientais associadas ao gradiente de hábitat oferecido pela região Iguaçu. Moradores da floresta, como *Panthera onca*, *Puma concolor* e *Tapirus terrestris* eram fortemente associados aos mais altos níveis de proteção e a cobertura florestal. Outras espécies demonstraram boa tolerância à alteração do hábitat e à presença de humanos. Entre elas, os carnívros *Cerdocyon thous*, *Eira barbara*, *Leopardus tigrinus*, *Herpailurus yagouaroundi*, *Nasua nasua* mas também, *Cebus apella*, *Hydrochaerus hydrochaeris*, *Cuniculus paca*, e *Dasypus novemcinctus*. *Leopardus pardalis* mostrou uma associação forte com a presença de água, e *L. wiedii* mostrou uma associação forte com a distância das estradas e consequentemente, com os seres humanos. As espécies sensíveis à alteração do hábitat têm papeis ecológicos muito importantes no ecossistema e sua disparição iria aumentar mais ainda o contexto perturbado. Como demonstrado aqui, tais espécies são confinadas às florestas as mais preservadas do PNI. A integridade ecológica e a conectividade do hábitat devem ser prioridades de conservação de forma a promover o fluxo genêtico e estimular a saúde populacional a long prazo.

PALAVRAS-CHAVES

Parque Nacional do Iguaçu, mamíferos, zona de amortecimento, alteração de hábitat, ACC.

INTRODUCTION

What causes a species to be found in one forest stand and not in another? When we understand the species' ecological requirements, it becomes easier to predict. However, many anthropogenic factors also enter the equation and often confuse ecologists by producing variable responses from species. Habitat selection and species ecological requirements have been the focus of many studies in South America (e.g. Chiarello, 1999; Goulart et al., 2009; Di Bitetti et al., 2010). Depending on species' adaptability, access to water or food or resting sites or certain type of microhabitat might be of vital importance, and the lack of access to it might jeopardize the species' survival. The biome, available food source, presence of competing species or predators, or climate may alter one species' response to variation (e.g. Di Bitetti et al., 2010). Resource partitioning is a scale-related concept and changes in resource availability do not impact two different species the same way (Orians, 1991).

Historically, the Atlantic forest used to cover 12% of Brazil (Brown & Brown, 1992). The Atlantic forest was a continuous forest ecosystem which occupied southern and eastern Brazil, the Argentinean province of Misiones and eastern Paraguay (Câmara, 2003). Ranta et al. (1998) estimated than even less was left, with only 7% of the original million squared kilometers of Atlantic forest, in the form of forest fragments in a largely agricultural landscape. It is the most remarkable biome of Brazil, considering its rate of endemic species and its species richness. It became one of the top five biodiversity hotspots (Mittermeier et al., 1998; Myers et al., 2000). Paradoxically, the Atlantic forest might be the richest biome species-wise, scientists are still identifying species and understanding their dynamics and yet, less than 12% remain (Ribero et al., 2009). Forest fragmentation stands among the main conservation issues affecting tropical forests, transforming enormous continuous forest areas into a matrix of pastures, agricultural lands with depleted soils and polluted waters, farms and altered forest thickets (Whitmore, 1997; Chiarello, 2000). While fragmentation diminishes the size of remaining forest fragments, it also isolates them, altering the forest ecological integrity at all levels (Bierregaard et al., 1992). It dramatically disturbs the natural balance and dynamics of the ecosystems, causes the extinction of an unknown number of species and forces ecological alterations of the surviving species (Keuroghlian et al., 2004).

Considering the severity of the reaction cascade, most Atlantic forest studies did focus on habitat loss and fragmentation (Galetti et al., 2009; Quadros & Cáceres, 2001). Another worsening factor is poaching which was also studied (Cullen et al., 2000; Di Bitetti et al., 2008). When they do not tackle fragmentation or poaching, studies consist in pioneer species monitoring (Chiarello & Melo, 2001; Goulart et al., 2009; Cassano et al., 2012), just like this one, in the 77-year old Iguaçu National Park, in southwestern Brazil. The Atlantic forest is as rich in fauna and flora as the Amazon biome (Valladares-Padua et al., 1997), and much remains to be discovered, documented and understood.

Initially, studies on multispecies in the Atlantic forest have focused on small mammals (e.g. Dalmagro & Viera, 2005); there is a scarcity of information on medium-sized species. On the other hand, studies also focused on charismatic large mammals such as jaguars (*Panthera onca*; e.g. De Angelo et al., 2013), pumas (*Puma concolor*; e.g. Paviolo et al., 2009) or lowland tapirs (*Tapirus terrestris*; e.g. Medici, 2010). This study is the first characterization of the mammal community occurring in the region of the Iguaçu National Park and its buffer zone. The Iguaçu National Park is often referred to as the last wildlife reserve surrounded by “a sea of soy crops” (Crawshaw et al., 2004). Surprisingly, the impact of forest fragmentation on local fauna has never been studied, nor wildlife distribution or composition. Economy usually dictates where efforts and resources are injected (Hawken, 1993). Tourism is the main input into local economy so recreational activities flourished in the Iguaçu National Park, i.e. boat or helicopter rides, ATVs, tree climbing, bikes, paddling. Eco-tourism is often mentioned and activities may be labeled as such, but no prior environmental impact study has ever been undertaken (D’Oliveira et al., 2002).

How do species respond to the landscape’s alteration? Which species venture out of the INP? How are species affected by habitat variation? We analyzed the relationship between the mammals’ distribution along gradients of habitat alteration. Our aim was to better understand species’ dynamics in the last and largest fragment of inland Atlantic forest in southern Brazil so that conservation strategies can be drawn and implemented.

MATERIAL & METHODS

Study Area

The Iguaçu National Park (INP; Fig. 1; 25° 22' 24"S, 54° 2' 33"O) was created in 1939 and comprises 1,852.62 km^2 of inland Atlantic forest in the Upper Paraná Eco region (Di Bitetti et al., 2003). It is the second oldest national park of Brazil after Itatiaia National Park. The INP became a UNESCO World Heritage Site since 1986 (MMA/IBAMA, 1999) and currently holds 80% of the remaining Atlantic forest of the entire Paraná state. The climate is classified as temperate subtropical with mean annual temperature of 20.7°C and 1712 mm of mean annual precipitations (Maack, 2012). July and August are the driest months of the year (Crespo, 1982). The predominant forest type is semi-deciduous inland Atlantic forest, with some Brazilian pine (*Araucaria angustifolia*) forests in the northeastern tip where altitude reaches ≥600m. The INP should not be seen as the pristine Atlantic forest for over 400 families lived in it and were only removed in the late 70s (Crawshaw et al., 2004). Meanwhile, lots of clearing and selective logging occurred within the park, especially in its westernmost section. Human settlements have brought in many invasive and exotic fruit and ornament plants species which thrived. The INP buffer zone is characterized by its extremely fertile soil, which is being striped and chemically-sprayed by prosperous extensive agro-businesses which annually produce genetically modified corn, wheat and soy, and cattle pastures. Ironically, while the legislation suggests enhanced protection and selected soil uses in agreement with the buffer zone ecological role (SNUC, 2000; see Chapter 6), the INP buffer zone was illegally used to grow and test Monsanto genetically-modified corn (Kenfield, 2008).

Camera-trapping

Data were collected using sixteen RECONYX PC900 camera units. This continuous camera-trap monitoring was conducted between September, 2012 and October, 2014, totalling 755 consecutive days, with 33 days per sampling station on average (see Chapter 1). Individual records were obtained from the total record number after applying a 60-minute filter (Table 1). If a given individual appeared twice within this period, it was considered the same individual and therefore was counted only once.

For each sampling station, the minimum distance to water and road, and a vegetation cover index were extracted using geoprocessing information. Each sampling station was characterized using 2013-2014 NDVI maps from the study area. Calculations were conducted using ENVI™ 5.2 and ArcGis™ 10.2 (see Mesquita, 2015). During field work, local information was recorded in each sampling station, microhabitat type, soil pH, air and soil temperature, forest litter and canopy closure were also recorded.

Data Analysis

An abundance index was calculated using the number of species detected per sampling station and divided by the total number of species (Table 2).

We performed a canonical correspondence analysis (CCA) in order to verify which environmental variables could explain the most variance in species distribution (Table 3). Explanatory variables were selected using stepwise forward selection to rank explanatory variables by importance (Table 4). Randomization tests, i.e. Monte Carlos permutation tests (n =1999) were used to verify the significance of the species-environment relationships (Oug, 1998). Multivariate analyses, permutation tests and biplot were performed using CANOCO 5.0 (ter Braak & Šmilauer, 1998).

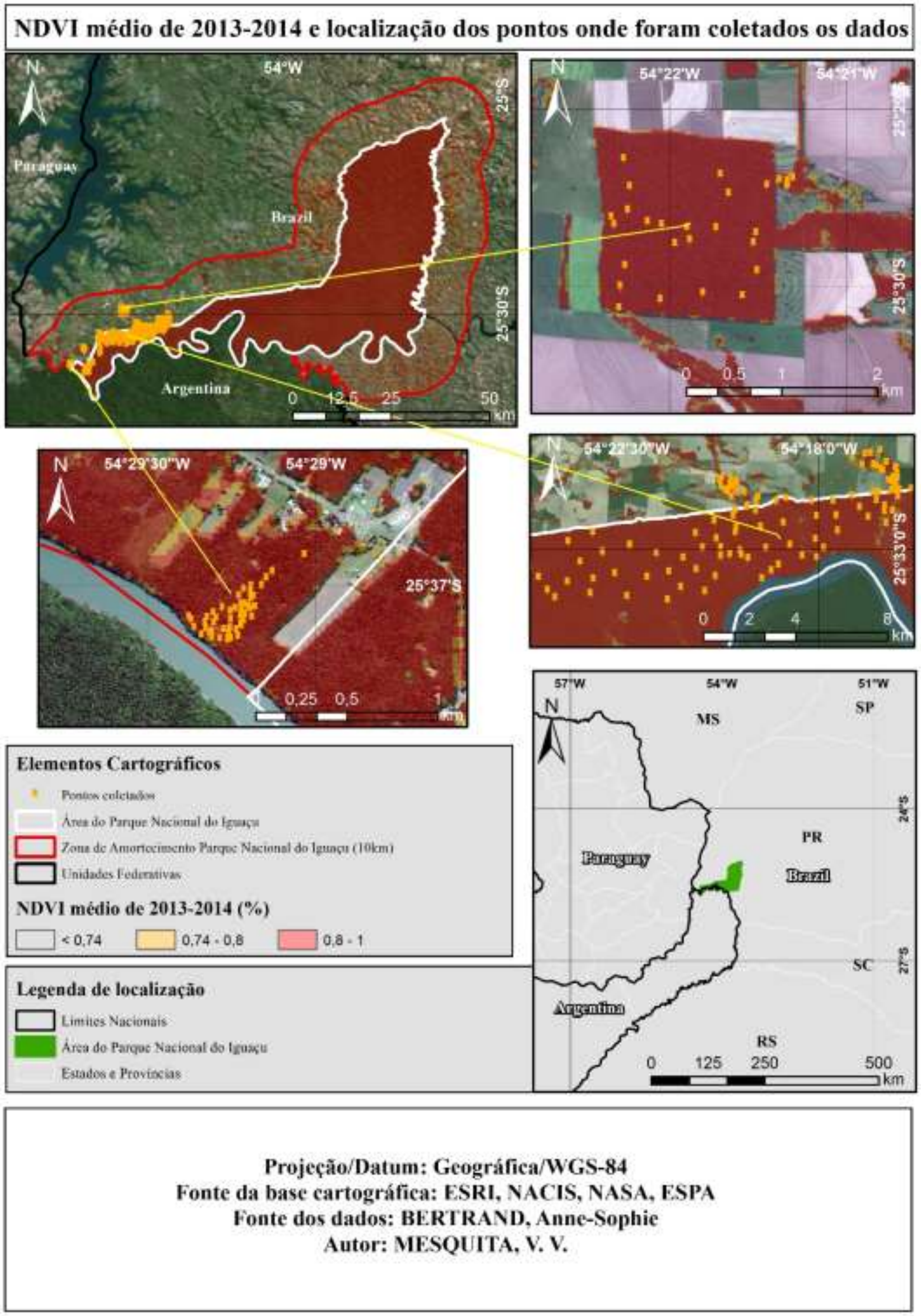

Figure 1. Iguaçu National Park location in southwestern Brazil and sampling effort (each yellow dot is a sampling station).

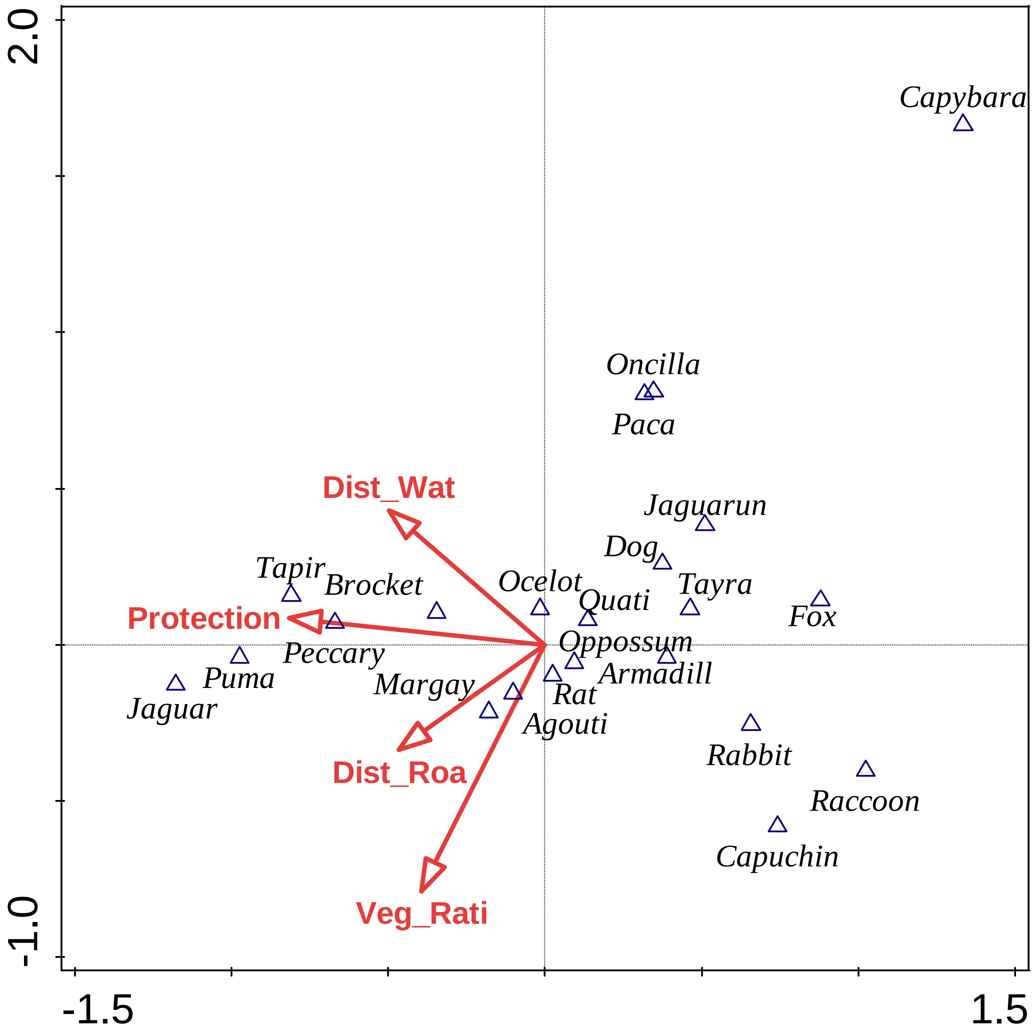

Figure 2. CCA biplot showing relationships between mammal species and environmental parameters (protection level, vegetation index, proximity to water and roads) in the region of the Iguaçu National Park, southwestern Brazil (Total inertia or total extracted variance: 2.917).

RESULTS

Thirty taxa were recorded during this monitoring, 13 were carnivore species and 17 were prey species (Table 2). Twenty-two of them were abundant enough to be retained for further analyses. Most frequently recorded species were collared Red Brocket (*Mazama americana*), Collared Peccary (*Pecari tajacu*), Azara's Agouti (*Dasyprocta azarae*), and South-American Coati (*Nasua nasua*). Species such as Lowland Tapir (*Tapirus terrestris*), collared peccary and red brockets, but also Puma (*Puma concolor*) and Jaguar (*Panthera onca*) were the most strongly associated with a higher level of habitat protection (Fig. 2).

Table 1. Sampling effort details for both zones (INP: Iguaçu National Park; BZO: Buffer Zone) and total.

	INP	BZO	Total
Sampling stations	81	108	189
Records number	4347	2993	7340
Presence number	530	495	1025
Mean/Station	6.31	4.54	
Number of taxa	31	24	
Detection rate	59.22	40.78	100.00

Most smaller carnivores species were associated with altered and/or open habitat of the INP buffer zone, i.e. Crab-eating Fox (*Cerdocyon thous*), Crab-eating Raccoon (*Procyon cancrivorus*), Tayra (*Eira barbara*), Dog (*Canis lupus*), Jaguarundi (*Herpailurus yagouaroundi*), and Oncilla (*Leopardus tigrinus*). Other species showed great tolerance to habitat alteration: Capybara (*Hydrochaerus hydrochaeris*), Spotted Paca (*Caniculus paca*), and Nine-banded Armadillo (*Dasypus novemcinctus*). Likewise, possums and small rodents were well represented inside and outside of the INP. Margay (*Leopardus wiedii*) was associated with a greater distance to road and was much more abundant within the park boundaries than outside (Table 2). Last, Ocelot (*Leopardus pardalis*) was strongly associated with the presence of water. Species found exclusively within the INP boundaries were puma, jaguar, Greater Naked-Tailed Armadillo (*Cabassous tatouay*), Giant Anteater (*Myrmecophaga tridactyla*), and Brazilian Porcupine (*Coendou prehensilis*). On the other hand, certain species were solely recorded outside of the INP, i.e. raccoons and Neotropical Otter (*Lontra longicaudis*). The ratio between the abundance of prey and carnivore species was larger for the INP, suggesting more prey available inside the park as compared with its buffer zone (Fig. 3).

Table 2. Capture records and frequency of occurrence in both zones (Iguaçu National Park: INP; Buffer zone: BZO) from September 2012 to October 2014, listing species included in the analysis.

Species	Common name	Area	%INP	%BZO	Nb. Rec.	Used in the analysis
Prey species						
	RODENTS					
Dasyprocta azarae	Azara's Agouti	Both	80.95	65.14	1169	Yes
Cuniculus paca	Spotted Paca	Both	8.33	5.50	24	Yes
Hydrochaerus hydrochaeris	Capybara	Both	2.38	5.50	26	Yes
Sciurus aestuans	Guianan Squirrel	Both	2.38	0.92	4	No
Coendou prehensilis	Brazilian Porcupine	INP	1.19	0.00	2	No
Rodentia	Rodents	Both	50.00	32.11	327	Yes
	LAGOMORPHS					
Sylvilagus brasiliensis	Tapeti	Both	16.67	23.85	152	Yes
	XENARTHRANS					
Dasypus novemcinctus	Nine-banded Armadillo	Both	54.76	51.38	403	Yes
Myrmecophaga tridactyla	Giant Anteater	INP	3.57	0.00	3	No
Tamandua tetradactyla	Lesser Anteater	BZO	2.38	3.67	5	No
Euphractus sexcinctus	Six-banded Armadillo	Both	1.19	0.92	4	No
Cabassous tatouay	Greater Naked-tailed Armadillo	INP	1.19	0.00	1	No
	UNGULATES					
Mazama americana	Red Brocket Deer	Both	84.52	42.20	513	Yes
Pecari tajacu	Collared Peccary	Both	67.86	16.51	326	Yes
Tapirus terrestris	Lowland Tapir	Both	47.62	2.75	113	Yes
	ARBOREAL SPECIES					
Didelpidae	Possums	Both	55.95	50.46	500	Yes
Cebus apella	Black Capuchin Monkey	Both	1.19	18.35	36	Yes
Carnivore species						
	FELIDS					
Leopardus pardalis	Ocelot	Both	13.10	10.09	30	Yes
Leopardus wiedii	Margay	Both	13.10	2.75	16	Yes
Leopardus tigrinus	Oncilla	Both	9.52	3.67	18	Yes
Herpailurus yagouaroundi	Jaguarundi	Both	4.76	7.34	12	Yes
Puma concolor	Puma	INP	8.33	0.00	8	Yes
Panthera onca	Jaguar	INP	3.57	0.00	5	Yes
	CANIDS					
Canis lupus	Domestic Dog	Both	11.90	13.76	64	Yes
Cerdocyon thous	Crab-eating Fox	Both	2.38	7.34	21	Yes
	PROCYONIDS					
Nasua nasua	South-American Coati	Both	61.90	56.88	418	Yes
Procyon cancrivorus	Crab-eating Raccoon	BZO	0.00	9.17	20	Yes

	MUSTELIDS					
Eira barbara	Tayra	Both	19.05	22.94	87	Yes
Galictis cuja	Lesser Grison	INP	1.19	0.00	2	No
Lontra longicaudis	Neotropical Otter	BZO	0.00	0.92	1	No

Table 3. Canonical Correspondence Analysis (CCA) showing eigenvalues, extracted variance and environment correlations for the first four axes (22 species; 4 environmental variables).

Statistic	Axis 1	Axis 2	Axis 3	Axis 4	Total[a]
Eigenvalues	0.1394	0.0490	0.0367	0.0174	0.2425
Extracted variance (%)	0.3744	0.2443	0.2089	0.1723	
Pseudo-canonical correlation	0.7159	0.4678	0.4467	0.3723	
Explained fitted variance*	57.49	77.69	92.84	100.00	

*cumulative

[a] Sum of all eigenvalues and percentage of explained variance of species data.

Table 4. Explanatory variable stepwise forward selection for the CCA.

Explanatory variable	Extracted Variance (%)	Contribution (%)	pseudo F-statistic*	Corrected *P*-value[a]
Protection	4.0	47.8	7.7	0.002
Veg_Ratio	1.6	19.8	3.2	0.006
Dist_Wat	1.6	19.3	3.2	0.002
Dist_Road	1.1	13.1	2.2	0.028

* ratio between the eigenvalue of the single constrained (canonical) axis and the average of the eigenvalues of the unconstrained (residual) axes

[a] Bonferroni correction applied.

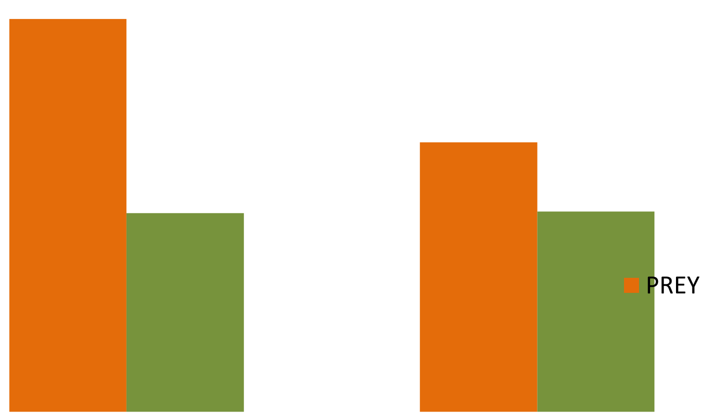

Figure 3. Mean abundance of prey and carnivore species in both zones: the Iguaçu National Park (INP) and its buffer zone (BZO).

DISCUSSION

Our study examined the relationships between the distribution of mammal species and habitat variables. The multivariate analyses showed clear associations between species and a habitat alteration gradient characterized by vegetation cover, distance to road, and level of protection. Certain species were strongly associated with most preserved forests, vegetation cover, or least amount of human-related disturbances (e.g. roads), such as jaguars, pumas, tapirs, collared peccaries and red brockets; other species were associated with altered, open and/or fragmented lands, i.e. quatis, capybara, fox, raccoon, tapeti (*Sylvilagus brasiliensis*), capuchin monkeys (*Cebus apella*), spotted paca, jaguarundis, oncillas, and quite logically, domestic dogs.

It is worth noting that domestic dogs were almost as common inside and outside the INP (see Chapter 1). This worrying phenomenon is not the INP's privilege as domestic dogs have increasingly been reported elsewhere in the past decade (Paschoal et al., 2012). They represent a human-activity byproduct impacting negatively preserved areas (Hughes & McDonald, 2013). In our analysis, dogs were negatively associated with distance to road and with the vegetation cover, suggesting their association with open lands and human settlements. The presence of this domestic species should not be overlooked as it has been demonstrated that while dogs may not be efficient predators (Oliveira & Cavalcanti, 2002), they displace species, they can act as intraguild competitors with other mesocarnivores (Paschoal et al., 2012), they may kill medium-sized mammals (Silva-Rodríguez & Sieving, 2011) and transmit domestic-borne diseases to wildlife (Fèvre et al., 2006). Also, they may be used for hunting (Paschoal et al., 2012), and are often correlated with illegal hunting and lack of law enforcement (Peres, 2000).

From our analysis, the other species associated with open lands and proximity to human infrastructures are known to be highly adaptive and disturbance-tolerant (e.g. coatis; Beisiegel, 2001). The smartest mammal species in the INP is likely the South-American coati as it tolerates very well the presence of humans. Coatis are known to adapt their behavior and requirements based on resource availability (Desbiez & Borges, 2010). In the INP, they have literally taken over the area dedicated to tourism around the spectacular waterfalls. They regularly make the headlines of local press with many attacks on visitors, especially around food courts. More than 150 attacks were reported during the first semester in 2014 (G1 PR, 2014). Coatis are witty carnivores and find ways to obtain food from many natural and human-made sources.

Several frugivorous species and most carnivore species showed great tolerance to habitat alteration. They all have in common great adaptability, changing their activity patterns, food choices and spatial/temporal resource use to fit external conditions. Capybaras and spotted pacas stand among the ubiquitous species as demonstrated elsewhere (Schaller & Crawshaw, 1980; Michalski & Norris, 2011) and both appeared in and out of the INP. Capuchin monkeys tend to be the primate species which copes best with current forest alteration trends (Chiarello & Melo, 2001). Moreover, while they have been associated with forested habitats (Emmons & Freer, 1997; Schaefer & Hostetler, 2003; Goulart et al., 2009), nine-banded long-nosed armadillos were also more abundant in the INP buffer zone than in the INP, maybe as a result of the absence of jaguars, which preferentially prey on them (Garla et al., 2001; see Chapter 1). Tapetis, or Brazilian rabbits, are often cited in the literature either as a prey item for cats (Wang, 2002) or as a species populating forest remnants (Chiarello, 1999; Srbek-Araujo et al., 2005; Tobler et al., 2008). In our study, tapetis were found more frequently in the INP buffer zone than in the park, and displayed a nocturnal activity pattern, both suggesting great tolerance to suboptimal habitats.

Furthermore, several carnivores also showed great adaptability to poor environments. While some believe that oncillas are declining and suffering from anthropogenic habitat alterations (Giordano, 2012), other studies demonstrated a high degree of adaptation (Oliveira et al., 2009), which could turn oncillas the top predators of the small forest fragments where other cat species have gone extinct (Oliveira-Santos et al., 2012). Oncillas have been consistently recorded in small Atlantic forest fragments (Chiarello, 1999; Chiarello & Melo, 2001; Oliveira et al., 2009). Oncillas have shown to adapt their activity pattern from nocturnal to cathemeral, according to the presence of other felids in order to reduce interspecific encounters (Oliveira-Santos et al., 2012). The presence of ocelot acts as a pressure onto other small cats' populations, phenomenon referred to as the "ocelot effect" (Oliveira et al., 2009). Ocelots were also recorded inside and outside the INP, and showed a clear association with watercourses, as noted by others (Cáceres et al., 2007; Goulart et al., 2009). Ocelots are tolerant to habitat variations, being reported in secondary vegetation (Jacob, 2002). In the present research, ocelots were even more abundant out of the INP boundaries than inside the park (see Chapter 1). Given the intraguild competition it exerts on other cat species, its wide prey selection (Oliveira et al., 2009) and its tolerance to habitat disturbance, the ocelot endures better the impacts of anthropogenic habitat alteration when compared to more sensitive forest dwellers (e.g. jaguar; Goulart et al., 2009; De Angelo et al., 2011).

Tayras are associated with forested habitats, but as human encroachment upon forested habitat increased, tayras adapted well and started using gallery forests, gardens, and plantations (Emmons & Freer, 1997; Presley, 2000). They showed strong arboreal habits and were frequently spotted by farmers around chicken husbandries. Another generalist species, the crab-eating fox, has been found to occupy any type of habitat, even open lands (Faria-Corrêa et al., 2009). It is described as a typical generalist species with a wide distribution in South America (Emmons & Feer, 1997), and to have very opportunistic habits (Eisenberg & Redford, 1999). The diversity and easy access to natural and human-originated food sources found in the INP buffer zone may explain why it was so common outside the park. The same is true for the raccoon, also depicted as a generalist, omnivorous, opportunistic and nocturnal terrestrial mammal (Gatti et al., 2006; Faria-Corrêa et al., 2009). Crab-eating fox and raccoon are sympatric in most of their ranges. In our study, they appeared associated with the same type of habitats, typically fragmented forest fragments inserted into a matrix of cereal crops and in the vicinities of farms with cattle, pigs or chicken husbandries.

On the other hand of the sensitivity spectrum stands the charismatic jaguar, whose sensitivity has been recently demonstrated at the genetic level (Roques et al., 2015). Jaguars have long been persecuted by humans (De Angelo et al., 2013). Our results corroborate their findings as jaguars were negatively associated with roads, protection level and little vegetation cover, which all are parameters of habitat fragmentation. Forest cover is crucial for large predators for ambushing, their characteristic hunting technique (Jackson & Jackson, 1996), and for raising their cubs. In our analysis, both pumas and jaguars showed the strongest associated with the level of protection. In their large-scale study, De Angelo et al. (2011) analyzed suitable habitat for both large felids occurring in the eco-region of interest, i.e. the jaguar and the puma. They found out that solely 66% of what is considered habitat by pumas is suitable to jaguars. Pumas are more adaptive to habitat alteration than jagaurs, and are also less targeted by poachers and cultural myths (Morato et al., 2013).

Our findings as for margays are very similar to what was presented by Goulart et al. (2009), i.e. the presence of the species was associated with the distance from roads, and therefore the distance from humans. It was associated with dense forest cover and narrower trails (as opposed to larger roads; Oliveira, 1998). They do occur in small forest fragments but trends suggest than they might be less adaptive than oncillas (Chiarello, 1999; Chiarello & Melo, 2001; Oliveira et al., 2009), not because of their arboreal habits, as it was documented that they mostly use the ground to travel (Oliveira, 1994; Bertrand & Rinaldi, 2007), but because of their arboreal food choices, which might not always be available in degraded areas (Oliveira, 1994; 1998).

Last, jaguars and pumas' all-time favourite prey appeared to be associated with large, well protected forested habitat with generous access to water points. It might be their size that causes the lowland tapirs to be very rarely seen outside of forested habitats (Table 2). We also know that lowland tapirs are highly sensitive to habitat alteration, loss and fragmentation and poaching (Medici et al., 2007). In the Amazon, Peres (1995) observed white-collared peccaries were relatively tolerant to habitat degradation as compared with their relatives, the white-lipped peccaries. This may explain why they still venture out of the INP in small numbers. Peccaries were found to be the main prey item for jaguars in the INP (Crawshaw et al., 2004). Red brockets ranked second in the same study, but red brockets are doubtlessly the most capable deer species to deal with human interference, being the species with the widest distribution in South America (Emmons & Freer, 1997). Our Argentinean colleagues have studied extensively the mammal assemblages of forest remnants on the other side of the Iguaçu river in the province of Misiones, and observed similar trends (Di Bitetti et al., 2008). While red brockets tend to be less numerous where conditions are unfavourable (i.e. poaching, predators, lack of habitats), they are more tolerant to hunting than any other mammal species (Bodmer et al., 1997), very likely because they are largely hunted all across their range (Hurtado-Gonzales & Bodmer, 2004). Our findings corroborate what is known about these three species' tolerance to habitat alteration as tapir are more sensitive than collared peccaries, which in turn are more sensitive to red brockets (Fig. 2).

Similarly, on the CCA biplot (Fig. 2), one of the main prey items for margays, rodents, appeared very close, suggesting they occupy similar habitats. It is logical that prey and predators appear close to each other for they are sympatric species. At the microhabitat scale, resource partitioning and use of time and space are contributing to species coexistence, which include competition, predation and avoidance (Goulart et al., 2009; Di Bitetti et al., 2010). Carbone & Gittleman (2002) showed that carnivore density was related to prey density. This implies that temporal responses of carnivore density to changes may be faster for smaller species than larger ones, given prey availability (e.g. rodents vs. tapirs). This corroborates the difference in prey abundance between the INP and its buffer zone (Fig. 3), with more prey in the INP. Chiarello (2000) showed that larger forest fragments tend to shelter larger populations than smaller ones. There were more predator species associated with degraded and/or open lands in our study, all being generalists with opportunistic behaviours, exerting a stronger pressure on the prey species outside of the park. Therefore, it is intuitive that we should find a larger prey base in the largest forest remnant with low predation pressure, as compared to the fragmented buffer zone, with many predators, less habitat and more competition. Small cats for instance are known to have a higher impact on small fragment prey base (Chiarello & Melo, 2001).

Fragmentation also affects plants within the forest remnants (Silva & Tabarelli, 2000), which, in turn, impact frugivorous species communities. In fragments <300 ha, many important plant sources for terrestrial mammals (i.e. Myrtaceae, Lauraceae, Sapotaceae and Rubiaceae) were reported missing elsewhere (Tabarelli et al., 2009). Azara's agouti, spotted paca, lowland tapirs and collared peccaries are all frugivorous (Chiarello, 1999). Human interference can and does provide other food sources (e.g. orchards, vegetable gardens and cereal fields), which might hide the direct impacts of fruit scarcity in nearby small, isolated fragments with strong edge effects (Stevens & Husband, 1998; Silva & Tabarelli, 2000).

Habitat size, connectivity and protection are the keynotes here. Less than 20% of the remaining Atlantic forest fragments are ≥20,000 ha (IBGE, 1993), which has been evaluated as the minimum patch size to ensure most mammalian species' long-term conservation (Franklin & Frankham, 1998; Chiarello 1999; 2000), hence conservation efforts should concentrate on creating biodiversity corridors to foster genetic flux (Pardini et al., 2005). In the Iguaçu region, efforts have started in the early 2000's to create from agricultural lands the Santa Maria corridor, connecting a 121 ha preserve to the INP and to the Itaipu Binacional's gallery forests. During the present study, we installed 26 cameras over the course of 5 months, which allowed collecting 435 sightings of 18 mammal species. No strict forest dweller was recorded (Bertrand, *unpublished*). However, it is likely that this 60-m wide forest segment may act as a transiting route from one fragment to another, just as Eucalyptus plantations did in the Passa-Cinco river basin in the state of São Paulo (see Dotta & Verdade, 2007).

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CHAPTER 3

BIGS CATS ON THE ROAD AND THE IGUAÇU NATIONAL PARK

ABSTRACT

When it comes to development, political decisions are rarely elaborated based on scientific findings, and most often, act against environmental conservation. Bill nº 7.123 from Deputy Assis do Couto in Brazil, which asks for the reopening of an old road that cuts through the core area of the Iguaçu National Park [INP], might just be a relevant example from which we could derive useful lessons if we are to maintain remnants of natural ecosystems for future generations. We present scientific and historical data available on wildlife in the INP and its vicinity to put that bill into ecological perspectives. Our analysis indicates fauna impoverishment with the predominance of ubiquitous and generalist species, permanent and omnipresent pressures of all kinds (i.e. poaching, fishing, palm-tree extraction), and the disappearance of the main top predator (i.e. jaguar). Altogether they characterize a destructive trend consistent over time. This trend generates long-lasting effects on wildlife and flora suggesting the INP is not fulfilling its function. This paper is a pioneer attempt at documenting the general ecological and historical situation of the most renowned national park in Brazil while evincing the lack of peer-reviewed publications on it. Last, it likely - and most importantly - exposes data contrasting with the overall perception provided by the tourist tour at the waterfalls. The story of the INP might just reflect what is happening everywhere else nature is seen as an impediment to "humans' progress".

KEYWORDS

Iguaçu National Park, jaguars, wildlife, road, politics, conservation.

CAPÍTULO 3

ONÇAS NA ESTRADA E O PARQUE NACIONAL DO IGUAÇU

RESUMO

No tema do desenvolvimento, as decisões políticas são raramente baseadas em dados científicos, e acabam, muitas vezes, prejudicando a conservação da natureza. No Brasil, um projeto de lei, que pede a reabertura de uma antiga estrada que cortava a parte intangível do Parque Nacional do Iguaçu [PNI], é provavelmente um exemplo perfeito, a partir do qual poderiamos criar consciência em relação aos remanescentes de ecossistemas naturais. Apresentamos aqui alguns dados históricos e científicos disponíveis sobre a vida selvagem no PNI e seu entorno para colocar a realidade em perspectiva. As nossas análises indicam um empobrecimento da fauna com a predominância de espécies generalistas e ubíquas, com pressões onipresentes e permanentes de todos os tipos, e o declínio do maior predador na unidade. Trata-se de uma tendência destrutiva consistente no tempo, gerando efeitos deletérios duradouros sobre a vida selvagem e a flora podendo comprometer os serviços ecológicos do PNI. Este artigo é uma tentativa pioneira de documentar a situação histórica e ecológica geral do parque brasileiro o mais renomeado e de mostrar a pobresa de material científico sobre o mesmo. Finalmente, e mais importantemente, expõe os dados que contrastam com a percepção geral obtida ao fazer o passeio turístico nas cataratas. A história do PNI apenas reflete o que está acontecendo em todos os lugares onde a Natureza é vista como um impedimento ao tal do “progresso”.

PALAVRAS-CHAVES

Parque Nacional do Iguaçu, Brasil, estradas, onças-pintadas, desenvolvimento humano.

The Atlantic forest is the biome which has suffered most since European settlement in Brazil (Ribero et al., 2009) with only 7% of the original forest cover remaining (Galetti et al., 2013), and holds the sad record of being the ecosystem the most threatened of Brazil (Dean 1997; Myers et al. 2000). In the state of Paraná, the largest continuous block remaining of this forest is protected in the 185000-ha Iguaçu National Park (INP; S 25° 41'; W 54° 26') under federal protection since 1938 (Conforti & Azevedo, 2003). However, the park is subject to heavy pressure from extensive, chemically-based agricultural crops in its surroundings, and from poaching and palm-tree extraction (Tabarelli et al., 2005). The INP surroundings are composed of a matrix of agricultural lands representing over 2500 km^2, under a specific environmental legislation which allegedly emphasizes human impacts buffering through the restriction of human activities and soil uses (Antunes, 2002). This could not be any further from the reality. Conventional agriculture applied in the INP's surroundings negatively impacts biodiversity, especially mammals, amphibians and aquatic ecosystems (Waynon & Finley, 1980). These practices applied on the INP buffer zone represent an undeniable pressure over the park's biological integrity, contaminating waters, soils and air (Tscharntke et al., 2005; Londres, 2011). Ironically, the INP is a World Heritage site since 1986 because of the scenic beauty of a magnificent curtain of 274 waterfalls sculpting the borderline between Argentina and Brazil.

Figure 1. Iguaçu National Park and Settler's Road (Estrada do Colono) location in southwestern Brazil.

The Settler Road (SR; in Brazilian Portuguese: "*Estrada do Colono*") refers to a 17.6 km-long unpaved road that was used for some time by locals and explorers between 1953 and 1955 (Fig. 1). They used the trail to access the Iguaçu River from the forest edge (MMA/IBAMA, 1999). It has been used until 1986 when it was closed based on federal justice order. It was then illegally invaded three times, in 1997, 2001 and 2003, causing violent oppositions between locals and the police (Dias, 2006). It has been a social, economic and political headache ever since and still makes the headlines today with the bill nº 7.123 written in April, 2010 by Federal Deputy Assis de Couto from the Workers' Party, asking for its reopening, as bill's author says it would promote regional development, tourism, education and nature conservation. Here we present summaries of ground data collection in the SR area, with emphasis on mammals and especially keystone felid species, and we discuss their implications if such a bill were to be approved.

READING THE BUSH

The first scientific monitoring incursion in the old SR occurred one year after its closure in 2001. As one could have expected, it was 21 hectares (12 x 17600 m) of environmental disaster, garbage and soil deep compaction due to the passage of heavy vehicles. Aggressive and invasive Guinea grass (*Panicum maximum*) had taken over the place. We registered and geo-referenced 78 location points of fauna evidence, on average one every 225.6 meters (Savi et al., 2002). The majority of species found during this expedition are listed as threatened of extinction in the Red Book of the Threatened Fauna in the State of Paraná (Mikich & Bérnils 2004) such as pumas (*Puma concolor*), tapirs (*Tapirus terrestris*), otters (*Lontra longicaudis*) and neotropical felines of small and medium sizes: ocelots (*Leopardus pardalis*), margays (*L. wiedii*), and oncillas (*L. tigrinus*).

Threatened and data-deficient species account for 32% of the total number of species occurring in the state of Paraná today. The main threats to all these species' survival are the very results of a road opening in a forested area: habitat fragmentation, habitat loss, poaching and road kills (Pires & Cademartori 2012). Straube and Urben-Filho (2002) studied the INP avifauna and registered in the SR the fifth official occurrence of the Ornate Hawk-Eagle (*Spizaetus ornatus*), a nationally threatened species according to the Brazilian Ministry of Environment. The same authors published an update on avifauna for the INP two years later, regretfully acknowledging the lack of avifauna data for the SR area and the whole INP, stressing that environmental crimes from locals were a negatively-impacting factor on nature conservation (Straube & Urban-Filho 2004).

The SR was first taken over by invasive Guinea grass, Wanderingjew Zebrina (*Zebrina pendula*), *criciúma* (*Arundinaria aristulata)*, creepers and pteridophytes. The presence of open-crowned tree species covered with invasive species was detected, along with new clearings created by the fall of trees on the SR's edges on both sides. These observations are common indicators of alterations like those associated with a pronounced edge effect, coinciding with the accelerated biological impoverishment of local ecosystems (Ribeiro et al. 2009). Invasive species quickly take over the space and the nutrient access in the area and inevitably favor biological losses in different temporal scales. In addition to the spatial isolation from local original ecosystems due to the forest landscape discontinuity, the pronounced edge effect in the INP's core area is, according to Fernandez (2000), "the forest's death from within". Director of the Protected Areas Department from the Brazilian Ministry of Environment, Sérgio Brandt, explained that the SR represents a barrier for many species, including bird species, which would not cross it, creating "an island within the INP." Road ecology has widely documented that the observed edge effect represents a barrier to fauna movements and to flora dispersion, accentuating even more the observed on-going fragmentation process (Ferreira 2000; Develey & Stouffer 2001; Coffin 2007).

In addition to the forest fragmentation caused by the SR at proximity of the INP core area and the soils and waters contamination by agrochemicals in its surroundings, illegal activities such as intense palm-tree extraction, fishing and poaching, worsen the observed ecological impoverishment and specialist species decline (see Chapters 1 & 2), as Crawshaw (1995) claims looking at species dynamics between jaguars and their favorite prey species, white-lipped peccaries (*Tayassu pecari*). Poaching aggravates the loss of genetic variability of species on the edge of extinction (Crawshaw 1995; Rosser & Mainka 2002). In 2008/9, during a mammal inventory funded by the Fundação Boticário de Proteção a Natureza, we found that humans were largely prevalent with 20% of all records (n = 1661 records), almost twice more abundant than the next most abundant mammal we found: the Brocket Deer (Bertrand, 2010). Domestic and hunting dogs accounted for 7% of total records while emblematic jaguars represented only 0.2%. Overall, 249 infringements were recorded, which included 0.7 poaching trail per investigated kilometer (Bertrand, 2010). Meanwhile, patrollers from the INP Law Enforcement Sector found ten active camping sites in the INP intangible area, close to the SR. Interestingly, active camping sites were also found in the intensive use area (waterfalls area), one being 400 m behind the domicile of the lieutenant of the Environmental Police's battalion. Clearly, impunity reigned and infringers were active and omnipresent. In a study published in 2011, Fragoso *et al*. (2011) analyzed qualitatively ten years of poaching data from the INP. Poachers usually go to the bush in 3-4 people, and preferentially hunt deers, agoutis, and birds. Most poachers were from Capitão Leônidas Marques, Capanema and Céu Azul (Fragoso et al. 2011), three municipalities in the vicinity of the SR and INP core area (Fig. 1). According to Ivan Carlos Baptiston, current INP Director and former head of the INP Law Enforcement Sector, offenders come from Capanema for two reasons: (1) lack of continuous presence of patrollers in the region, and (2) quick access to the INP by the Iguaçu River by the southernmost part of the SR.

The Atlantic forest is the Brazilian biome with the most conservation units. This particularity can be seen as tangible attempts to deter habitat and biodiversity loss (Pinto et al., 2009). However, most conservation units in Brazil do not achieve their mission, lacking skilled staff, equipment and inadequate resources distribution (Vallejo, 2002), and the INP is no exception.

We returned to the SR four years later, forest re-growth was noticeable even though the colonizing grass was still dominant, reaching three meters of height, making it difficult for pioneer tree species to thrive. Ruts made by former bridges' implosion were already covered by vegetation. We found 14 big cats' feces on the main road, suggesting its use by these keystone species. We also found many evidence of palm-tree extraction in the southernmost section of the SR. Palm-tree extraction is on-going in areas close to Capanema, Matelândia, Missal and surroundings. In December 2008, six men from Capanema were caught in the act. In one day, these six men had cut down 700 palm-trees, devastating a large area of forest easily reached from Capanema.

The INP largest continuous monitoring was conducted between 2012 and 2014 with camera-traps. We collected ca. 248,000 photographs of wildlife. We recorded the presence of over 30 mammal species in 6,190 trapping nights (see Chapter 1). The most represented animals were opossums (*Didelphis* spp.), agoutis, rodents, nine-banded armadillos, coatis, red brockets, white-collared peccaries, and tayras, all being alteration-tolerant species (Pires & Cademartori, 2012). Five feline species were registered in small numbers: ocelots, oncillas, jaguarundis, pumas, and margays. While most species listed above are generally present in anthropized areas, the presence of specialized carnivore species such as ocelot and jaguarundi indicate the ecological importance of this area (Indrusiak & Eizirik, 2003).

During this monitoring, we have lost three cameras to infringers. The omnipresence of offenders in this federal conservation unit, confirmed through unmistakable evidence of palm tree extraction and poaching, has been consistent over time (Paviolo et al., 2008; Bertrand, 2013). Such situation is a contributor to jaguars decline. Among over 247 000 photographs, we solely had three photograph sequences of this species, an adult female bearing a radio collar (which was not working any longer) and two other young adults (Bertrand, 2013). Sadly, the INP emblematic jaguar was estimated on the verge of extinction in the Upper-Paraná Atlantic Forest eco-region (Paviolo et al., 2009). It is estimated that less than 250 adult individuals are occurring in the entire biome (Galetti et al. 2013). Scientific historical data available for the INP are presented in a doctoral thesis from George Schaller's pupil, wildlife biologist, senior researcher and employee of the Brazilian Ministry of Environment, Dr. Peter Crawshaw Gransden Jr. (1995). INP jaguar density between 1985 and 1995 was 3.7 adults/100 km^2. Considering an adult female has 1.5 cubs per litter which she raises during two years, the total density estimated for the whole INP area would be 7.5 jaguars/100 km^2 or a total of 64 adults or 134 animals, all age combined (Crawshaw, 1995). By the end of his research, all jaguars monitored by Peter were dead, either by poachers, or road kill or because of livestock depredation. After 600 monitoring days, the cumulative curve for jaguar mortality reached 100% vs. only 24% for ocelots (Crawshaw, 1995). Dr. Paviolo's camera-trap work in the Parque Nacional del Iguazú (the Argentinean side of the INP) between 2004 and 2010 brought more light to the big cats' timeline in this eco-region. He determined a jaguar density ranging from 0.53 to 0.85/100 km^2 (Paviolo, 2010). Animals move evenly from one side of the park to the other, which means that Paviolo's findings are likely to be valid for the whole 'Iguazu' preserved area. From the broader perspective of the Green Corridor, which includes the green areas of Argentina and Brazil (i.e., 10.528 km^2), jaguar population was estimated of 33 and 54 individuals (Paviolo, 2010). The INP Carnivores Project, which started the same year in the INP in Brazil, reported only 6 to 12 jaguars for the Brazilian INP based on a 72-camera-trap monitoring (INP Counsel, 2012).

DIRECT AND INDIRECT THREATS

The data available for the Iguaçu region indicate that many big cats were killed by humans, may it be poaching, road collisions, trophy hunting, unexpected encounters with poachers, or livestock depredation. Like in many other areas where cities' expansion reaches wilderness areas, myths and traditions strongly rooted into local cultures and belief systems have taken a heavy toll on large cats (Conforti & Azevedo, 2003; Michalski et al., 2006; Colchero et al., 2010). This reinforces the idea that public awareness campaigns and environmental literacy programs are much needed to replace local community ignorance by knowledge and sensitization, so that locals can start treating carefully the regional natural treasures that are still present (Azevedo, 2008).

On a habitat level, De Angelo et al. (2011) conducted a regional analysis on ecological requirements for both large cat species. They found out that less than 15% of the whole Upper-Paraná Atlantic Forest eco-region was favorable to the presence of jaguars and pumas. In fact, the jaguar population is not considered a viable population anymore by local researchers (Paviolo et al., 2008). We know that large cats tend to use preferentially human trails (Paviolo et al., 2009) and we found latrines (territorial marking) for both species in every SR expedition, confirming the need to maintain this area preserved. The INP is indeed one of the last rare places characterized as jaguar habitat.

IMPLICATIONS FOR CONSERVATION

This paper provides a coarse overview of the pressures exerted by human activities in and around the INP as well as big cats population information in this part of their range. Monitoring the INP's 185,000 hectares simultaneously is logistically challenging and financially prohibitive. We did not intend here to numerically characterize infringements in the INP. Given the data available, one can only underestimate the pressures suffered by the INP. Nonetheless, ground data indicate the ecological importance of the INP as it still shelters specialist species (e.g. jaguars, anteaters; see Chapter 2) with specific requirements and as it represents 80% of the Atlantic forest left in the entire state of Paraná (Crawshaw et al., 2004). The SR historic clearly depicts the negative impacts of roads in forested landscapes.

Based on what is known on the inland Atlantic forest and its rich wildlife, this first assessment suggests fauna impoverishment with the predominance of ubiquitous and generalist species, intense and omnipresent environmental pressures along with the disappearance of the major top predator (i.e. jaguar; more details in Chapter 2). There is a time-consistent destructive trend ever since the first investigations were conducted in the area. With environmental crimes continuing or worsening, we might observe an even more drastic simplification of the animal community mostly composed of generalist species. If nothing changes, the INP may become Redford's dreaded 'empty forest' (Redford 1992).

Datasets do corroborate the fact that the current trend of violations and exploitation in the INP remains high. This paper is an attempt at publishing important information that usually remains hidden in technical reports and graduate studies' theses. Datasets are periodically passed onto the INP managers and to Brasília via SISBIO (on-line platform for the Brazilian Ministry of Environment), but the problem remains unsolved. Situation complexity is blatant with politics' agendas, industrial lobbies interests, unsustainable resources' exploitation and established environmental crime impunity, all rooted in local habits and regional mentalities. Confronting these findings to the political context initially described, common sense will bring about the answer to the following questioning: if it is currently not possible to protect the 440 km of INP perimeter from daily assaults from all sides, what will the destruction of 21 more hectares of opening/edge create?

When put in perspective the current biodiversity situation, Couto's bill, whose objective is to alter the National System of Conservation Units to institute "parkways" and therefore legalize road opening in Brazilian forest remnants, starting with the SR (Baptiston & Garcia, 2010), is an offense to common sense and demonstrate that legislation serves economic and lobby interests much rather than world heritage. If the INP is to maintain its World Heritage title and its "one of the seven World Wonders" ranking, no road should ever be open within this conservation unit and efficient strategies should be defined and implemented to mitigate and minimize these pressures on INP's biodiversity in the best delays.

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CHAPTER 4

OCCURRENCE OF GEOFFROY’S CAT *(Leopardus geoffroyi)* IN THE IGUAÇU REGION, SOUTHWESTERN BRAZIL. SURPRISE OR INEVITABLE RESULT?

ABSTRACT

Geoffroy’s cats are known to be associated with open areas and shrub lands in South America. Yet, a Geoffroy’s cat was found in the Upper Paraná Atlantic Forest Eco-region, near Foz do Iguaçu, Brazil. Later, DNA analyses conducted on feces samples in the same area reinforced the evidence of the concealed presence of the species. We discuss here the possible reasons of its occurrence in this region.

KEYWORDS

Geoffroy’s cat, Brazil, Iguaçu region, Atlantic forest, habitat change.

CAPÍTULO 4

OCORRÊNCIA DO GATO-DO-MATO-GRANDE *(Leopardus geoffroyi)* NA REGIÃO IGUAÇU, SUDOESTE DO BRASIL: SURPRESA OU RESULTADO INEVITÁVEL?

RESUMO

Os gatos-do-mato-grandes são conhecidos por ser associados com áreas abertas e terras arbustivas na América Latina. No entanto, o gato-do-mato-grande foi encontrado na eco-região da mata Atlântica do Paraná superior, próximo ao município de Foz do Iguaçu, Brasil. Em seguida, análises de DNA foram realizadas em amostras de fezes coletadas na mesma área e reinforçaram a prova da presença oculta da espécie. Discutimos aqui as possíveis razões para a ocorrência desta espécie nesta região.

PALAVRAS-CHAVE

Gato do mato grande, Brasil, região Iguaçu, floresta Atlântica, alteração do hábitat.

There are six felid species known to occur in western Paraná, Brazil: jaguar Panthera onca, puma Puma concolor, ocelot Leopardus pardalis, margay Leopardus wiedii, oncilla Leopardus tigrinus, and jaguarundi Puma yagouaroundi (Crawshaw, 1995; Chebez, 1996). In February of 2006, a male Geoffroy's cat Leopardus geoffroyi (Fig. 1) was captured in the border of an agricultural area in Foz do Iguaçu, Paraná, Brazil (Fig. 2). Fire fighters brought it to ITAIPU Binational Wildlife Refuge where it has been kept in captivity. This species' closest confirmed occurrence sites are located ca. 300 km toward the west and south in Argentina and Paraguay (Lucherini et al. 2008) or 600 km southward, in Rio Grande do Sul state, southern Brazil (Trigo et al., 2013). Another visual sighting was also earlier reported in the eastern part of the Rio Grande do Sul state but there was no confirmed evidence (Margarido & Braga, 2008). Two hypotheses were initially proposed to explain the species presence in this area: (1) it may have escaped from local illegal captivity, or (2) land alteration has provided suitable habitat for this species to colonize the area from existing populations in nearby Paraguay and Argentina.

The process of resource exploitation – through logging, agriculture and urban sprawl has greatly reduced the original vegetation cover in the Upper Paraná Atlantic Forest region during the last six decades (Strauber & Urben-filho, 2002). The resulting landscape contains forest fragments ranging from 0.3-185,000 ha, riparian forests, agricultural fields, ranches/farms, and urban development.

At the time of the cat's capture, we were studying mammals' response to habitat alteration, with particular focus on felids. Between 2008 and 2010, presence signs such as feces and latrines, scratches and predation signs of felids were recorded and georeferenced over 400 km of investigated poaching and game trails in and surrounding the Iguaçu National Park. A total of 49 scat samples were collected and sent to the American Museum of Natural History, New York, for analysis as part of the Global Felid Genetics program led by Salisa Rabinowitz for species identification. Genetic methodology consists in amplifying extracted DNA from scat samples using six primer sets spanning four mitochondrial gene regions that have proven effective in discriminating carnivore species. The four mitochondrial genes used were: cytochrome oxidase b, ATP-6 synthase, 12S rRNA, and 16S rRNA (see details in Caragiulo et al. 2013). Of the 49 scat samples analyzed, 31 could be positively identified and ten were positively identified twice as belonging to Geoffroy's cat. The remaining were 14 ocelot scats, 5 puma scats, one jaguar scat and one small feral cat scat.

All Geoffroy's cat feces were found on the forest edge or buffer zone of the Iguaçu National Park (Fig. 2). The buffer zone is an agricultural matrix composed primarily by soy-corn-wheat fields, eucalyptus plantations and forest remnants. According to the scats distribution, Geoffroy's cat was the predominant species in the non-forested landscape including areas with human alteration.

The possibility that Geoffroy's cats are colonizing areas near the Iguaçu National Park from the bordering populations in Paraguay and Argentina should be considered. The reviewed literature clearly illustrates the Geoffroy's cat ability to adapt to a wide range of situations including habitat conversion

Figure 1. Geoffroy's cat male specimen captured in Foz do Iguaçu, Paraná, Brazil in 2006. From left to right, fore paw, rear paw, right profile and tail views. Microchip ID: 00-0629-EB9E. Credit: Anne-Sophie Bertrand © 2006.

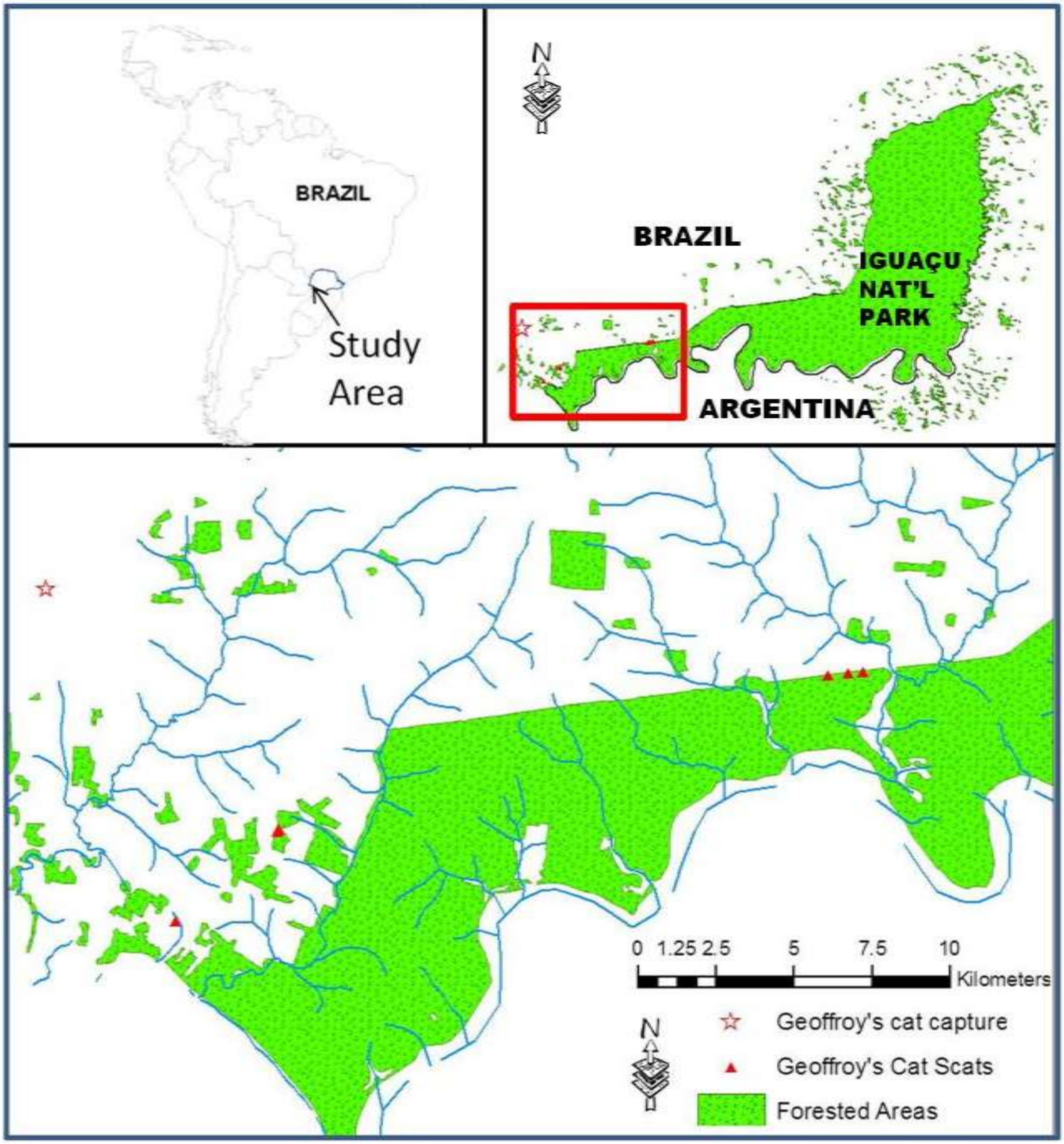

Figure 2. Capture location of a Geoffroy´s cat individual and locations of scats genetically confirmed to be of Geoffroy's cats, Iguaçu, southern Brazil.

and fragmentation (Manfredi et al., 2006; 2011; Pereira et al., 2012). With the increase of open lands providing more suitable habitat in this region and given Geoffroy's cat's ecological plasticity and opportunistic behavior, it could be migrating to western Paraná from neighboring populations. While this is coherent with our discovery of a live Geoffroy's cat along with 10 scat samples in Foz Do Iguaçu, we cannot however discard the hypothesis the captured individual being an escapee from illegal captivity.

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CHAPTER 5

NEOTROPICAL SPOTTED CATS SPECIES DISCRIMINATION USING MORPHOMETRICS

ABSTRACT

Leopardus cat species identification can be performed using footprints. We performed a discriminant function analysis on a data set of 52 Neotropical Spotted Cats (NSC) individuals from a Brazilian wildlife reserve. We isolated five morphometrics that wildlife biologists can use to identify the author of a track or footprint, i.e. total length, heel pad width, negative space (Lead toe to the front lobe of heel pad length), outer toe area and heel pad area. Our technique properly classified 94.2% of the individuals into the correct species, and 86.5 % of cross-validated groups were correctly identified. By relying on measurable variables rather than the observer's ability to identify a species from a footprint, this technique will increase correct identification during track surveys, which will in turn help increase the accuracy of population inventories.

KEYWORDS

Morphometrics, *Leopardus pardalis, L.tigrinus, L. wiedii*, footprints, Brazil.

CAPÍTULO 5

DISCRIMINAÇÃO DE GATOS PINTADOS NEOTROPICAIS UTILIZANDO MORFOMÊTRICOS

RESUMO

A identificação das espécies do gênero *Leopardus* pode ser realizada utilizando pegadas. Realizamos uma análise de função discriminante com um banco de dado de 52 Gatos Pintados Neotropicais (GPN) de um refúgio biológico brasileiro. Identificamos cinco morfomêtricos que os biólogos da vida selvagem podem utilizar para identificar o autor de uma pegada, i.e. cumprimento total, largura da almofada traseira, espaço negativo (distância entre a ponta do dedo líder e o lobo frontal da almofada traseira), a superfícia da almofada traseira e dos dedos os mais externos. A nossa técnica classificou com sucesso 94.2% dos indivíduos nas espécies corretas, e 86.5 % dos grupos contra-validados foram corretamente identificados. Recorrendo a essas variáveis mensuráveis ao invés da habilidade do observador em identificar uma espécie com base em pegadas, esta técnica contribuirá a aumentar o número de identificações corretas durante os levantamentos de pegadas, o quê contribuirá a aumentar a exatidão dos levantamentos populacionais.

PALAVRAS-CHAVES

Morfomêtricos, *Leopardus pardalis, L.tigrinus, L. wiedii*, pegadas, Brasil.

INTRODUCTION

Brazil shelters six felid species, three of which belong to the *Leopardus* genus (Oliveira & Cassaro 1999). We will refer to these species as Neotropical Spotted Cats (NSC) as they all present spotted fur patterns, i.e., Ocelot (*Leopardus pardalis*), Oncilla (*L. tigrinus*), and Margay (*L. wiedii*; Figure 1). NSC are found in similar habitats, usually tropical and subtropical rainforests and dense vegetation areas (Oliveira & Cassaro, 1999). While larger felids are key species to the balance of ecosystems (Terborgh, 1990), smaller felid species are critical in controlling small prey populations (Konecny, 1989). The growing anthropogenic habitat alterations, e.g. forest fragmentation, poaching, water/soil pollution, affect all Brazilian ecosystems and compromise their ecological services (Haines et al., 2005; Inskip & Zimmermann, 2009). Habitat disturbance is a common root cause to the NSC species declines (Caso et al., 2008; Oliveira et al., 2008; Payan et al., 2008).

Figure 1. Comparison picture showing morphological specificities of each NSC species considered in this article. From left to right: *Leopardus tigrinus*, *L. wiedii*, and *L. pardalis*. Individuals are all captive adult males. Photograph was taken at the Itaipu wildlife refuge facilities.

NSC discrimination can be challenging as they have many similar attributes, e.g. habitats, morphology, behavior, ecology (Wang, 2002), especially Margays and Ocelots (Haemig, 2001; Oliveira et al., 2009). Haemig explained these similarities by the narrow phylogenetic links between them. Recent phylogenetic studies allowed separating these close-relative species (Eizirik et al., 1998) and even reported hybridization cases (Trigo et al., 2008).

Implementing conservation projects on specific NSC species is difficult for several reasons. Solitary nocturnal habits and dense, often impenetrable habitat make NSC sightings rare events. Each data collection method has its pros and cons (Silveira et al., 2003). Telemetry studies are costly and generally based on a very small number of individuals (e.g. Konecny, 1989). The reliability of camera-trapping data depends on the ability of the observer to distinguish species on photographs (Dillon & Kelly, 2008). When time allows, spoor surveys remain one of the most affordable methods for detecting and identifying felid species using footprints and track sets (Lewinson et al., 2001; Gusset & Burgener, 2005). However, results may vary depending on substrate quality, weather conditions and locomotion speed (Simonetti & Huareco, 1999) and this may interfere with the observer's ability to determine a species by its tracks (Smallwood & Fitzhugh, 1995; Grigione et al., 1999). Therefore, the reliability of data collected by any of these methods may be questionable (Frey, 2006). Using a similar approach to what Lewinson et al. (2001) and more recently, García et al. (2010), have done with free-ranging and captive pumas (*Puma concolor*), the objective of this study was to verify the possibility of discriminating NSC species by a limited number of foot morphometrics, using a computer-based technique rather than relying on an observer's visual identification abilities.

MATERIAL AND METHODS

Study area — The Itaipu Binacional's Wildlife Refuge is a facility sheltering several endangered species from inland Atlantic forest. It is located 30 km north of Foz do Iguaçu, state of Paraná, southwestern Brazil.

Data collection — We used a dataset of 52 captive adult specimens, genetically originating from the Iguaçu region. We drew the right fore foot outlines on a plastic board placed above footprints in sandy soil of 13 Ocelots, 21 Oncillas and 18 Margays. We then used SigmaScan Pro™ to accurately obtain morphometrics from these outlines (Lewinson et al., 2001). We used linear, angular and area measurements as well as total length and width (Figure 2; Smallwood & Fitzhugh, 1993; Gigrione et al., 1999; Lewinson et al., 2001; García et al., 2010). In total, we collected measurements of fifteen morphometric variables for each footprint (Appendix S1).

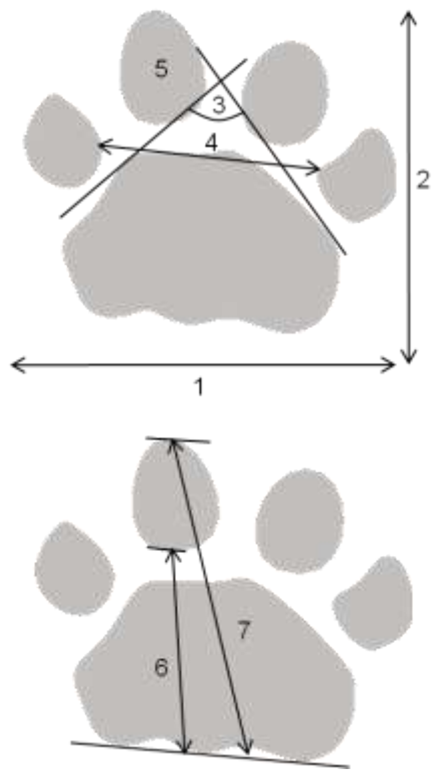

Figure 2. Linear and angular measurements characterizing NSC footprints. 1: Total width, 2: Total length, 3: Angle between inner toes, 4: Outer toe spread, 5: Width and length of lead toe, 6: Heel to lead toe length, 7: Negative space (Lead toe to the front lobe of heel pad length). The area of heel pad and each toe was also measured.

Statistical analyses — We tested normality using Kolmogorov-Smirnov test (García et al., 2010) and homogeneity of variance using Box's M test. As the purpose of a discriminant function analysis (DFA) is to predict membership in naturally occurring groups (Tabachnick & Fidell, 1983), we used it to identify the variables that could best categorize NSC individuals into species groups based on a selected set of morphometrics. Thus, we applied DFA to our data set of 52 individuals for which we calculated 15 distinct morphometric variables from their right fore feet. We evaluated the standardized discriminant function coefficients to identify the predictor variables which explained most of the linear classification functions obtained. We verified the NSC species assignment using Wilk's Lambda test, matrices values and locations within the scatterplot (Lewinson et al., 2001). Among the initial list, we selected the morphometrics the most correlated to the two first discriminant functions from the structure matrix. We then conducted another DFA with the reduced set of morphometrics (DFA_2). The probability of misclassification of uncategorized cases was assessed using cross-validation (Hill & Lewicki, 2005). We used SPSS™ 15.0 to perform all statistical analyses.

Variable selection – We evaluated the predictor variables that explained the most of the discriminant functions. We eliminated redundant predictor variables to reduce matrix ill-conditioning. We selected the variables with the largest absolute correlation with the discriminant functions in order to select a reduced number of morphometrics usable to discriminate NSC species and develop an effective identification tool for wildlife biologists.

Score calculations — The discriminant coefficients of each morphometric of the model equation used to compute discriminant scores for each case are determined by the following discriminant function equation:

$$L = b_1x_1+b_2x_2+\ldots+b_nx_n + c$$

where L is the latent variable formed by the discriminant function, b_n are the discriminant coefficients and c is a constant.

RESULTS

Using 15 morphometric measurements (Appendix S1), the first discriminant function explained 91.5 % of total variance, and 100 % of it could be explained by the two first canonical variables. All variables but one (i.e. negative space, Table 1) were strongly correlated to the first discriminant function. The five morphometrics that contributed the most to species discrimination were total length, heel pad width, negative space, outer toe area and heel pad area. In DFA_2 (Figure 3), 95.8 % of total variance was explained by the first canonical discriminant function and the remaining with the second. DFA_2 model allowed 94.2 % of original grouped cases to be correctly classified while 86.5 % of cross-validated groups were correctly classified.

Table 1. Standardized discriminant scores (*n* = 52 cases).* Largest absolute correlation between each variable and discriminant functions. † Ln transformed variables. Variable code key: PADA: Heel pad area; D1A: Inner toe area; D2A: Second toe area; D3A: Third toe area; D4A: Outer toe area; PADW: Heel pad (maximum) width; PADL: Pad length; D3W: Third toe width; D3L: Third toe length; PADD3: Heel to lead toe length; NEGSP: Negative space (Lead toe to the front lobe of heel pad length); D1D4: Outer toe spread; LENGTH: Total length; WIDTH: Total width; D2D3: Angle between inner toes.

	Function	
	1	2
LENGTH	0.803*	0.031
D4A†	0.783*	-0.177
PADW†	0.749*	0.130
PADA†	0.732*	0.045
D2A†	0.720*	-0.033
D1A†	0.700*	-0.107
PADL	0.696*	-0.058
D3A†	0.690*	-0.260
D3L†	0.642*	-0.357
D1D4	0.639*	0.129
WIDTH†	0.619*	0.069
PADD3	0.595*	0.129
D3W†	0.583*	-0.168
D2D3	0.269*	0.043
NEGSP	0.062	0.385*

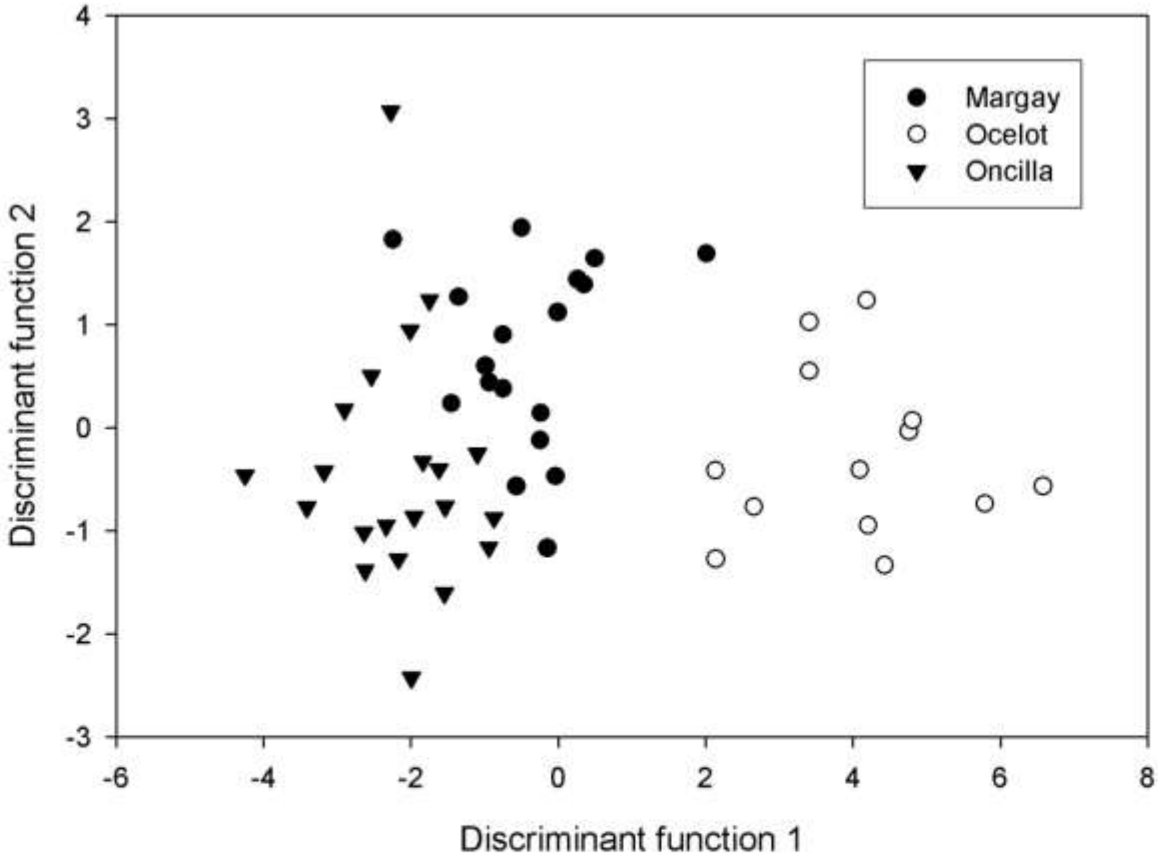

Figure 3. Scatterplot showing model performance in discriminating NSC individuals by species (n = 52).

DISCUSSION

The classification model obtained with the five morphometrics most correlated to the two discriminant functions failed to classify correctly 5.8 % of the original grouped NSC individuals. Two of the five selected morphometrics have been reported to best discriminate puma individuals by Smallwood and Fitzhugh (1993), i.e. the outer toe spread and the heel pad width. Interestingly, Gigrione et al. (1999) found that area measurements best discriminate individuals of a same species, with the exception of the heel pad area. This may suggest that heel pad area varies very little among individuals of a same species, which might explain why this morphometric is a component of our species classification model. When trying to discriminate males from females, García et al. (2010) found that the heel pad width, the inner toe area, the second toe area and the heel pad area provided the best results. Our data set contained both females and males of all three NSC species. Both the heel pad area and width contributed significantly to discriminate among species, suggesting that sexual size dimorphism also accounts for some of the species discrimination. Last, Lewinson et al. (2001)'s discriminant model for puma individuals included the outer toe spread, the heel to lead toe length and the angle between inner toes, which does not corroborate our findings for NSC species discrimination but their research focuses on pumas' rear feet.

Misclassification of cross-validated groups accounted for 13.5 % of cases, most of which occurred between Margays and Oncillas. Hybridization between NSC species has been reported, especially where distribution ranges overlap due to environmental stress (e.g., food scarcity; Oliveira et al., 2008; Trigo et al*.,* 2008). The morphometrics used to construct the classification model may need to be reviewed as hybridization progressively becomes a common event in NSC genetics. Meanwhile, the current model could help field NSC identification in 86.5 % of cases, as long as footprints are clear enough to extract measurements for the five selected morphometrics. The application of such tool could thus improve the reliability of field NSC inventories used for conservation purposes (Frey, 2006).

Management implications

If the simple outline collection method and identification tool presented here were used in some of the many shelters and refuges worldwide, much needed data could quickly be available to wildlife biologists. Method can be applied to other species by developing a discriminant model based on footprints collected from adult captive specimens of the species of interest, prior to field collection. Obviously, field data collection is totally dependent upon the quality of substrate and weather conditions. However, when these conditions are favorable, species abundance can then be estimated from track surveys in the field (Rodriguez et al., 2000) and used to inform conservation strategies anywhere they are required.

While NSC species have been receiving more and more attention for the past two decades, their population statuses and extinction risks could not be clearly determined due to the lack of field data (Oliveira et al., 2008; Payan et al., 2008). Our field experience and the number of misidentifications found in educational and vulgarization science materials suggest a high probability for spreading erroneous information about these cats. Without a reliable, observer-independent identification tool, the IUCN Red List may be presenting inaccurate information on these confusing NSC species and their actual population statuses might be quite different from what they are thought to be (Rodrigues et al., 2006). Margays are listed as 'nearly threatened' by the IUCN with habitat destruction as one of the main threats (Payan et al., 2008). Yet in 2007, during telemetry monitoring in the study area, we found Margays in moderately to heavily-altered habitats (unpublished data). Oliveira et al. (2009) conducted a telemetry study in the region of Emas National Park and also found that ocelots and other small cats used pasture and agricultural lands. Di Bitetti et al. (2010), who used camera-traps to look at habitat use and species occupancy patterns, also found that NSC, especially Margays and Oncillas, were relatively resilient to habitat disturbances. Lastly, one could rightfully question the validity of an identification tool based on tracks for the arboreal Margay species. Nonetheless, telemetry monitoring and camera-trapping have confirmed that Margays actually use the ground to move around and only occasionally use tree canopy (Konecny, 1989; Di Bitetti et al., 2010).

Typical NSC behaviors do not normally allow direct observations. Footprints or track sets therefore represent a convenient tool for inferring population attributes and dynamics (Balmé, 2005). The identification procedure presented here is innovative as it simplifies and increases the reliability of NSC species identification based on footprints. It does not rely on observation abilities, but rather on objective morphometrics. It can be used by anyone with some computer skills and greatly minimizes misidentification risks.

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Appendix S1. Right front foot measurements (in cm) collected on 52 captive Neotropical Spotted Cats. Itaipu Binacional wildlife refuge, Brazil. Code key: PADA: Heel pad area; D1A: inner toe area; D2A: Second toe area; D3A: Third toe area; D4A: Outer toe area; PADW: Heel pad (maximum) width; PADL: Pad length; D3W: Third toe width; D3L: Third toe length; PADD3: Heel to lead toe length; NEGSP: Negative space (Lead toe to the front lobe of heel pad length); D1D4: Outer toe spread; LENGTH: Total length; WIDTH: Total width; D2D3: Angle between inner toes.

Common name	Latin name	Gender	PADA	D1A	D2A	D3A	D4A	PADW	PADL	D3W	D3L	PADD3	NEGSP	D1D4	LENGTH	WIDTH	D2D3
Margay	Leopardus wiedii	F	2.441	0.472	0.491	0.520	0.550	2.083	1.686	0.696	1.010	2.010	0.841	1.776	2.900	3.164	86.303
Margay	Leopardus wiedii	F	1.973	0.404	0.388	0.410	0.441	2.013	1.414	0.614	0.803	1.774	0.444	1.424	2.900	2.724	67.326
Margay	Leopardus wiedii	M	2.136	0.434	0.447	0.429	0.413	1.928	1.595	0.558	0.905	1.949	0.409	1.804	2.900	3.212	90.938
Margay	Leopardus wiedii	F	2.340	0.554	0.539	0.584	0.505	2.078	1.583	0.689	0.967	2.237	0.761	1.850	3.300	3.318	82.214
Margay	Leopardus wiedii	M	2.160	0.397	0.513	0.476	0.452	2.006	1.563	0.697	0.895	1.797	0.353	1.794	2.611	3.200	83.319
Margay	Leopardus wiedii	M	2.762	0.676	0.634	0.554	0.639	2.178	1.883	0.754	0.754	2.948	1.167	2.104	3.900	3.718	68.513
Margay	Leopardus wiedii	M	2.508	0.639	0.697	0.576	0.460	2.057	1.747	0.689	1.057	2.084	0.590	2.082	3.445	3.500	78.109
Margay	Leopardus wiedii	F	2.500	0.490	0.506	0.509	0.437	2.213	1.920	0.730	0.956	2.191	0.435	1.633	3.124	3.300	68.827
Margay	Leopardus wiedii	M	3.099	0.692	0.758	0.846	0.637	2.102	1.914	0.864	1.167	2.202	0.280	1.582	3.700	3.106	62.806
Margay	Leopardus wiedii	F	2.162	0.550	0.571	0.619	0.545	1.885	1.515	0.748	1.011	1.961	0.532	1.459	3.117	2.900	76.047
Margay	Leopardus wiedii	M	2.006	0.578	0.581	0.609	0.541	2.012	1.461	0.755	0.975	1.723	0.410	1.744	2.700	3.247	85.124
Margay	Leopardus wiedii	M	2.431	0.600	0.654	0.480	0.504	1.970	1.774	0.660	0.863	2.143	0.479	1.810	3.106	3.300	89.699
Margay	Leopardus wiedii	F	2.292	0.613	0.544	0.595	0.473	2.002	1.641	0.732	0.991	1.834	0.399	1.483	2.989	2.978	77.704
Margay	Leopardus wiedii	F	2.435	0.526	0.553	0.677	0.533	2.125	1.708	0.827	1.075	1.904	0.358	1.823	3.100	3.198	84.686
Margay	Leopardus wiedii	M	1.423	0.324	0.337	0.345	0.336	1.604	1.203	0.526	0.731	1.879	0.882	1.335	3.000	2.438	47.228
Margay	Leopardus wiedii	F	2.232	0.467	0.518	0.554	0.472	2.043	1.617	0.706	0.967	1.942	0.469	1.651	2.900	3.011	69.706
Margay	Leopardus wiedii	M	2.856	0.526	0.786	0.652	0.566	2.321	1.723	0.804	0.999	2.284	0.621	1.845	3.600	3.316	78.163
Margay	Leopardus wiedii	F	1.697	0.425	0.433	0.357	0.404	1.773	1.358	0.606	0.755	1.799	0.484	1.660	2.600	2.892	68.739
Ocelot	Leopardus pardalis	F	7.646	1.238	1.843	1.833	1.296	3.689	2.762	1.337	1.752	3.067	0.239	2.477	4.900	4.729	90.508
Ocelot	Leopardus pardalis	F	4.938	1.032	1.309	1.150	1.012	2.893	2.537	1.057	1.359	2.536	0.081	1.950	4.200	3.960	81.978
Ocelot	Leopardus pardalis	F	5.043	0.976	0.995	1.046	0.936	2.969	2.483	0.960	1.327	3.013	0.805	2.685	4.207	4.600	85.743
Ocelot	Leopardus pardalis	M	7.535	1.503	1.496	1.704	1.559	3.657	2.937	1.253	1.648	2.930	0.576	2.901	4.800	5.321	94.870
Ocelot	Leopardus pardalis	F	5.716	1.193	1.716	1.483	1.274	3.154	2.568	1.105	1.558	2.844	0.334	2.381	4.700	4.539	80.286
Ocelot	Leopardus pardalis	M	7.119	1.252	1.179	1.420	1.392	3.689	2.697	1.192	1.566	2.856	0.278	2.872	4.500	5.215	96.983
Ocelot	Leopardus pardalis	F	4.698	1.069	1.057	1.029	0.946	3.078	2.321	0.894	1.444	2.554	0.416	2.258	3.913	4.300	80.834
Ocelot	Leopardus pardalis	F	3.805	0.868	0.935	0.907	0.822	2.507	2.090	0.958	1.193	2.279	0.288	2.148	3.473	4.000	88.230
Ocelot	Leopardus pardalis	M	5.505	1.105	1.131	1.360	1.070	3.163	2.544	1.100	1.508	2.617	0.210	2.683	4.086	4.800	95.860
Ocelot	Leopardus pardalis	F	3.631	0.807	1.118	1.070	0.883	2.438	1.921	0.941	1.411	2.523	0.642	2.034	4.000	3.935	77.115
Ocelot	Leopardus pardalis	M	3.623	0.846	0.944	1.082	1.057	2.759	2.050	0.941	1.459	2.700	0.718	2.378	4.000	4.321	93.503

Ocelot	Leopardus pardalis	F	3.676	0.853	0.813	0.986	0.879	2.557	1.993	0.956	1.318	2.343	0.497	1.871	3.900	3.655	83.107
Ocelot	Leopardus pardalis	F	5.537	1.278	1.302	1.271	1.224	3.242	2.483	1.047	1.527	2.176	0.600	2.559	4.105	4.600	89.783
Oncilla	Leopardus tigrinus	F	1.484	0.382	0.379	0.418	0.381	1.589	1.292	0.610	0.817	1.488	0.200	1.422	2.300	2.534	78.746
Oncilla	Leopardus tigrinus	M	1.967	0.522	0.472	0.448	0.427	1.833	1.521	0.637	0.907	1.637	0.263	1.525	2.700	2.875	81.600
Oncilla	Leopardus tigrinus	M	1.601	0.328	0.402	0.443	0.457	1.675	1.386	0.581	0.929	1.839	0.439	1.229	2.800	2.403	61.986
Oncilla	Leopardus tigrinus	M	2.421	0.598	0.518	0.617	0.486	2.045	1.622	0.737	1.027	1.859	0.171	1.569	2.977	3.000	69.086
Oncilla	Leopardus tigrinus	M	2.075	0.511	0.492	0.504	0.477	1.839	1.619	0.639	0.972	1.756	0.290	1.364	2.900	2.717	80.577
Oncilla	Leopardus tigrinus	F	1.279	0.331	0.415	0.338	0.323	1.460	1.290	0.556	0.780	1.500	0.331	1.219	2.400	2.261	64.899
Oncilla	Leopardus tigrinus	F	1.842	0.503	0.377	0.443	0.386	1.832	1.412	0.625	0.908	1.973	0.542	1.407	3.050	2.727	65.928
Oncilla	Leopardus tigrinus	M	1.516	0.399	0.533	0.486	0.438	1.552	1.359	0.728	0.850	1.597	0.251	1.355	2.600	2.686	79.772
Oncilla	Leopardus tigrinus	M	2.008	0.505	0.468	0.586	0.436	1.868	1.486	0.720	1.009	1.685	0.212	1.408	2.900	2.716	73.481
Oncilla	Leopardus tigrinus	M	1.733	0.465	0.447	0.547	0.474	1.717	1.437	0.730	0.942	1.565	0.282	1.382	2.800	2.735	66.192
Oncilla	Leopardus tigrinus	M	1.444	0.445	0.355	0.446	0.352	1.662	1.202	0.653	0.854	1.866	0.635	1.434	2.700	2.665	63.670
Oncilla	Leopardus tigrinus	M	1.844	0.379	0.451	0.538	0.520	1.798	1.384	0.690	0.957	1.820	0.427	1.484	2.800	2.810	76.288
Oncilla	Leopardus tigrinus	F	1.718	0.349	0.335	0.516	0.407	1.608	1.466	0.704	0.930	1.783	0.331	1.433	2.900	2.653	57.864
Oncilla	Leopardus tigrinus	M	1.861	0.462	0.385	0.516	0.508	1.846	1.382	0.664	0.970	1.858	0.397	1.549	3.000	2.894	69.944
Oncilla	Leopardus tigrinus	F	1.595	0.325	0.397	0.427	0.377	1.570	1.278	0.671	0.851	1.539	0.322	1.274	2.600	2.497	64.988
Oncilla	Leopardus tigrinus	F	1.966	0.436	0.561	0.490	0.387	1.770	1.503	0.644	0.983	1.858	0.368	1.268	3.000	2.407	58.490
Oncilla	Leopardus tigrinus	F	0.908	0.324	0.293	0.318	0.251	1.239	1.023	0.473	0.704	1.520	0.536	1.249	2.400	2.073	69.345
Oncilla	Leopardus tigrinus	F	1.562	0.342	0.373	0.396	0.326	1.637	1.251	0.612	0.768	1.551	0.296	1.380	2.400	2.551	87.513
Oncilla	Leopardus tigrinus	F	1.403	0.419	0.368	0.404	0.402	1.559	1.321	0.640	0.767	1.680	0.458	1.104	2.700	2.412	56.655
Oncilla	Leopardus tigrinus	F	1.092	0.292	0.267	0.287	0.269	1.321	1.016	0.474	0.734	1.484	0.576	1.623	2.200	2.628	80.008

CHAPTER 6

FIRST DOCUMENTED REPORTS OF GIANT ANTEATER (*Myrmecophaga tridactyla*) AND GREATER NAKED-TAILED ARMADILLO (*Cabassous tatouay*) AND THE XENARTHRANS OF THE IGUAÇU NATIONAL PARK, PARANÁ, BRAZIL

ABSTRACT

Five xenarthran species appear on the list of mammals whose presence has been proved by sighting reports, road kills or hair samples in the Iguaçu National Park. We conducted the first long-term camera trap monitoring in the region, including in the park's buffer zone. We confirmed the presence of four of these five species, with the first photographs of Giant Anteaters for this 77-year old park. We also detected the presence of the Greater Naked-tailed Armadillo (*Cabassous tatouay*), which was not previously reported for the park. Giant Anteater sightings are described and habits are discussed. They exhibit strong adaptation skills.

KEYWORDS

Brazil, Giant anteater, Iguaçu National Park, Greater Naked-Tailed Armadillo, Paraná, Xenarthrans.

CAPÍTULO 6

PRIMEIROS REGISTROS FOTOGRÁFICOS DE TAMANDUA BANDEIRA (*Myrmecophaga tridactyla*) E DO TATU-DE-RABO-MOLE-GRANDE (*Cabassous tatouay*) E OS XENARTROS DO PARQUE NACIONAL DO IGUAÇU

RESUMO

Cinco espécies de xenartros constam na lista oficial de mamíferos para o Parque Nacional do Iguaçu, para os quais a presença foi confirmada através avistamentos, exemplares atropelados ou análise de pêlo. Realizamos o primeiro monitoramento de armadilhas fotográficas de long prazo na região, inclusive na zona de amortecimento. Confirmamos a presence de quatro dessas espécies, inclusive os primeiros registros fotográficos de tamandua bandeira para o parque, que já comemorou 77 anos de existência. Detectamos também a presença do tatu-de-rabo-mole-grande (*Cabassous tatouay*), que não constava na lista para o parque. Os registros de tamandua bandeiras são descritos e os hábitos são discutidos. Demonstram alta faculdade de adaptação.

KEYWORDS

Brasil, Paraná, Parque Nacional do Iguaçu, *Myrmecophaga tridactyla*, *Cabassous tatouay*, Xenartros.

INTRODUCTION

The official non-flying mammal species list for the Iguaçu National Park accounts for 48 species, which include five xenarthrans: the Six-banded Armadillo or Yellow Armadillo (*Euphractus sexcinctus*), the Seven-banded Armadillo (Long-nosed Armadillo, *Dasypus septemcinctus*), the Nine-banded Armadillo (*Dasypus novemcinctus*), the lesser Anteater (*Tamandua tetradactyla*) and the Giant Anteater (*Myrmecophaga tridactyla*). Species presence was confirmed on sightings, road kills, or through trichology (i.e., big cats' scat content analysis; MMA, 1999). Lacking more proficient techniques, hair analysis has been popular in science at first, but its reliability has been questioned many times. Nowadays, microscopic hair identification is not held as a definite proof when compared to DNA or photographic evidence (Foran et al., 1997; Farrel et al, 2000; Sahajpal et al., 2009; Robinson, 2010). Any hard evidence can only benefit a species' conservation.

All the above cited xenarthrans but the Giant Anteater are listed as 'Least Concern' by the IUCN. However, much remains unknown regarding the species dynamics, ecology, distribution and trends (Abba et al., 2014; Anacleto et al., 2014; Loughry et al., 2014; Miranda et al., 2014a). The Giant Anteater is listed as 'Vulnerable' based on local extinctions, road kills (Cáceres et al., 2010), and habitat loss due to fire (Prada & Marinheiro-Filho, 2004) and human-oriented soil use (Miranda et al., 2014b). It has some sort of conservation threat status at all scales in every location where it is known to occur (Miranda et al., 2014b). This paper presents findings on xenarthrans extracted from a broader mammal inventory conducted in the Iguaçu National Park (INP) region, southwestern Brazil, between 2012 and 2014. We used camera-traps to record over 30 different non-flying mammal species. This paper is the first peer-reviewed confirmation through photographic records of the presence of four out of the five xenarthran species of the INP list. We also confirmed the presence of the Greater Naked-tailed Armadillo, also listed as 'Least Concern' by IUCN (Gonzalez & Abba, 2014). We present ecological information for all recorded xenarthran species occurring within the park's boundaries and in the forest remnants of its buffer zone. Conservation issues are discussed.

MATERIAL & METHODS

Sixteen motion-triggered cameras were used to conduct a broad survey in the region of the Iguaçu National Park between September 2012 and October 2014. We randomly displayed the cameras across the Iguaçu National Park and its 5-km direct surrounding zone. On occasions, cameras were placed close to active burrows. In each sampling station, data recorded consisted of latitude and longitude coordinates, habitat type, microhabitat, canopy closure and soil type. Reconyx rapidfire PC900™ cameras automatically recorded air temperature, date, time while taking 15 pictures in near-video sequences. Cameras were set in a bursting mode taking one photograph per second until the animal leaves the detection zone.

RESULTS

Sampling effort accounted for 6,190 sampling days in 216 different sampling locations covering 80 km^2 of the westernmost section of the Iguaçu National Park and its buffer zone, from São Miguel do Iguaçu to Foz do Iguaçu (Fig. 1; see Chapter 1 & 2). This pioneer monitoring is the first long-term monitoring in the region, with 755 consecutive days, totaling 247,693 photographic records. Each sampling location was monitored for 33 days on average. Three of our 16 camera units were stolen, and possibly destroyed, by fishermen and poachers, who represent a direct cause of xenarthran population declines based on IUCN reports (Miranda et al., 2014a; Miranda et al., 2014b). From 7,681 animal individual records, 540 (7.0%) were of xenarthrans, accounting for 6.3% of INP records (*n* = 216) and 14.9% in the buffer zone (*n* = 324). The Nine-banded Armadillo was largely predominant with 97.4% of all xenarthran records (*n* = 526), followed by the Lesser Anteater (1.1%, *n* = 6). The remainder is four Yellow Armadillos, three Giant Anteaters (0.56%; Fig. 2) and one Greater Naked-Tailed Armadillo (Fig. 3). These last two species were solely found within the INP boundaries while the Yellow Armadillo was only found in the buffer zone. The Lesser Anteater was found both inside and outside the INP. Records' description, including date, time, weather, soil type and habitat features, is summarized in Table 1. Activity pattern from the four Giant Anteater photographs were compared to existing peer-reviewed material (Fig. 4).

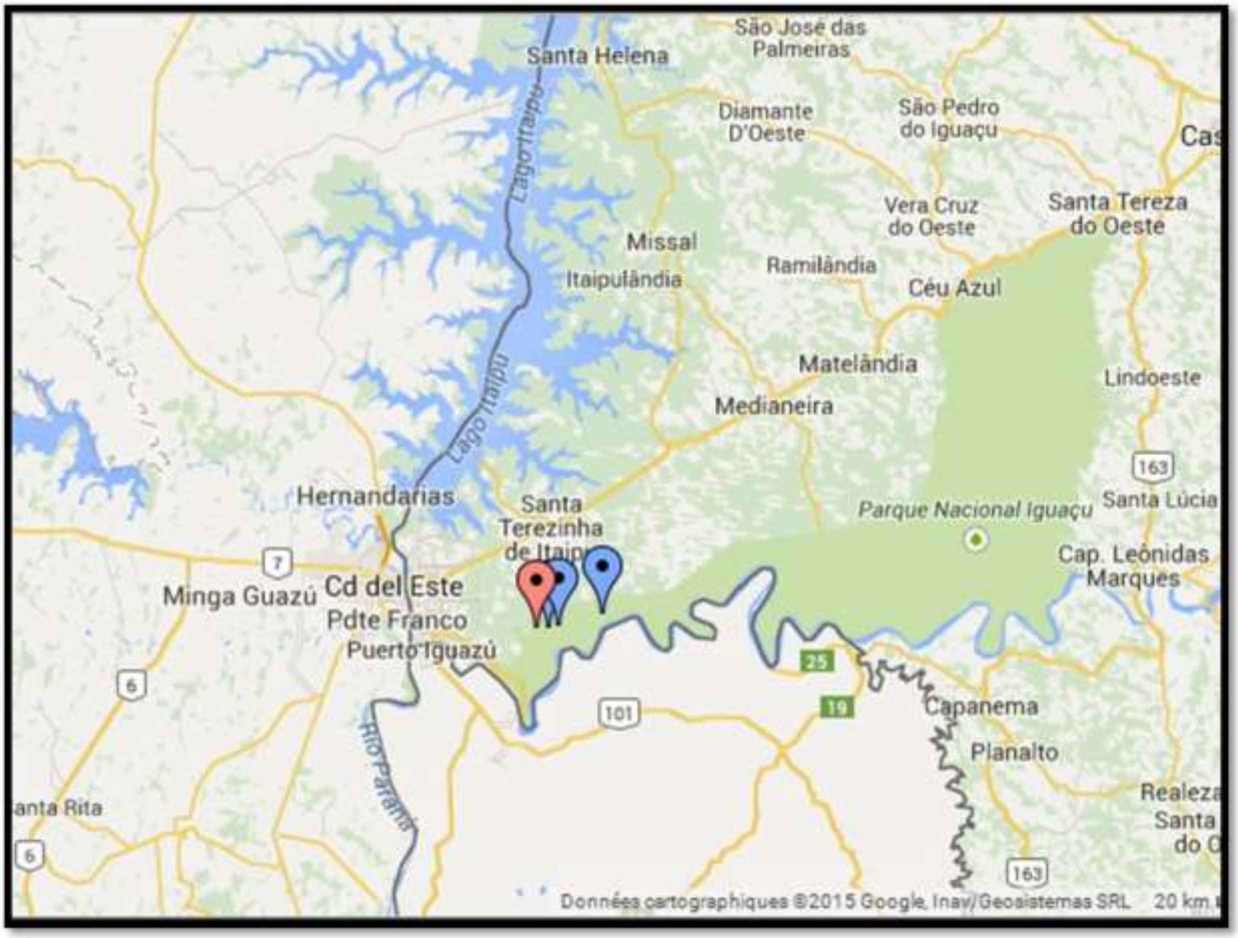

Figure 1. Locations of confirmed occurrence for both xenarthran species: *Cabassous tatouay* (n = 1, red); *Myrmecophaga tridactyla* (n = 3, blue), Iguaçu National Park, Paraná, Brazil.

Table 1. Latitude, Longitude, Date, time, habitat and microhabitat, soil and weather conditions (precipitation, mean temperature and relative humidity) of each occurrence for both xenarthran species (C. t.: *Cabassous tatouay*; M. t.: *Myrmecophaga tridactyla*).

Species	Lat	Long	Date	Time	Habitat	Micro habitat	Canopy (%)	Soil Type	Precip. (mm)	Temp. (°C)	R. H. (%)
C. t.	-25.56995401	-54.41980172	03/05/2014	02:08	Subtropical Rainforest	Alluvial	80	Rhodic Paleudalf NVef2	0.0	26.3	74.6
M. t.	-25.55244044	-54.33033647	04/06/2013	16:22	Subtropical Rainforest	Palmtree dominated	80	Rhodic Paleudalf NVef2	0.0	17.4	90.5
M. t.	-25.56747439	-54.38930852	31/03/2014	18:50	Tropical Rainforest	Riparian	95	Typic Haplorthox LVef1	29.6	23.8	87.6
M. t.	-25.56911004	-54.40301646	24/04/2014	11:20	Tropical Rainforest	Palmtree dominated	90	Typic Haplorthox LVef1	0.0	19.5	85.0

Figure 2. Near-sequence video of one of the three photographic records of a Giant Anteater (*Myrmecophaga tridactyla*) in the Iguaçu National Park, Paraná, Brazil, on March 31 2014.

Figure 3. Photographic record of a Greater Naked-tailed Armadillo (*Cabassous tatouay*) on May 3rd 2014, in the Iguaçu National Park, Paraná, Brazil.

DISCUSSION

Confusion of taxonomic identification for the Greater Naked-tailed Armadillo has made their conservation status and distribution uncertain (Gonzalez & Abba, 2014). More solid evidence is needed to document the species' distribution and ecology. Unmistakable Giant Anteaters benefitted from more interest from scientists and more data is available (Miranda et al., 2014a). They appear to choose different habitat types according to temperature suggesting thermoregulation sensitivity as they feed exclusively on low-caloric foods (i.e. antes and termites; MacNab, 2000). During colder days, they were seen sun bathing in open areas, while they rested in forest shade in hotter days (Medri, 2002; Sampaio et al., 2006). They have also been seen bathing in water ponds to regulate their body temperature on hot days in Bolivia (Emmons, 2004). In one of our three photographic records, the animal was on the edge of a river within the INP early at night on a hot day (Table 1). However, their bathing habits are puzzling as they also bathe in the middle of cool nights, and during the dry season where they are unlikely bothered by flies. Furthermore, they do not share the characteristics of other bathing and wallowing mammal species.

Emmons et al. (2004) hypothetized bathing as a parasite treatment attempt, or maybe simply for their own enjoyment.

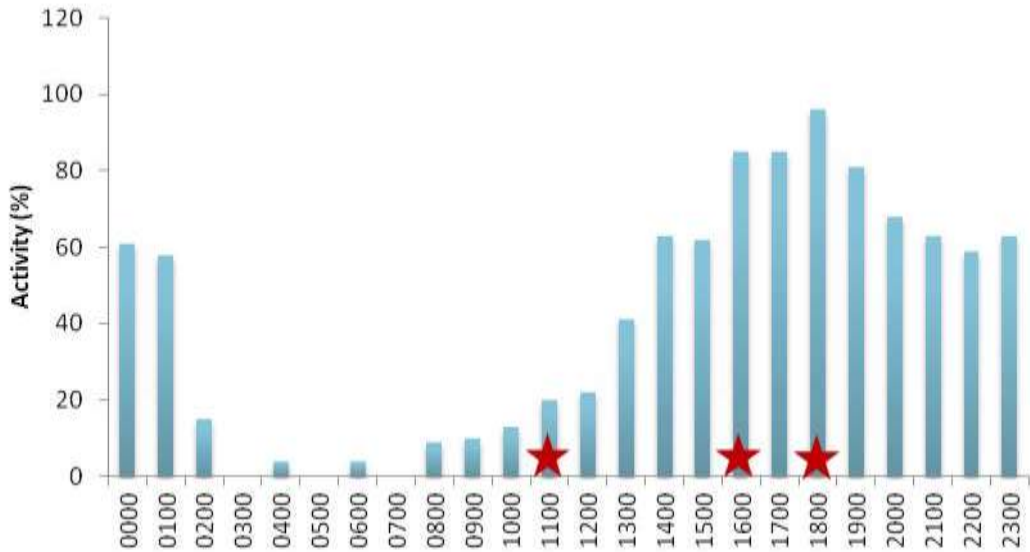

Figure 4. Giant anteater activity pattern and the three recorded occurrences (red stars), adapted from Shaw et al. (1987).

Their activity pattern is also influenced by other climatic conditions than solar exposition. In Venezuela and in Argentina, they were exclusively nocturnal during the hot season (Shaw et al., 1987; Di Blanco et al., 2009). All xenarthrans but the Yellow Armadillo usually display nocturnal habits (Zimbres et al., 2013). While all our xenarthran records were indeed between 7 PM and 3 AM, two of the three Giant Anteater records occurred during the day. Nonetheless, this does not conflict with what had been previously documented by Shaw et al. (1987; Fig. 4).

Habitat preferences remain unclear as they use open and forested lands in uncertain proportions. In a study conducted in Serra da Canastra in the Brazilian *Cerrado* (Minas Gerais; Shaw et al., 1987), individuals preferred scrublands over other habitat types. However, some individuals had none of this common habitat type in their home range. Giant anteaters also favored riparian forests over termite-rich scrublands.

The limits of Giant Anteaters' distribution range in South America are also periodically altered by sporadic sightings or roadkills. In Honduras, it was thought to be extirpated in the 90s but few sightings have recently been reported (McCain, 2001; Reyes et al., 2010). One sighting was also reported in Costa Rica in 1989 (Timm et al., 1989), and another individual was killed by a hunter in Nicaragua (Koster, 2008), all suggesting extension or maintenance of their current distribution.

Much still needs to be understood on Giant Anteaters' behavior. In the Brazilian Paraná state, they adapted to pine plantations feeding on the leaf-cutter ants (Braga et al., 2014). In *Cerrado*, it showed resilience to human-caused habitat alteration, like fire and habitat loss (Shaw et al., 1987), remaining present where most natural habitat has been converted to soy crops (Klink & Moreira, 2002). Existing knowledge on Giant Anteater's ecology and behavior depicts strong adaptation skills to local contexts. Individuals from captivity were released in the wild since 2007 as part of a reintroduction project in the Iberá Natural reserve, Missiones, Argentina. Four out of six released individuals managed to survive (Di Blanco et al., 2009).

CONCLUSION

The first photographic confirmations of a Greater Naked-tailed Armadillo and Giant Anteaters are a positive contribution to the INP mammal list. Considering the conservation significance of the Giant Anteater and the lack of information on the species in this part of its range, more hard evidence and ground data reports help piecing together recent occurrence reports and/or behavioral variations' descriptions from different locations in South America (Koster, 2008; Jimeno & Amaya, 2009; Braga et al., 2010; Hack & Krüger, 2013). Only time will tell whether human-caused landscape transformation actually impacts negatively Giant Anteaters (Parera, 2002; Superina et al., 2010), or if they are an exception to the rule and are not actually declining, as Shaw et al. (1987) suggested. The species proved to cope in unexpected ways to human-imposed pressures and contexts (Young et al., 2003; Braga et al., 2014). Rather, we suggest the species may simply be outsmarting our ability to measure and comprehend their resilience, reminding us to remain humble as we make assumptions from limiting tunnel-vision perspectives (Greenwald, 1980; Klaczynski & Narasimham, 1998).

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CHAPTER 7

BAIT LAMINA AND SOIL USE: PIONEER TRIAL IN ATLANTIC FOREST, BRAZIL

ABSTRACT

Soil organisms are responsible for the maintenance of soil biological integrity, which is inevitably impacted by soil use and management. Soil organisms' feeding activity has long been used to assess soil biological integrity in a wide array of environmental issues. The bait lamina method has rapidly gained popularity given its many advantages over other methods. We tested this method in inland Atlantic forest, in the Iguaçu National Park and its buffer zone, comparing the park, its edge and its buffer zone. The park's edge had less feeding activity than the two other zones. The buffer zone had less feeding activity deeper in the topsoil layers than the park, suggesting alterations from intense agricultural practices and a pronounced edge effect on the remaining forest fragments. Bait lamina is a robust technique for a quick assessment but benefits from being used in conjunction with other techniques assessing nutrient cycling and carbon sequestration.

KEYWORDS

Bait lamina, feeding activity, Atlantic forest, Brazil.

CAPÍTULO 7

BAIT LAMINA E USO DE SOLO: TESTE PIONEIRO EM MATA ATLÂNTICA, BRASIL

RESUMO

Os organismos do solo são responsáveis pela manutenção da integridade biológica do solo, que inevitavelmente é impactada pelo uso e manejo do solo. A atividade alimentar dos organismos do solo é utilizada na avaliação da integridade biológica. O método Bait Lamina rapidamente tornou-se popular por conta de suas vantagens sobre outros métodos. Testamos a validade deste método em mata Atlântica do interior, no Parque Nacional do Iguaçu e sua zona de amortecimento, comparando o parque, sua borda e zona de amortecimento. A beira do parque presentou menor atividade alimentar que as duas outras zonas. A zona de amortecimento tinha menos atividade alimentar em profundidade comparado ao parque, sugerindo alterações oriundas das práticas agriculturais intensivas e um efeito de borda pronunciado nos remanescentes florestais. O bait lamina é uma técnica robusta para avaliações rápidas, porém ganha a ser utilizada associada a outras técnicas avaliando ciclos dos nutrientes e fixação de carbono.

PALAVRAS-CHAVES

Bait lamina, atividade de alimentação, mata Atlântica, Brasil.

INTRODUCTION

Microorganisms play a key role in the maintenance of soil health and quality (Filip, 2002), and are therefore expected to be affected by land use or management (Peixoto et al., 2006). When compared to physical or chemical indicators, microbial-based indicators of soil quality are believed to be more dynamic with potential use for early signals of soil degradation or improvement (Aboim et al., 2008). In fact, along with other measurements, feeding activity has been considered one of the functional parameters usable for biological integrity assessment of soils (EA, 2002; ISO, 2012).The bait lamina method is a microbial-based method which was developed by Von Törne (1990). It allows a simple and fast way to quantify feeding activity of soil microorganisms, and therefore reflects the complex processes of organic matter decomposition (Helling et al., 1998). This PVC probe is 160 x 8 x 1 mm, perforated with sixteen 1.5 mm-diameter holes, spaced by 5 mm. Bait lamina is an integrative method which informs about biological activity occurring in the forest litter and in the superficial layers of the subsoil. This process is strongly influenced by the organic matter decomposition, and by the synthesis and degradation of hummus (Kratz, 1998). The bait is inserted into these holes and soil organisms feed on it while it is vertically inserted into the ground. After the sampling period, emptied holes are counted and are used to obtain an estimate of the soil microorganisms' feeding activity. The assumption of this method is that the bait will disappear from the holes due to microbial and micro-biogenic processes which are strongly influenced by the feeding activity of soil invertebrates (Kratz, 1998).

Each bait lamina test provides the data necessary to: (1) quantify the biological activity in the sampling station (Von Törne, 1990; Kratz, 1998), (2) determine the vertical distribution of soil biological activity (Filzek et al., 2004; Hamel et al., 2007), (3) obtain a graphic distribution of soil biological activity at the landscape scale (Kratz, 1998).

As for litter decomposition assessment, the bait lamina method have several advantages over other soil sampling techniques like litterbags, mini-containers, cotton strip assay, and wheat-straw degradation. Numerous studies consistently demonstrated that bait lamina is a steady approach to assess the soil functional integrity (Kula & Römbke, 1998; Paulus et al., 1999; Gestel et al., 2003; Knacker et al., 2003; Hamel et al., 2007). More recently, bait lamina probes have become more popular given their qualities and have been used in a several researches in Brazil (Podgaiski et al., 2011, 2014; Niemeyer et al., 2012; Musso et al., 2014). Overall, they are considered a good tool to rapidly assess the soil organisms' feeding activity as indirect information on litter decomposition (Ghaley et al., 2014).

In addition, the bait lamina method offers several practical advantages. It is simple to use and cheap, it does not require taxonomic knowledge; it is a time-efficient field protocol; it is not invasive as no soil or vegetation is removed or displaced; experimental error is easy to estimate; bait composition may be adapted to place or problem; and it is re-usable.

It has been used in forest ecosystems to monitor pollution gradients (Förster et al., 2009; Niemeyer et al., 2012). In agro-systems, one can determine the influence of chemical or organic fertilizer, soil compaction, fungicide or other chemical application, etc. (Edwards & Stafford, 1979; Reinecke et al., 2002). It has also been used in urban systems to monitor composting process, assess pollution gradients and measure altered soil recovery (Hartley et al., 2008; Piola et al., 2009; Niemeyer et al., 2012). The method is versatile and can be used to quickly address most environmental questions, as long as it is about litter decomposition and not nutrient cycling (Ghaley et al., 2014).

This soil assessment was part of a broader wildlife monitoring across a gradient of habitat alteration in the Iguaçu National Park (INP) and its buffer zone in the Iguaçu region (see Chapter 1 & 2). We defined three zones: INP, INP's edge, and Buffer zone. This pioneer work aimed at testing the bait lamina method in inland Atlantic forest and assessing changes in the soil organisms' feeding activity in the three different zones.

MATERIAL AND METHODS

Study area

This short-term investigation was conducted in the region of the Iguaçu National Park (INP), Paraná, southwestern Brazil. The INP (S25° 61' W54° 48') accounts for 185,262.5 ha. It was created in 1939 and was added on the World Natural Heritage list by UNESCO in 1986 due to its scenic beauty. Monitoring was conducted in one of the two predominant forest types with trees reaching 35 meters in height, i.e. semi-deciduous forest in the western half of the INP. Temperatures vary from -1°C to +53°C, mean annual precipitation for INP is 1,700 mm and rain is evenly distributed year-round. The buffer zone is composed of a mosaic of agricultural lands, pastures and farms with small scattered thickets, from which economically valuable species were removed (MMA/IBAMA, 1999). The INP buffer zone accounts for ca. 2560 km^2. Legally, the buffer zone is defined as "the surroundings of a conservation unit, where human activities are subject to norms and specific restrictions, with the greater purpose to minimize negative impacts over the unit" (bill 9.985/2000). However, current use of the INP buffer zone is predominantly conventional agriculture with less than 20% of forest cover (Mesquita, 2015). Agricultural practices are mostly extensive conventional agriculture, which traditionally implies soy-wheat-corn annual crop rotation. The INP edge is marked by a drastic habitat change for the forest is brutally stopped by a dirt road with heavily compacted exposed soil (Fig. 1). The buffer zone's forest fragments are located around headwaters, rivers and brooks as they are the only ones protected by law.

Figure 1. From left to right: Iguaçu National Park, edge and buffer zone and their contrasting habitat characteristics.

Experimental design

We installed 432 bait laminae in 27 sampling stations to collect information on soil communities' feeding activity. Sampling effort accounted for 876 sampling days (from September to January). At each sampling location, we installed a quadrant of 4 x 4 bait lamina probes. In order to characterize habitat varitation, sampling stations were separated by approximately one kilometer and scattered in three habitat zones: inside the park, on the park's edge and outside of the park. Sampling stations located in the buffer zone were located into forest remnants of varying size. Bait laminae remained inserted into topsoil for 18-26 days on average.

Cellulose accounts for up to 70% of the organic C compounds in plants and stands therefore among the most important components of the decomposition cycle (Gestel et al., 2003). We used a food mix composed of microgranular cellulose, oat bran and active charcoal in 70:27:3 proportions, respectively (Von Törne, 1990; Römbke et al., 2006; Podgaiski et al., 2011; Niemeyer et al., 2012).
After exposition to soil fauna, the probes were removed and emptied holes are counted. The proportions and depth of emptied holes were calculated for each bait lamina quadrant (Hamel et al., 2007).

Lab soil protocols

In each sampling station, two surface soil samples were collected (i.e. 500-g plastic bag and a 3.5 x 9 cm soil core) and processed within 24 hours at the lab. Lab measurements included soil pH, soil moisture content, organic matter content and maximum water holding capacity. Soil pH was obtained from a soil-water 1:5 suspension with a pHmeter (FAO, 1984; ISO, 1994). Soil moisture content was obtained by comparing sample weight before and after drying at 105°C during 15 h. Organic matter content was determined by weight loss after ignition of dried soil samples at 430°C during 2 h (ISO, 2008). Maximum water holding capacity was obtained after soil core samples were immersed in deionized water for 2 h, and drained on absorbent paper over sand for 2 h. Samples were then weighed before and after being dried during 15 h at 105°C (ISO, 2008).

Statistics

Correlations among soil microorganisms' feeding activity and soil abiotic factors were assessed through Spearman's rank correlations. As mean feeding activity rates were not normally distributed, we compared them between the three zones using non parametric Kruskal-Wallis and Mann-Whitney tests with Bonferroni post-hoc tests (Von Törne, 1990; Araújo et al., 2008). All analyses were performed using SPSS™ 15.0.

RESULTS

We looked at the data collected from bait lamina protocol and habitat and soil variables at a local scale in 27 sampling stations. Spearman's correlation analysis revealed moderately strong positive and negative correlations with bait exposure time (ρ = 0.588, α= 0.000), air moisture (ρ = 0.556, α= 0.003), maximum water holding capacity (ρ = -0.495, α = 0.043), live vegetation cover (ρ = -0.464, α= 0.015) and dry vegetation cover (ρ = 0.422, α = 0.028).

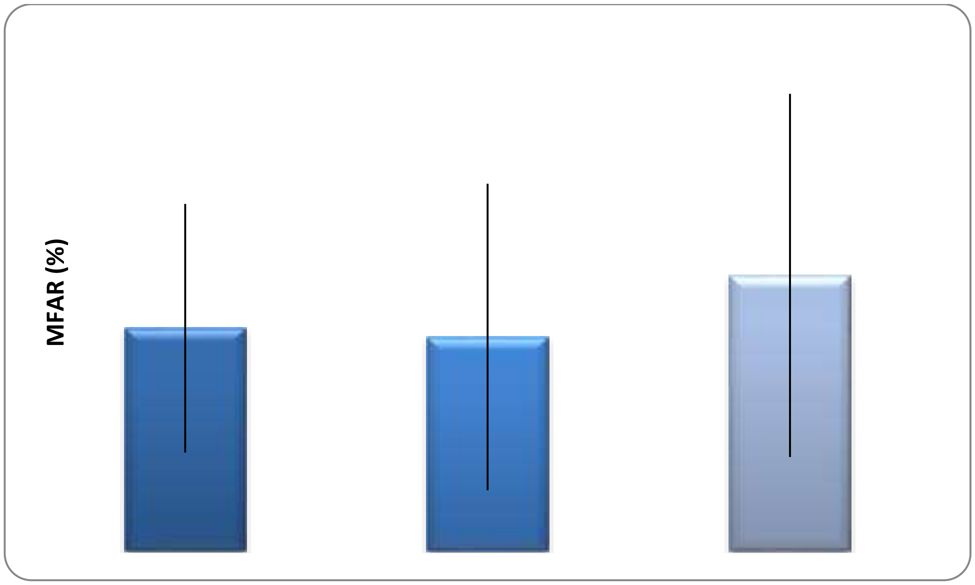

Figure 2. Mean feeding activity rates and standard deviations per zone.

When comparing mean feeding activity rates (MFARs) among zones based on our 27 sampling locations, we obtained no statistically difference between MFARs among zones (*p* = 0.446, α = 0.05; Fig. 2).

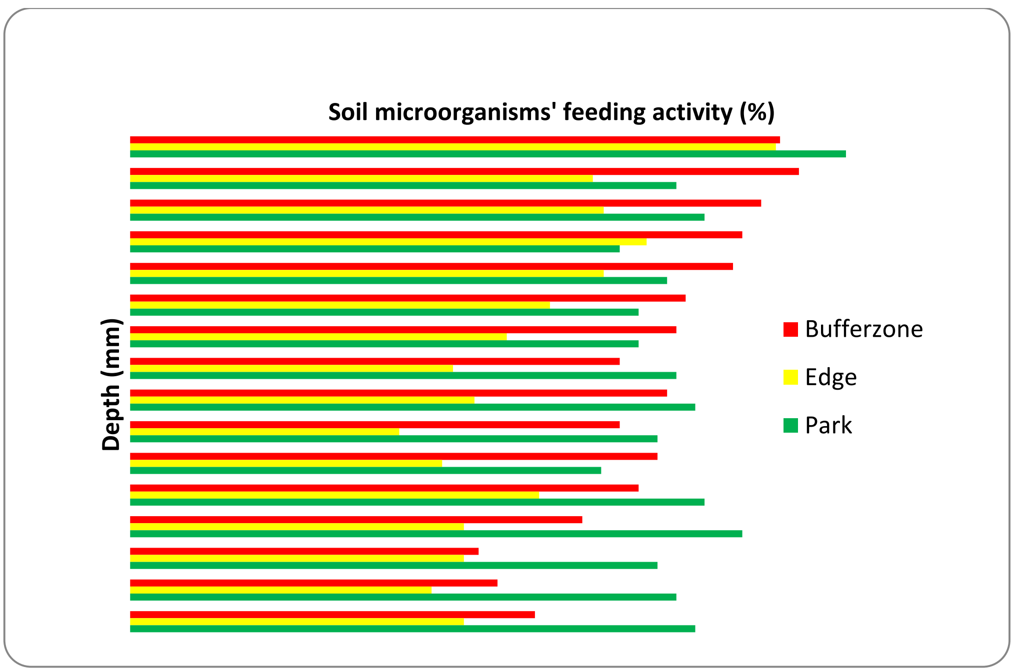

Figure 3. Mean feeding activity rates in depth in the three zones.

However, when we looked at the depth feeding activity was happening, the difference was significant. Mann-Whitney tests revealed that a significant difference is observed for both the buffer zone and the INP and compared to the park's edge (p = 0.000, α=0.01), which had less feeding activity than the two other zones (Fig. 3).

DISCUSSION

Correlations to habitat characteristics

We found that the feeding activity was correlated to bait lamina's exposure time, maximum water holding capacity, relative air moisture, dry and live vegetation cover. The longer the probes remain in the soil, the more time they are exposed to soil organisms, and therefore the more bait apertures can be consumed (Gestel et al., 2003; Gongalsky et al., 2008). Römbke et al. (2006) were among the first to test bait laminae in tropical ecosystems in the life-rich Brazilian Amazon. They reduced the traditional 10-day exposure time to four days the exposure time as after two days, most bait holes had been emptied and obviously, no significant difference was found with longer exposures. In temperate climates, exposure time typically ranges between 10 to 20 days (Römbke et al., 2006). Other studies conducted in other Brazilian ecosystems used different exposure times, like 14 days in Santo Amaro, Bahia (Niemeyer et al., 2012); 9, 16, 22, 29 and 65 days in Campos do Sul (Podgaiski et al., 2011), and 10 days in Brazilian savanna *Cerrado* (Musso et al., 2014). Gongalsky et al. (2014) mentioned that certain microorganisms could not perforate the bait under short-term exposition (i.e. 14 days) suggesting leaving the probes inserted in soil for longer periods of time. In our study, exposure time averaged 26 days ensuring optimal bait exposition.

Water holding capacity, air moisture and forest litter cover are all associated with climate and soil moisture which are known to directly affect soil biological activity and organic matter decomposition (Von Törne, 1990; Römbke et al., 2006; Simpson et al., 2012). Podgaiski et al. (2014) showed that canopy openness increases the effects of abiotic factors on feeding activity rate. Also, feeding activity decreases with low soil moisture (Römbke et al., 2006; Simpson et al., 2012). Both attributes are associated with deforested areas, which may explain why the INP edge area is the most influenced zone among our three zones due to the sudden canopy opening (Fig. 1).

The forest litter conditions also affect directly the soil functional parameters. Both live vegetation cover and maximum water holding capacity were negatively correlated to MFAR while dry vegetation cover and soil moisture were positively correlated to MFAR. Excess water can act as a limiting factor to soil organisms' feeding activity like during rainy seasons (Musso et al., 2014). Birkhofer et al. (2011) have also found a steady correlation between MFAR and vegetation cover during their study in Germany despite other tested potential influences (i.e. fertilizer, cattle grazing), suggesting this assumption as a general rule beyond regional differences.

The most interesting finding here was the contrast of the non significant difference in MFAR between the three zones and the highly significant MFAR difference when the depth is taken into account. Feeding activity was found more intense in the INP and in the buffer zone compared to the park's edge, suggesting that the transition between forested and agricultural lands affects the capacity soil communities have to keep up with soil processes. The unfavorable climatic conditions and abrupt habitat transition between the INP and its buffer zone, may very well be responsible for the lower feeding activity observed in this zone. Contrasts in soil microbial activities between forests and open lands have been demonstrated elsewhere (Stefanowicz et al., 2010; 2012). In addition to habitat features, agrochemicals used may also impact soil communities in many ways and to different levels of toxicity (see for instance, Renaud et al., 2002; Piola et al., 2009; Förster et al., 2011). However, sampling in the buffer zone was conducted in forest remnants, which provide more favorable conditions (i.e. increased organic matter, cooler and moister soils and vegetation cover) to soil fauna as compared to crops and pastures. In turn, it increases soil communities' feeding activity.

Human activities exert an inevitable pressure on soil functions and structures causing the feeding activity to be more intensive in deeper levels in the INP than in the two other, more altered zones. While sampling in the buffer zone offered more favorable conditions to soil communities due to some forest fragmented cover, the amount of pressures added to an overwhelming edge effect could potentially explain the differences in feeding activity levels between the park and the buffer zone as observed by Simpson et al. (2012). Reduced soil moisture due to higher soil temperature, and bare soil exposition and compaction are the legacy of conventional agricultural lands, which are highly detrimental to soil communities, compromising their resilience in the remaining small fragmented remnants (Vasconcelos et al. 2007, Simpson et al. 2012).

Who is eating?

While Kratz (1998) claimed the method could not allow the identification of taxonomic groups feeding on the strips, several studies later looked in more depth at this question successfully (Kula & Römbke, 1998; Helling et al., 1998; Paulus et al., 1999; Gestel et al., 2003). Perforation rates depend on both the activity and density of soil communities, with springtails, enchytraeids and earthworms apparently playing an important role (Gestel et al., 2003). Gongalsky et al. (2008) identified enchytraeids and lumbricids as being the main bait consumers, and also, collembolans and mites to a lesser extent. In all cases, logic implies that different baits will attract different soil organisms (Hättenschwiler et al., 2005).

Bait composition

Different baits were reported to be used in the literature. The original version developed by Von Törne (1990) was composed of cellulose, wheat bran and active charcoal in the same proportions of the ones used in this study. As stated earlier, cellulose is a major plant organic component, and for this reason, it should be the main bait component (Chew et al., 2001). Other scientists elsewhere in the world adapted the bait to the local (e.g. Helling et al., 1998; Geissen & Brümmer, 1999; Förster et al., 2009). In a crop comparison study conducted in Canada, Hamel et al. (2007) tested several baits and observed no significant impact of bait type over the recorded feeding activity. Nevertheless, Helling et al. (1998) and Reinecke et al. (2002) did find a significant preference for some bait types. Last, the original mix of microgranular cellulose, cereal bran and active charcoal (70:27:3) was successfully used in different ecosystems (including in Brazil) in varying weather conditions (Reinecke et al., 2002; Kratz, 1998; Gestel et al., 2003; Römbke et al., 2006). For these reasons, we decided to use the original mix and obtained satisfying results.

Method Reliability

Overall, we agree with Römbke et al. (2006), the bait lamina method is a satisfying approach to soil biological evaluation in tropical ecosystems, this study being the first bait lamina testing performed in inland Atlantic forest. Several papers compared the usefulness of bait lamina and other methods such as litterbags or mini-containers, and all agree on the bait lamina's qualities and weaknesses (Helling & Larink, 1998; Paulus et al., 1999; Gestel et al., 2003; Ghaley et al., 2014). It is an easy and quick protocol to derive a coarse idea of the soil organisms' activity, and provides sufficient data quality for statistic evaluation. Kula & Römbke (1998) also compared bait lamina to other soil methods, and found that bait lamina was one of the most practicable methods. In an overall risk assessment, the data obtained are the most appropriate as they perform fine in both model ecosystems and in the field.

Its simplicity comes from its relatively crude reading. It is a simple 'yes' or 'no' test. It informs neither the identity nor the number of eaters (Helling & Larink, 1998), and a reasonable amount of uncertainty exists for the reading as watery soil may penetrate bait holes and/or bait might fall, creating potential bias in readings. All tests are inevitably sensitive to environmental conditions by their very nature, and all would fail in very dry or very wet conditions. If the research focus is on soil-based ecosystem functional aspects, not only forest litter decomposition process should be looked at, but nutrient cycling and carbon sequestration should also be included in the assessment as they are of equal relevance (Ghaley et al. 2014). Any assessment, whether it is at local or global scale, does require taking into account the strengths and weaknesses of each and every method applied.

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CHAPTER 8

THE IGUAÇU NATIONAL PARK'S ECOLOGICAL SERVICES: A QUICK ASSESSMENT USING MICROBIOLOGICAL WATER ANALYSIS

ABSTRACT

We collected water samples from the São João River in the Iguaçu region, located in the southern part of the state of Paraná. We completed a microbiological analysis of these water samples in order to assess Iguaçu National Park's ability to filter water, or rather measure its ability to eliminate pathogenic organisms caused by anthropogenic disturbance around the park (i.e. conventional farming and cattle grazing). Our analysis included: bacterial counts, *Escherichia coli* and total coliforms; detection of pathogens (i.e. *Acanthamoeba* sp. e *Cryptosporidium* sp.); detection of pathogens' eggs, viable cysts and larvae; abiotic factor measurements, i.e. temperature, pH and turbidity. Samples were collected from the headwaters until the point where the São João River flows into the Iguaçu River. Our results showed the presence of pathogens at all sampling points.

KEYWORDS

Bacteriological analysis, water, Iguaçu National Park, ecological services, pathogens.

CAPÍTULO 8

OS SERVIÇOS ECOLÓGICOS DO PARQUE NACIONAL DO IGUAÇU: UMA AVALIAÇÃO RÁPIDA POR ANÁLISE DO PERFIL MICROBIOLÓGICO DO RIO SÃO JOÃO

RESUMO

Amostras de água do rio São João no sudeste Paranaense na região Iguaçu foram coletadas visando efetuar análises microbiológicas e assim, avaliar um dos serviços ecológicos do Parque Nacional do Iguaçu, que é a sua capacidade em filtrar a água, ou seja sua capacidade em eliminar patógenos decorrentes das atividades humanas em seu entorno (i.e. agricultura convencional e criação de animais). Foram realizadas as seguintes análises: quantificação de bactérias, Escherichia coli e coliformes totais; detecção de presença de microorganismos patogênicos (i.e. Acanthamoeba sp. e Cryptosporidium sp.); detecção de presença de ovos, cistos viáveis e larvas de patógenos e determinação de fatores abióticos (i.e. temperatura, pH e turbidez). As amostras foram coletadas desde a cabeceira do rio São João até sua foz no rio Iguaçu. Os resultados indicaram a presença de patógenos em todos os pontos de amostragem do rio.

PALAVRAS-CHAVES

análises bacteriológicas, água, Parque Nacional do Iguaçu, serviços ecológicos, patógenos.

INTRODUCTION

Today there is no longer an ecosystem that is not threathened by human development (Hawken, 1993; Pimm & Raven, 2000). The risk of degradation and destruction increases along with pressure created by high economic growth goals which are based on crescent exploration of natural resources. This type of threat is frequent in developing countries like Brazil, where people's health is directly related to ecosystem health, and communities depend on biodiversity and natural resources to survive (Skole & Tucker, 1993).

Being one of the five hotspots of biodiversity worldwide (Myers et al., 2000), the Atlantic forest is known internationally for its high conservation priority worth (Vasconcelos, 2002; Webb et al., 2005). This ecosystem shelters many endemic species, including more than 26 mammal species and 160 bird species (BDT, 1996). No less than 53% of tree global diversity; 64% of all palmtree species and 74% of Bromeliaceae are found here (Valladares-Pádua et al., 1997). The Atlantic forest is as rich as Amazonia in terms of fauna and flora. The Atlantic forest is Brazil's most threatened ecosystem, mainly because human colonization first occurred in this biome. Today there is less than 7% of the Atlantic forest's original distribution remaining, in sparse fragments along the Brazilian coast (Valladares-Pádua et al., 1997).

The Iguaçu Nacional Park [INP] represents the largest and last remnant of Interior Atlantic forest protected by federal law in Southern Brazil. The INP (S25º41'; W54º26') is located in the Atlantic forest ecoregion of Upper Paraná, on Brazil's border with Argentina and Paraguay. It represents more than 185.000 ha of semi-deciduous forest, and includes an altitude gradient of 400 m, creating mixed rainforest, characterized by the presence of *Araucaria* trees (*Araucaria angustifolia*) in higher regions (i.e. the INP northern tip). Temperatures range between -1ºC and +53ºC. Annual average rainfall is 1,700 mm. The area surrounding INP is a mosaic landscape, composed of variable sized farms, crops and small disturbed forest fragments, from which economically valuable species have been removed (IBAMA, 1999). Forest remnants are mainly secondary successional or regenerational forest, dominated by *tacuara* bamboo and strongly influenced by edge effect (IBAMA, 1999). The INP contains forest microsystems of high ecological value. These microsystems vary by soil type and altitude. They contain many pioneer species as well as many endemic and endangered species, e.g. *cabreúva* (*Myrocarpus frondosos*), *peróba* (*Aspidosperma polyneuron*), *ipê-roxo* (*Tabebuia heptaphylla*), *ipê-amarelo* (*Tabebuia chrysotricha*) and *jaracatiá* (*Jaracatia spinosa*).

The INP was declared a World Heritage site in 1986 by UNESCO, given its scenic beauty and outstanding ecological value. It was then added to the list of 'endangered' heritage in 1999 because of the "Settler's Road", which was cutting through the INP core area over 18 kilometers from north to south, linking the municipalities of Serranópolis do Iguaçu and Capanema (see Chapter 3). Although this road has been closed by the local authorities several times (and today remains closed), the anthropogenic pressure exerted on INP's biodiversity and its surroundings has been increasing constantly since 1999 regardless of the INP's Management Plan (IBAMA, 1999).

The INP annually receives thousands of tourists that come to admire the Iguaçu Falls. The park also suffers pressure from being located in an agricultural matrix, predominantly extended monocultures of corn, soy and wheat, all of which require conventional cultivation methods including large amounts of chemical input. Rio Grande do Sul and the state of Paraná are two of the biggest grain producers in the country. Paraná uses almost 9.000.000 ha to produce more than 30 million tons of cereal, grain and oilseed, making it the most productive state in Southern Brazil (IBGE, 2011). Nonetheless, there are no studies investigating the impact of human activities on the biological integrity of the INP. Such work should be among the first steps for characterizing the environmental impact of human development in the Iguaçu area.

Considering the conventional cultivation methods, pesticides used in the INP surrounding areas are varied, e.g. herbicides, fungicides, insecticides, spreader, adjuvants, pheromones, etc. In 2009, they totalled almost 8 million liters and 8 million kilos of commercialized pesticides just in the municipalities near the INP. These municipalities include: Capitão Leônidas Marques, Santa Lúcia, Lindoeste, Santa Tereza do Oeste, Céu Azul, Matelândia, Medianeira, Serranópolis do Iguaçu, São Miguel do Iguaçu, Santa Terezinha do Itaipu and Foz do Iguaçu (SEAB, 2009). Considering just in the Iguaçu, glyphosate and atrazine were the most commercialized pesticides in 2009, with more than one million liters and 120.000 liters sold, respectively. As a result, the extensive conventional cultivation in the INP's buffer zone produces an undeniable pressure against the biological integrity of the park, including contaminating water, soil and air. Although there are alternative methods which do not require the use of chemical inputs (e.g. permaculture, agroecology; e.g. Melão, 2010), this agricultural approach is not well known nor widely used among regional farmers. Finally, many of them do not adopt the legal methods required by the federal government (Law 7802/89, Law 9974/00 and Decree 3.694/00), which provide information about the final destination of pesticide packages from the discarded chemical inputs. Many agrochemicals are illegally acquired from Paraguay (e.g., 2-4 D). The inadequacy of the chemical packaging and disposal further worsen environmental damage. Moreover, Paraná is the 10th largest state in Brazil in terms of cattle herd size, containing more than 10 million heads (EMBRAPA, 2005), resulting in the pollution of groundwater, erosion, ruts, gullies, and soil compaction (Fidalsky, 1997).

The INP's ecosystems present obvious disfunctional symptoms that impact human well-being (Rapport et al., 1998). Organic substances from livestock activities flow from rivers and creeks which form part of the hydrographic basin of the Iguaçu River and ultimately enter the federal conservation unit. Soil properties play a fundamental role in absorbing pollutants, but organic matter appears to be the primary factor in the absorption process (Spadotto et al., 2001). Other agricultural practices exacerbate this problem, e.g. inadequate and excessive use of pesticides on crops, removal of native vegetation, destruction of riparian forests and vegetation surrounding springs, etc.

The hypothesis that pollution of the São João River by livestock and agricultural activities in INP's buffer zone contributes to the proliferation of pathogens was proposed in order to investigate threats to the park's ecological services. Ecological services refer to natural vital processes that guarantee the integrity and life of organisms living there (Tonhasca, 2004). The goal of this pilot project was to verify through microbiological testing whether INP still has one of its main ecological services, which is water filtration (Daily et al., 2000), i.e. elimination of potential pathogens entering the federal conservation unit.

MATERIAL AND METHODS

Study area

In order to assess the INP water purification ecological services, we chose to perform the analysis in this study on the São João River (25°32'S; 54°25' W, Figure 1) due to the following characteristics:

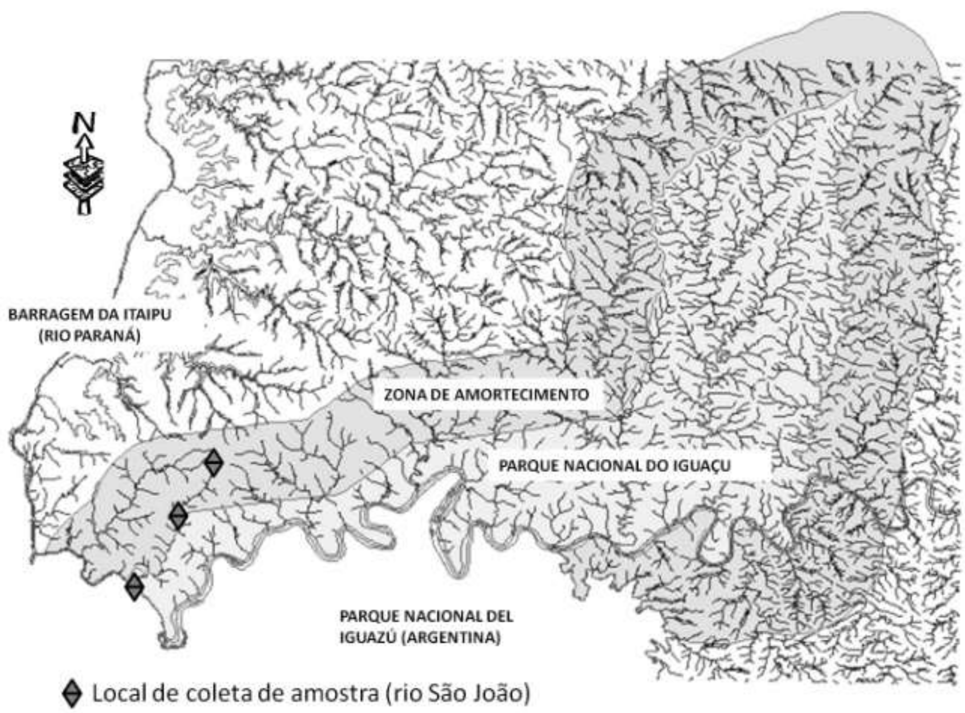

Figure 1. Study area map showing the INP, its buffer zone (*zona de Amortecimento*) and sampling location (black diamond shapes) in the São João River, near Foz do Iguaçu, Paraná, Brazil.

The São João River is 20 km long and rises in depth up to 10 km and encompasses part of the INP's buffer zone. It drains into the Iguaçu River, next to the old hydroelectric plant near to the INP's headquarters. The São João River's headwater contains a few water springs, which show advanced erosion caused by cattle. This phenomenon is quite evident, being visible from satellite images. Since it is a river that rises and drains in the study area, it is assumed that the physical and biological changes discovered here are a direct result of events occurring in the same area. The physical-chemical and bacterial analyses (n = 11) were performed from water samples (n = 9), from three sampling stations in the São João River, which were: (1) the river's head, (2) transition between the buffer zone and the INP, and (3) the São João River's mouth where it empties into the Iguaçu River. All the samples were collected on the same day (October 26th, 2010) under ideal weather conditions: clear sunny sky and after a period of over seven days without rain.

Analyses

The samples were collected in sterile flasks, preserved in a cooler filled with ice and brought to the lab on the same day to be processed through filtering membranes. The analyses were conducted at the Itaipu Binacional's environmental laboratory. The following analyses were conducted for each sample: bacteria count, coliforms, totals, and *Echerichia coli*; detection of pathogenic microorganisms, i.e. *Acanthamoeba* sp. and *Cryptosporidium* sp.; detection of eggs, cysts and pathogenic larvae; and temperature, pH and turbidity measurements.

Standard Operating Procedures − The membrane filtration technique consists of filtering 100 mL of water through a sterile mesh membrane filter, with 47 mm diameter, a porosity of 45 µm, and filtration equipment with filter-holder previously sterilized. Bacteria were captured on the membrane surface because they are wider than the membrane mesh. Bacteria were then transferred to a Petri dish containing Endo Agar and Salmonella-Shigella growth media (S.S.). The dishes used for the research of total coliforms and *Salmonella* sp. were incubated in a greenhouse at 36°C ± 0.5, the dishes used for the examination of *Escherichia coli* were incubated in a greenhouse at 44°C ± 0.2 for 24h. The growth medium diffuses into the membrane through capillary action. The bacteria develop characteristic and unique colonies, which allow for biochemical identification. Colonies are counted on a magnifying glass (Santos, 1999). In the Endo medium, the colonies had a coloration varying from pink to dark red with a metallic green sheen. On the other hand, the S.S. medium colonies were colorless, cream or black. For identification, the species developed in the Endo and S.S. growth media were transferred to the Rugai medium (Santos, 1999) in which the biochemical characteristics (e.g. gas or H_2S production, reaction to indol, etc.) allowed for genus and species identification. Finally, bacteria count was conducted in colony-forming units by 100 mL (CFU.100 mL-1).

Cryptosporidium sp. research – To verify the presence of this pathogen, we used a filtering membrane to filter 1 mL of water, which was then placed in 5 mL of deionized water. After 2.5 minutes of centrifuging, 15 µL of sediment was deposited onto a glass slide. We used Kinyoun staining (Santos, 1999) to carry out the procedure. The fixing was carried out with methyl alcohol covering the same slide for a few minutes. Then the smear is covered with basic fuchsine at 6% (94% mL of basic fuchsine + 6 mL of phenol) for 6 minutes. After rinsing the slide with deionized water, it was discolored with 70% ethylic alcohol for 2 minutes. Methylene blue was used as a stain (0,3 g of Methylene blue + 100 mL of sterile deionized water).

Acanthamoeba sp. research – The analysis of this pathogenic microorganism was described by Santos et al. (2009). A BHI growth medium (Brain Heart Infusion) was deposited in 0.4 mL of sediment and was then incubated at 35°C for 48 hours. Afterwards, the growth was examined on a slide along with Trypan blue, a vital stain that appears green when the protozoan is in a viable form. In the event of membrane being ruptured, the stain would then be blue.

Eggs, cysts and larvae research – We centrifuged test tubes containing the water samples for 5 minutes at 25000 rpm. We discarded the supernatant and stirred the bottom of the sterile tubes. Using a micropipette, we placed 100 µL of lugol on a slide and observed with a microscope.

Hydrogen potential – The pH was measured in the laboratory by electrometric method (Santos, 1999).

Temperature – The water temperature was measured at the collection site with a digital thermometer (accuracy: 0.1°C).

Turbidity – The turbidity was measured at the laboratory using Jackson's nephelometric method which determines turbidity by measuring the scattering and absorption of light by particles suspended in water, and is measured in Nephelometric Turbidity Units (NTU).

RESULTS AND DISCUSSION

Regarding abiotic factors, the data that stood out most was obtained at the head of the river. Temperature was the highest in this location, which could be attributed to greater exposure caused by partial loss of riparian forest around the springs. Other notable factors include a more acidic pH and a lower turbidity at the head of the river in comparison with the other sampling areas (Table 1).

Table 1. Temperature (ºC), turbidity (NTU) and pH of samples (average values obtained from tree samples of each sampling area).

Zone	Temp.	Turbidity	pH
Head	21.4	5.5	5.9
Transition	19.9	7.3	7.0
Mouth	20.9	6.4	7.5

In the transition area and at the mouth of the river, pH was neutral and all samples met the requirements defined by the Ministry of Health (Decree n° 518/04), remaining between 6.0 and 9.5. Turbidity of samples from transition area and river's mouth were high, suggesting many organic matter particles in suspension. For the bacterial analysis, the thermotolerant coliform *Escherichia coli* was found in 100% of the samples (Fig. 2).

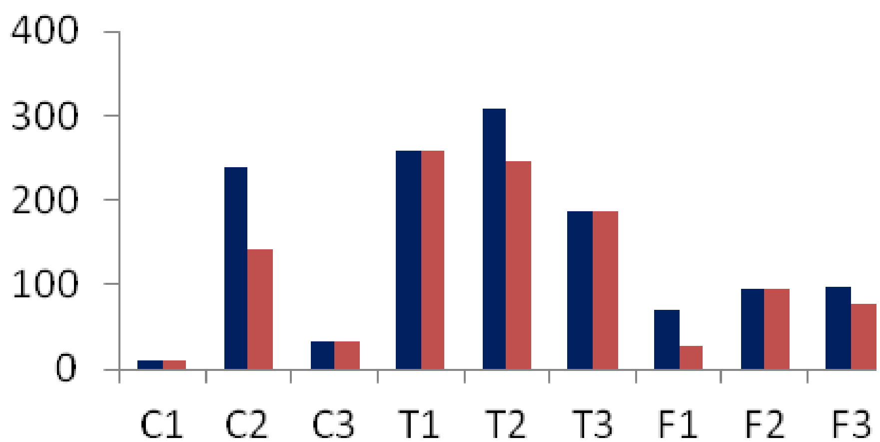

Figure 2. Bacteria count (unit: Colony-forming unit, CFU.100 mL-1, medium: Endo 44ºC) of thermotolerant coliforms (blue) and *Escherichia coli* (pink).

Other bacteria were found in varying proportions, the most common being *Citrobacter* sp., found in 100% of the samples (Fig. 3) and *Proteus vulgaris*, found in 66.7% of the samples. *Enterobacter* sp. was found in 33.3% of the samples, corresponding to samples from the head and the mouth of the river. *Edwardsiella* sp. was found in 11.1% of samples, all them exclusively from the head of the river.

The bacterium *Salmonella* was found in samples collected at the head of the river and in the transition area between the buffer zone and the INP (Table 2).

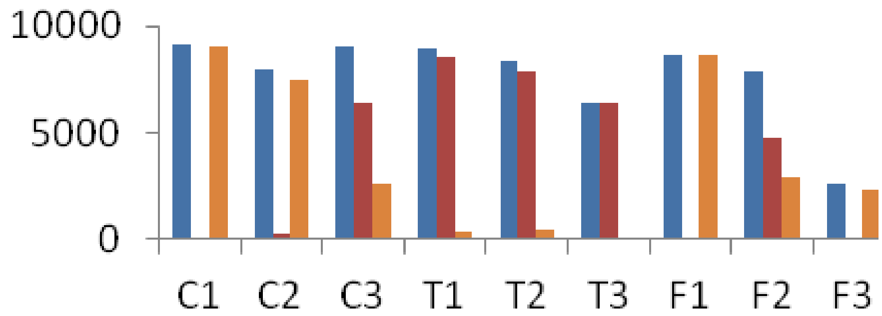

Figure 3. Bacteria count (CFU.100 mL-1, Endo 37ºC) of Total Coliforms (blue), *Citrobacter* sp. (red) and *Escherichia coli* (yellow).

A viable form of protozoan *Acanthamoeba* sp. was detected in 100% of samples, and oocysts of *Cryptosporidium* sp. were found in two samples from the headwaters of the river (Table 2). Rotifers and oocysts of *Eimeria* sp., an intestinal parasite that may compromise the renal system of the host (Yabsley et al., 2002), were found in two samples from the headwaters of the river.

Table 2. Detection of *Salmonella* sp., viable cysts of *Acanthamoeba* sp., *Cryptosporidium* sp., eggs, cysts and larvae (OCL) of microorganisms.

Samples	C1	C2	C3	T1	T2	T3	F1	F2	F3
Salmon.	A	A	**P**	**P**	A	**P**	A	A	A
Acantha.	P	P	P	P	P	P	P	P	P
Crypto.	A	**P**	**P**	A	A	A	A	A	A
OCL	**P***	A	**P****	A	A	A	A	A	A

A: absent; P: Present; P*: Eimeria sp.; P**: rotifers mite

Several results suggest a potential risk of microbiological contamination for the rural population because they use the spring water for consumption. It is noted that most of the contamination occurred in the samples from the springs of São João River's head.

The risks for public health by infection with detected organisms have been well-documented. *Escherichia coli* is a bacterium widely found in the intestinal tract of warm-blooded animals. Some groups of *E. coli* are responsible for diarrhea (i.e., the "Traveler's disease"), urinary tract infections, enteritis, neonatal meningitis, septicemia and pneumonia hospital, septic arthritis, endocarditis, and abscesses in the liver and brain (Tavares, 2002; Jawets & Levinson, 2005). The most susceptible age groups to contamination by *E. coli* are the young and the elderly, with symptoms ranging from severe stomach cramps to diarrhea and vomiting (CDC, 2008).

The genus *Citrobacter* is often found in the intestinal microbiota of humans, but three species; *C. freundii*, *C. diversus* and *C. amalonaticus*, are responsible for urinary tract infections, meningitis, brain abscesses in newborns, hospital infection and septicemia (Tavares, 2002).

The presence of *Salmonella* must be monitored because it also presents a serious risk to public health. Some authors suggest the use of *Salmonella* sp. as an indicator of pollution because it survives longer than coliforms (Cetesb, 1993). Most infections by *Salmonella* sp. are caused by *Salmonella enteric*, of which there are 2400 serotypes (Tortora et al., 2005). Often infection originates from animals rather than humans (Pelczar et al., 1997). Humans may become infected by eating poultry, eggs and other contaminated products (Alcocer et al., 2006) or by ingesting water that has been contaminated by human and animal wastes (Levinson & Jawetz, 2005). *Salmonella* is also a threat to wild mammal species, as they can harbor the parasite without showing clinical signs (i.e. asymptomatic vectors; Liebana et al., 2003).

The presence of *Cryptosporidium* sp. in samples from the head of the river is of great concern because Cryptosporidiosis is generally associated with water contamination (Robertson et al., 1992; Smith, 1998). Lima and Stamford (2003) pointed out that the microorganism *Cryptosporidium* sp. in its oocyst shape, is resistant to chlorination and filtration treatments (Markel et al., 2003) and is very likely underrated. Another study that compared various detection methods observed that detection by specific staining underestimates the presence of the pathogen (Greca 2010). Thus, it is likely that more oocysts were present in samples than those reported, given the fact that the method used does not necessarily guarantee their detection. *Cryptosporidium* sp. is an intestinal parasite of various vertebrates. It has been mentioned that due to the high genotypic similarity between these parasites (e.g. > 98%; *Cryptosporidium parvuum, C. hominis, C. canis, C. felis*), cross-infection between pets and humans is likely (Greca, 2010). Up until the late 1990s, only one species, *C. parvuum*, seemed to present risks to human health. Since then however, human infections by other species of *Cryptosporidium* have been reported (Pedraza-Diaz et al., 2000), greatly increasing the risks to humans. The genus is recognized worldwide due to high rates of mortality related to the presence of oocysts in water (Garrido, 2005). The first cases of Cryptosporidiosis were reported in 1967 in the United States. Since then, this infectious disease has been identified in 60 countries, including Brazil. The symptoms include prolonged diarrhea, vomiting, abdominal pain, nausea, and less commonly anorexia, fever and malaise. The disease can be fatal for immuno-compromised people as well as HIV carriers (Wiebbeling et al., 2002), whereas in other cases, the symptoms of the parasitic disease may last for an average of only ten days. In most cases, however, the established infection leads to death (Muller, 1999; Lima & Stamford, 2003). The potential danger of Cryptosporidiosis to public health (Heller et al., 2004) reinforces concern about the occurrence of *Cryptosporidium* eggs in the headwaters of the rivers.

Lastly, pathogenic organism *Acanthamoeba*, found in all sampling locations, has been isolated in several environments, e.g. public water supplies, air conditioning ducts, surgical instruments and still waters (Khan, 2006). This microorganism has the ability to host other bacteria (e.g. *Legionella*, Nwachuku & Gerba, 2004) and is responsible for causing keratitis, a serious eye disease (Khan, 2006; Jung et al., 2008) whose main symptoms include blurred vision, intense pain, photophobia, and overall discomfort (Obeid et al., 2003). In the 1980s, several studies were conducted demonstrating the occurrence of this pathogen in the feces of wild species, specifically reptiles, birds and small mammals (Jadin et al., 1973 *apud* Nwachuku & Gerba, 2004).

The Decree 518 of March 25, 2004 establishes the procedures and responsibilities for the control and surveillance of water quality for human consumption and its potability standards. Bacterial analyses made on drinking water samples from a neighborhood in Foz do Iguaçu by Cruz (2009) revealed that the criteria used for assessing the quality of drinking water in Decree 518/04 may be insufficient to determine whether or not the water is suitable for consumption, as it does not take into account pathogenic microorganisms dangerous to public health as detected and described here. If they had been considered in the study conducted by Cruz (2009), about 97% of the water samples would have been classified as unfit for consumption, while only 33% are classified as such according to the Decree 518/04. Another study with analyses performed in the Tamanduá River in Foz do Iguaçu is consistent with this observation (Pinto et al., 2010). The contamination by fecal and total coliforms in large quantities in the visiting area of the INP has also been reported in a previous study (Vaz et al., 2008).

The presence of *Cryptosporidium* oocysts in water samples from the head of the river is somewhat surprising since these pathogens are found in urban areas such as wells, surface water, water sources of supply, sewage, and even in the feces of animals and humans (Heller et al., 2004). Although several detection methods already exist (Lima & Stamford, 2003), there is a lack of information on how to monitor water intended for human consumption. However, rising concern has resulted in the inclusion of *Cryptosporidium* sp. research to determine the potability in the Decree 1.469 of the Ministry of Health in 2000. Nevertheless, the premise of the Decree 518/04 only suggests using turbidity as an indication of risk of presence of cysts and oocysts in treated waters (Heller et al, 2004). No significant relationship was found between the density of *Cryptosporidium* and water physico-chemical parameters (Muller, 1999).

There are already existing reports of wild mammals in the Atlantic forest and other Southern Brazilian ecosystems contaminated with *Cryptosporidium* oocysts (Dall'olio & Franco, 2004; Oliveira et al., 2008). There are records that the protozoan infects more than 150 species of mammals (Fayer et al., 2001), where more than 21 species (specific to hosts) have already been described (O'Donoghue, 1995). Moreover, there have been cases of human infection by *Cryptosporidium* species involving other genotype-groups (Pedraza-Diaz et al., 2000).

The ubiquity of *Cryptosporidium* oocysts as well as other pathogens in the São João River is a threat to wildlife populations as well as human health. The presence of *Acanthamoeba* in water intended for consumption may contribute to the survival and proliferation of other pathogens and this relationship may actually increase their virulence (Nwachuku & Gerba, 2004).

CONCLUSION

All samples presented a large diversity of bacteria and contamination by pathogenic microorganisms in their resistant and viable forms. There is a growing concern for public health due to the presence of these pathogens. Although the present study represents a small sampling effort, our results corroborate what has already been published on this subject. We believe that the presence of these pathogens in the INP presents non-negligible risks to public health and to the INP's biological integrity. We conclude that the ecological service of water filtration has been partially compromised. Future quantitative research on the presence of pathogenic microorganisms is required in order to measure the risk incurred by the local population.

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GENERAL CONCLUSION

The general purpose of this research project was to characterize the situation of the Iguaçu National Park in southwestern Brazil and discuss its conservation situation. On order to achieve my goal, I have adopted a multi-disciplinary and multi-scale approach and conducted the following monitoring: track surveys (>500 km, since 2006); DNA scat analyses (64 samples, 2009 and 2015); soil bait-lamina tests (432 stations, 2012-2013); water bacteriological analyses (81 tests; 2010) and camera trapping (6,190 trapping days, 193 stations; 2012-2014).

For the first time in the INP whole existence, we provided much needed wildlife numerical information which can be used by natural resources managers to derive conservation and management strategies.

Results consisted in:

(1) density estimates for 17 mammal species inside the park and in its buffer zone using the Random Encounter Model (REM) method, as described by Rowcliffe et al. (2008);

(2) mammals' response to habitat alteration over the gradient offered by the Iguaçu region, but also, with the historical case study of the multi-openings and closings of the Settler's Road in the INP core area, offering a temporal perspective, which has had historical, social, economical and ecological consequences;

(3) soil ecological integrity assessment through micro-organisms' feeding activity levels and depths;

(4) water quality assessment with regard to INP's ecological services and public health.

In nine years, I have gathered more information that I could ever fit in an academic document. Research gaps are all the INP ever had. However, I have done all I could to depict here the INP current global situation by providing species' density estimates and by analyzing how species responded to habitat variations at the species and trophic guild levels. Bonus material include first photographs of two xenarthran species in the INP, i.e. the Giant Anteater and the Greater Naked-Tailed Armadillo. Track surveys and volunteer-based partnerships with local conservation actors allowed me to develop a measuring tool to discriminate the too-often confusing Neotropical spotted cats. The DNA analyses performed on carnivore scats in collaboration with the Global Felid Genetics Program at the American

Museum of Natural History of New York allowed revealing the concealed presence of Geoffroy's cat, an open-land species. The species was not known to occur in the region. Last, quick ecological assessments on soils and waters brought more information on how habitat change impacted the inland Atlantic forest ecosystem.

In terms of applied conservation, the findings presented here provide extremely useful insights to define timely management and conservation strategies at the regional level. The following conservation recommendations are based on almost a decade of living conservation at the regional level while analyzing the root causes of each specific phenomenon.

Most striking results include a) the disappearance of the jaguar; b) the subsequent highly populated jaguar prey species within the INP; c) the thriving populations of ubiquitous, generalist, opportunistic species such as coatis, tayras, opossums and capuchin monkeys in and out of the park; d) the omnipresence of domestic dogs; e) soil's degradation in the INP region; and f) water contamination with pathogens.

a) <u>Low Top Predator Density</u>

Such trend is characteristic of what happens in disturbed and/or disconnected forest ecosystems where specialist species suffer and disappear as opportunistic generalists thrive (Redford, 1992). We observe an assemblage simplification where alteration-sensitive species are not present any longer and where ubiquitous species are taking over ecological niches left vacant (Bertrand, 2010). The prey-predator population balance has inevitably shifted. A quick conservative calculation of the prey biomass required to maintain an adult jaguar for a year allowed demonstrated that the INP shelters jaguar food for over 2,500 jaguars while it is estimated that it only shelters 6 to 12 individuals (INP Counsel, 2012). Added to the jaguars' scarcity (Paviolo et al., 2008), recent bamboo seed production in the INP have likely caused steep increases in rodent populations (Murúa et al., 2003; Sage et al., 2007); both phenomena benefited smaller sympatric carnivore species. Intraspecific competition among carnivores now also includes domestic dogs, which were found in the same proportions in and outside the INP (see Chapter 1).

While we did not have enough camera-trap data to extract density estimates for both INP top predators, i.e. jaguars and pumas, existing information in the literature allow drawing population trends for both species.

Emblematic jaguars

While Crawshaw (1995) had estimated 3.70 ind./100 km^2 in 1995 for the INP, which roughly means 68 individuals for the whole park, the recent INP Carnivore Project conducted a 72 camera-trap monitoring on jaguars between 2010 and 2012, and reported a dwindling 0.29 ind./100 km^2 for the whole 2,000 km^2 area (INP Counsel, 2012). Root causes of regional jaguar population's severe decline are anthropogenic. During his PhD study, Crawshaw observed a 100% mortality rate of monitored animals due to poachers, road collisions or livestock depredation (Crawshaw, 1995).

Between 2002 and today, Argentinean researchers Mario Di Bitetti and Agustín Paviolo have extensively studied big cats using camera-traps and later on, telemetry in the Green Corridor, with most sampling effort focused on the Iguazú National Park and the Uruguaý provincial park in Argentina. While they had estimated 40 individuals in 2005 for the whole 9,400 km^2 Green Corridor, which extends from the southern tip of the state of São Paulo in Brazil to the south of the Misiones province in Argentina, and while they and others had doomed the species to inevitable short-term extinction (Paviolo et al., 2008; Zanin et al., 2015), most recent findings suggest that the population might have recently increased to 71 individuals, since the last monitoring using both camera-trapping and telemetry data (Paviolo, *pers. comm.*). In 2008, the same researchers published density estimates for the Iguazú National Park, the 65,000 ha-Argentinean counterpart of the INP. As survey effort increased between 2004 and 2006, density estimates also did, going from $0.49 \pm 0.16/100$ km^2 - $1.07 \pm 0.33/100$ km^2 to 0.93 ± 0.2 - $1.74/100$ km^2 $\pm$ 0.34/100 km^2.

However, these numbers are not suggesting a viable population according to molecular analyses (Galetti et al., 2013). Biesiegel et al. (2012) estimated to fewer than 250 individuals the total population for the Atlantic forest biome. Habitat loss and fragmentation in Atlantic forest is the worst of all Brazilian biomes, with less than 12% of its original distribution remaining today (Ribeiro et al., 2009). It has the smallest protected area size (431 km^2) and the smallest mean population (10 individuals; Sollmann et al. 2008) as opposed to the Amazon where we have the largest protected area (10.993 km^2) and the largest population size (311 ind.). Regionally, the species has been looked at as 'on the brink of extinction' (Cullen et al., 2005; Paviolo et al. 2008; Morato et al., 2013). Among the pressure factors, jaguars have lost lives to livestock depredation, poaching, and poor or fragmented habitat (Crawshaw et al., 2004; Galetti et al., 2013).

Sympatric pumas

While our camera-trap monitoring did not allow for obtaining reliable density estimates given the small number of records (n = 7), previous track surveys allowed obtaining relative abundance of over 28 species (Bertrand, 2006; Bertrand, 2009). Track surveys proved to be efficient for diurnal species and ungulates (Munari et al., 2011). In the present study, pumas ranked in 7th (n = 313; Bertrand, 2006) and

5th position with 8.65% of total records (n = 1661; Bertrand, 2009), suggesting a good representation among the animal assemblage. More recently, in the 4-year long INP Carnivore Project, pumas were recorded in 54% of the 37 sampling sites of a camera-trap monitoring and puma occupancy increased between 2009 and 2013 (Silva, 2014).

Peer-reviewed papers providing population estimates for pumas used to be scarce given the difficulty individual recognition represents and the high cost involved with monitoring activities (Azevedo et al., 2013). Since 2008, several papers were published, providing a good overview of puma's ecology, its activity patterns, abundance and densities (e.g. Trolle et al., 2007; Paviolo et al., 2009; Negrões et al., 2010). Density comparisons between North and South America would be a little use as habitat and subspecies' characteristics radically differ. In South America, estimates are available for Chilean Patagonia (2.5-3.5 ind./100 km^2), Bolivia (5-8 ind./100 km^2) and Venezuela (0.019 ind./100km^2). In Argentina, Kelly et al. (2008) estimated 0.5-0.8 ind./100 km^2 using an observer-based identification process of camera-trapping photographs. Two years later, in the Argentinean INP counterpart, Paviolo et al. (2010) divulged 1.55-2.89 ind./100 km^2 for the most preserved areas of the Iguazú National Park, while finding only 0.3-0.74 ind./100km^2 in the most intensely disturbed areas. Given the fact rivers do not represent a physical barrier to animal movement, Paviolo's (2010) findings are likely to apply to the Brazilian INP (Azevedo et al., 2013).

Pumas are highly adaptive and hence are the mammal species the most widely distributed on the American mainland, being found from southern Alaska to Tierra del Fuego in Patagonia (Culver et al., 2000; Caragiulo et al., 2013). However, it suffers - like all other life forms on the planet - from human-caused disturbances such as habitat destruction and fragmentation (Kelly et al., 2008), poaching (Azevedo et al., 2013), retaliation after livestock depredation (Conforti & Azevedo, 2003), and trophy hunting (Azevedo et al., 2013). According to the conservative factor provided by Frankham (1995), the effective puma population for Brazil is 3.489 individuals (Azevedo et al., 2013).

Given its high adaptability, it is not clear what parameters most influence its behavior. Human-caused pressures and natural limiting factors add to the conundrum. On the other hand, pumas' ecology has been widely explored and elements such as jaguar avoidance and competition, competition with ocelots, and correlation with prey abundance and activity patterns are well known (Emmons, 1987; Schaller & Crawshaw, 1980, Sunquist & Sunquist, 2002). Moreover, pressures vary in nature and intensity depending on the location. For instance, in Pantanal, trophy hunting is the first mortality cause (Azevedo et al., 2013). In the INP area, major pressures exerted onto puma populations are poaching, habitat loss and fragmentation, retaliation for livestock depredation and road kills (De Angelo et al., 2011).

The three recurrent root causes to the top predator decline are poaching, roads and deforestation. Applied conservation strategies to tackle such issues are summarized below.

** *Poaching* – Poaching mitigation has been a serious and life-threatening issue for anyone around the INP. Currently, efforts have been made to increase patrolling along the 440 km of INP edge and repression field expedition to catch infringers in the act. However, I believe that efforts should now most importantly focus on education and raising awareness among the people, particularly, the communities living right on the park's edge called "*lindeiros*". Those people should be looked at the INP keepers and should be treated accordingly.

** *Roads* – Roads have invaded and fractioned wildlife habitat causing the death of over 475 million animals every year; about 400 million small mammals, amphibians, birds, and reptiles, and 75 million larger mammals, such as Manned Wolf (*Chrysocyon bachyurus*), felines, and large herbivores. Just in Brazil, about 15 animals are killed every second on the roads. Strategies such speed reduction systems, fencing, wildlife passages have been explored elsewhere, showing promising results (Nauderer, 2014). In the INP, we are currently testing ultrasonic whistles (Scheifele et al., 1998) in both parks (Brazil and Argentina sides of the Iguaçu Park) in a double-blinded test.

** *Deforestation* – The removal of forest cover has two direct impacts: not only does it fragment and disconnect the remaining forested areas but it also distances remnants further away. Habitat fragmentation and connectivity go hand in hand. Generally, the only connective cover remaining between conservation areas are riparian forests. However, riparian forests are often located in private properties, where they do not benefit from the protection they deserve. Efforts should be concentrated in connecting with *lindeiros* and recuperating the integrity of riparian forests. Furthermore, wildlife corridors, such as the Santa Maria corridor (Gris, 2012) which connects the INP to the Itaipu Binacional dam's lake (see Chapter 2), should be prioritized. Also, human activities in the INP buffer zone should be oriented towards agroecology and permaculture. Such framework allows sustainable yields while preserving the balance of existing natural resources. It increases vegetation cover and promotes ecological integrity of the exploited ecosystems (Perfecto & Vandermeer, 2010).

b) <u>High Prey Density</u>

Tapirs, red brockets, collared peccaries, agoutis and other rodents, armadillos and opossums were found in great numbers in the INP. The two main reasons for it are the absence or rarefaction of the jaguar, which was discussed above, and the bamboo seed production, which provided sudden enormous amount of highly-energetic food to small prey populations. While the herein several measures can be taken to promote jaguar conservation, native bamboo species producing seeds every 25 to 30 years and its impact on the prey-predator populations fits in what mathematical ecology calls the Lotka-Volterra cycle (Bahar & Mao, 2004).

c) <u>Invasion and Overpopulation of Generalists</u>

Exception made of large cats, most carnivore species showed great adaptability to the presence of humans and the habitat disturbances associated with it. Most populous species were ubiquitous and generalist species, suggesting that the INP animal assemblage is progressively losing its specialists to the benefit of more adaptive species. In this section, wild coatis and domestic dogs are the most targeted. The reasons underlying this trend also include the absence and rarefaction of jaguars and top predators in general. A direct consequence of this is that the disappearance of these forest specialists leaves their ecological niches vacant. In addition, human-dominated environments often provide easy food sources and are less prone to predator attacks. On top of the solutions previously suggested, human impacts in the INP region could be reduced by adopting environment-friendly attitudes in the recreational area in order to difficult access to food. In the INP buffer zone, easy food sources are free ranging livestock, chicken husbandries, and pigsties. Success in reducing the amount of prejudice has been shown by using one or more of the following measures: animals safely gathered in closed infrastructures at night, electrical or thorn fences around these structures, protecting shepherd dogs, bells, visual stimulations, high frequency alarm systems, pyrotechnics or even rubber bullets (Pitman et al., 2004).

The INP buffer zone is mainly characterized by the discrepancy between what the legislation says it should be used for and how it is actually exploited. Legally, the buffer zone is the direct proximity of a conservation unit where human activities are controlled by specific norms and restrictions whose purpose is to minimize negative impacts over the preserved area (Bertrand & Garcia, 2010). In other words, it is supposed to act as a buffer to damaging soil uses so the ecosystem will more likely remain balanced, in the limit of its resilience, which – in turn – will prevent overpopulations of ubiquitous generalists. Efforts should concentrate on promoting and enhancing the soil use and activities befitting the buffer zone's definition to avoid such imbalances. Conservationists should show support and provide educational material to *lindeiros* during this adaptation process.

Last, dogs should be equipped with subcutaneous microchips. Chip reading of free-ranging dogs in preservation areas would ensure owner's accountability for their actions, which in the short-term would promote self-responsibility (Slater et al., 2008).

d) <u>Soil degradation</u>

Soil is a living organism which has been abused by unsustainable practices (Lebel, 2003). In the context of the present study, the main problem is conventional agriculture and pastures. We have shown in Chapter 7 the importance of vegetation cover and the consequence of its removal on soil's ecological integrity. The composition of ground vegetation is related to soil chemical properties (Hawkes et al., 1997; Wilson et al., 2001).

In the western world, people emigrated from rural areas to cities in the 60s, leaving the lands to huge machines and tons of chemicals. In the 80s, NGOs showed their first concerns about newly adopted agricultural practices and already suggested agroecology as a sustainable alternative (Altieri et al., 1996). Sustainable agrosystems were already seen as “a version of sustained yield, which was defined as a condition to harvest biomass from a system in perpetuity because of the ability of the system to renew itself or to be renewed is not compromised’ (Gliessman, 1997, p. 13). Since farmers tend to judge themselves by comparison with other local farmers’ performance, organic vs. conventional farmers is a very unfair situation. Government solely supports conventional farmers, having both government and farmers hooked on subsidies from international agro-businesses and corporations. At the landscape level, the few organic farms are inserted into a highly disturbed agriculture matrix, which results in discouraging yields during the first two years of organic farming, most often leading to severe depression or even suicide attempts from organic farmers (*pers. obs*.).

The regional scale is where efforts to change mentalities should concentrate. Organic farmers should be helped at this scale to gather into associations and functional groups to strengthen their initiatives (Fernandes & Woodhouse, 2008).

Natural and sustainable solutions exist to revert soil contamination using fungi, which are robust organisms, more tolerant than bacteria or enzymes to high concentrations of pollutants (Evans & Hedger, 2001). In addition, while manual removal seem the only solution to invasive Moso bamboo (*Phyllostachis edulis*) in the INP in which about 15 hectares are estimated to be dominated by the species (I. Baptiston, *pers. com*.), mycorrhizae stand as potential allies to forest restoration from invasive species (Jeffries et al., 2003).

<u>e) Water pollution and pathogen contamination</u>

As explained in Chapter 8, natural ecosystems have a natural ability to purify waters. In the INP region, the main culprit for pollution and contamination is agro-farming. Therefore, this is also where solutions can be applied. By switching to organic farming and permaculture practices, drastic changes would occur in a very short amount of time (Beeman, 1994). Also, adequate fencing should keep livestock off of headwaters to prevent the appearance of pathogens. During informal visits to farms on the INP edge, I have seen pigsties installed on headwaters, contaminating the purest waters with copious amounts of manure. Clearly, ignorance is at stake, and should be substituted by knowledge from local conservationists. A 2002 technical report form the US demonstrated that 69% of rivers, streams and brooks contained persistent detergent ingredients, and 66% contained disinfectants (Stone et al., 2014). As an example of applied conservation action, I have conducted practical workshops in several farms showing *lindeiros* how to make their own cleaning products using solely natural, cheap, and biodegradable ingredients. This taught me how simple and efficient solutions can be, and also helped me to better understand the friction between *lindeiros* and local authorities/park managers.

LIMITATIONS

During the camera-trapping monitoring, camera units were not located on roads and trails in order to limit the number of units lost to poachers. On one hand, it allowed the project to continue for 24 months with only three cameras stolen, but on the other hand, we gathered very few records of large cats, which preferentially use such features (Paviolo, 2010).

Also, we would have collected more data and potentially obtain density estimates for more species may we have had more cameras. We worked with a limited number of PC900 Reconyx cameras, primarily because of their cost. However, they are worth every penny for they have shown great resistance to the INP challenging climate, and stand as good candidates for further inventories. Most importantly, it is our hope that monitoring such as ours will be replicated and extended with more equipment so that more population estimates can be obtained and used to draw sound conservation and management strategies.

CONCLUSION & TAKE-HOME MESSAGE

Truly, the Achilles tendon in conservation lies in the fact that decision makers are often not aware of the actual situation of their direct natural environment. They are not aware of its vital importance hence they simply have no interest in it. Everyone knows that all ecosystems suffer from human-caused pressures, and that the Earth is suffocating because of humans' linear thinking tendency (Hawken, 1993; Kay & Schneider, 1995). Yet, this is not enough to trigger change and the generally accepted economy model does not include what it calls "externalities". It refers to the effect of production or consumption of goods and services imposes costs or benefits on others which are not reflected in the prices charged for the goods and services being provided (Khemani & Shapiro, 1993). The result of this intentional omission is the exploitation of unsustainable energy sources and the annihilation of any attempt (e.g. patents) at using sustainable sources, all in the name of short-term monetary profits.

In industrialized countries, education reaches more people and is of better quality than in developing countries (Heyneman & Loxley, 1983; Buchmann & Hannum, 2001). In Brazil, most politics have only primary education, some are illiterate, and few possess a university degree (Calil, 2010). Education levels define awareness levels (Lane et al., 1988). As the international Media portrays Brazil, politics and crime are two popular fast lanes for fat pay checks. Leaders are far too busy swerving money from an overcomplicated administration to care about the environment and what is done with it.

To contextualize this rationale, Dr. James Karr, Environmental Health specialist from the University of Illinois, provided rigorous definitions that are worth remembering here. Ecological integrity is the “sum of the physical, chemical and biological integrity” (Karr & Dudley, 1981). And biological integrity was defined as “the capacity to support and maintained a balanced, integrated, adaptive biological system having the full range of elements (genes, species and assemblages) and processes (mutation, demography, biotic interactions, nutrient and energy dynamics, and metapopulation processes) expected in the natural habitat of a region” (Karr & Chu, 1999). Goldman (2010) relevantly reminds us that “the resources we use, the species we see and the food we eat are all ecosystem processes: the biological, chemical, physical interactions between ecosystem components (e.g. species, water and soils)”. Furthermore, as technology and awareness evolve, scientists have progressively started to perceive the fractalian nature of the universe (Iovane et al., 2004). The human population beats records of incidence of cancers and a wide array of degenerative diseases (Campbell, 2006). We are polluting our bodies with chemicals we do not control. We are relating to the earth the way we relate to our bodies (Shape & Irvine, 2004). In Western society, we aim for fast fixes for everything, and usually rely on chemicals and technology to solve our health problems (e.g. chemotherapy, Cairncross & Macdonald, 1988), forgetting our very nature and the purpose of the human experience. The human body and earth are extremely perfect and complex systems, which possess a wonderful ability to repair themselves from damages of all sorts. Yet, their resilience is finite (Lenton et al., 2001).

As shown by quantum physics, the physical body can restore its integrity by applying higher level of vibrations. Everything in the universe is energy. All is vibrating energy. High levels of vibrations include the purest sentiment a human being may experiment, i.e. Love. It is a very similar vibration to light. When applied on the body, high frequencies can heal the body (e.g. Shi et al., 2010), the mind (Crowe & Scovel, 1996) or the soul. By fractal, the same is true for the planet and the Iguaçu region. What this region needs, what Earth needs, what each human being needs is a quantum leap of faith and love. If someone – consciously or unconsciously – cruelly lacks self-love, that person is emotionally unavailable. Therefore, any conscious attempt at having that person convinced of the need to change lifestyle or practices towards more sustainable ones, would have very low success rates. Conservation happens simultaneously with the on-going paradigm shift (Wu & Louks, 1995).

I intentionally included data I collected prior to my enrolment date at the University of Aveiro as my research focus has always been the same since my arrival as a guest researcher in the INP in 2006. The information contained in this thesis is only a portion of what one person can gather, analyze and publish in a 4-year frame for the sake of nature conservation. There is much more information I wish to process and share with the scientific and the global communities. In Brazil, there is a saying: “What the eyes do not see, the heart does not feel”. After all these years spent in the INP bush and visiting *lindeiros*’ farms, analyzing the land, waters and animals for as much as a volunteer scientist can do on a 1972-beetle, and after scratching my head anxiously seeking solutions to the INP conservation conundrum, I have finally understood that we had it all wrong all along. It is the other way around: “What the heart does not feel, the eyes do not see”. Therefore, at the regional level, the most efficient conservation action will likely be a human reconnection between *lindeiros* and conservation actors. As my experience has shown me, *lindeiros* crave to be heard, considered, helped and involved. When a heart-based relationship is established, trust sets in and people are much more receptive to awareness campaigns (Huy, 1999).

One of the major outputs of this PhD thesis is its huge wildlife photograph collection. A selection was made to share a fraction of the unspeakable beauty of what inhabits the *lindeiros*’ backyard. An even smaller number of photographs was already presented during the week of the environment in June 2015 in the INP visitor center in a tri-national joint initiative (i.e. carnivore specialists from Brazil, Argentina and Paraguay). However, I intend to promote a much larger reconnection campaign, which does not target foreigners but locals from the entire region, starting with the 14 cities located on the INP edge. This campaign will consist in an itinerant photograph and near-video sequences exhibit. Moreover, we will elect the closest poaching trail on the INP edge and convert it together into an educational trail; one trail per municipality, for which *lindeiros* will be the caretakers. It is a matter of touching hearts and through them, reaching consciences. By providing the adequate tools and knowledge in a collaborative and nurturing way, both hearts and ecosystems can heal.

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