Manejo e Conservação de Carnívoros Neotropicais

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Manejo e Conservação de Carnívoros Neotropicais

I Workshop de Pesquisa para a Conservação de Carnívoros Neotropicais

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Apresentação

Este livro é, em parte, resultado do I Workshop sobre Pesquisa e Conservação de Carnívoros Neotropicais, realizado em maio de 2003, na cidade de Atibaia/SP. Estamos satisfeitos com o resultado final, o qual devemos aos nossos colaboradores, registrando aqui a nossa eterna gratidão pelo envolvimento e vontade ímpar que tiveram em contribuir para a concretização deste trabalho, o que nos faz concluir que estas eram realmente as pessoas certas para a presente empreitada.

Apesar do volume de informações e da qualidade técnica do livro, evidenciados em cada um de seus capítulos, persiste uma lacuna de conhecimentos para a maioria das espécies de mamíferos da ordem Carnivora de nossa fauna. No Capítulo 2, vimos que a maioria das 26 espécies foi pouco estudada, sendo que, para algumas, não possuímos nenhum tipo de informação. O impacto desta falta de conhecimento para a conservação das espécies de animais em questão, não saberíamos precisar.

Quando lemos o Capítulo 3, que começa relatando a análise de risco de contaminação do planeta Marte por organismos terrestres, fica evidente a necessidade de informações exatas para análises de risco precisas. Mandaríamos para Marte uma espaçonave contaminada com organismos terrestres? A variante conservacionista deste tipo de análise é o PHVA (Análise de Viabilidade Populacional e de Habitat), que será mais exata, quanto mais informações precisas tivermos sobre a espécie em estudo e seu habitat. Cabe salientar que este tipo de análise tem também a finalidade de auxiliar nas estratégias de manejo a serem adotadas, de forma que possamos preservar a espécie e/ou habitat em estudo. Na carência de informações, corremos o risco de subestimar ou superestimar ameaças e, até mesmo, estabelecer estratégias que não estejam condizentes com a realidade. Isso pode ser particularmente negativo em três aspectos: 1) uso inadequado dos escassos recursos disponíveis; 2) agravamento dos riscos e ameaças e; 3) descrédito por parte da sociedade.

Sugere-se que ações de conservação e manejo levem em consideração a existência de diferenciações genéticas entre as regiões geográficas (ver Capítulo 4), sejam elas caracterizadas por uma Unidade de Manejo (UM) ou uma Unidade Evolutiva Significante (UES). Da mesma forma, a identificação e a delimitação destas Unidades requerem uma gama de conhecimentos que incluem desde o padrão de distribuição de uma dada espécie até a caracterização da variabilidade interindividual observada ao longo da distribuição (ver Capítulo 4). As coletas de amostras representativas para as espécies ou grupos de interesse, podem contribuir, sobremaneira, na definição destas Unidades (ver Capítulo 5). Assim, esforços integrados na obtenção de amostras podem acumular informações relevantes. Uma alternativa para esta integração é a formação de um banco de amostras biológicas, que assuma o papel integrador e ao mesmo tempo apresente regras claras para a disponibilização das amostras à comunidade científica (ver Capítulos 14 e 15).

A definição das UM ou UES também contribuiria para o estabelecimento de Unidades de Conservação (UC), cuja finalidade é preservar amostras intactas de ambientes naturais e, conseqüentemente, de biodiversidade. Considerando que apenas uma pequena parcela do território brasileiro é composto por área de proteção integral (ver Capítulo 6), é premente a obtenção de informação que subsidie, de forma realística, a implantação de novas áreas de conservação e, neste caso, levando em consideração a preservação da fauna de mamíferos da ordem Carnivora, haja vista que a conservação de carnívoros de maior porte demanda unidades de conservação com áreas maiores do que as que existem atualmente. Cabe salientar, que devemos avaliar a paisagem como um todo, caracterizando os fragmentos de ambientes existentes que possam, de maneira efetiva, preservar uma "metapopulação mínima viável" (ver Capítulo 7). Deve-se considerar que parâmetros ecológicos e de dinâmica populacional são fundamentais neste processo. Obviamente, como já mencionado, nos defrontamos com a escassez de informações, mesmo quando consideramos as espécies mais estudadas, como: a onça-pintada e o lobo-guará. Diante desta situação, podemos afirmar que, no momento, estamos incapazes de estabelecer um conjunto de unidades de conservação que preserve uma espécie de mamífero carnívoro. Um exemplo disso, é que informações elementares como a lista de espécies da fauna que ocorrem nas UCs brasileiras é, na sua maior parte, desconhecida. Lembramos, apenas, que tal informação é fundamental para a elaboração do plano de manejo de qualquer UC. Outro aspecto importante, é que o acúmulo de informações também permitiria avaliar a eficácia do atual sistema de criação de UCs na conservação de nossa biodiversidade.

De maneira auxiliar, a efetividade de áreas protegidas em manter populações viáveis de uma dada espécie pode também ser medida, numa primeira abordagem, se boas estimativas do tamanho e/ou densidade das populações de interesse e suas tendências forem obtidas (ver Capítulos 8 e 9). A escolha da melhor metodologia para determinação dos índices populacionais vai depender de fatores como: biologia da espécie e habitat em estudo. Uma síntese de aplicabilidade dos métodos para carnívoros neotropicais é apresentada no Capítulo 9. Infelizmente, não há informações sobre as tendências populacionais de mamíferos da ordem Carnivora de nossa fauna, sendo urgente o estabelecimento de programas de monitoramento de longo prazo.

Apesar da escassez de informações para a maioria das espécies de mamíferos da ordem Carnivora, algumas espécies têm sido mais estudadas, porém, ainda assim, são pouco conhecidas, tanto para o aspecto de sua biologia quanto para padrões populacionais ou principais ameaças. Na medida que a falta de conhecimento é vista como uma barreira para o planejamento de estratégias conservacionistas, não há impedimento para a utilização de ferramentas que possam garantir, pelo menos, a preservação de informação genética in vitro (ver Capítulos 14 e 15). No entanto, deve-se atentar para os cuidados necessários, a fim de evitarmos contaminação e/ou transmissão de agentes infecciosos por meio de amostras biológicas que compõem um banco genômico. A preservação ex situ de informação genética conta também com o auxílio de coleções zoológicas, seja de animais vivos ou mortos. No primeiro caso, há de se reconhecer o papel educativo que podem exercer, além da possibilidade de utilizarmos os animais, para fins científicos, obviamente respeitando-se premissas éticas. Para que possam cumprir, de maneira adequada, o papel de mantenedor de diversidade genética, as instituições zoológicas devem realizar cruzamentos ordenados, submetendo-se às recomendações de planos de manejo (ver Capítulo 16). Realmente, com a constante destruição e fragmentação de “habitats”, a conservacão ex situ parece ser um meio seguro contra a extinção. Mas é isso que queremos? Apenas observar animais em zoológicos, em museus ou em tubos criogênicos?

Mesmo diante do pouco conhecimento que detemos, é consenso que a destruição e fragmentação de “habitat” é a principal ameaça à conservação da fauna silvestre, trazendo, ainda, ameaças como: introdução de espécies exóticas, incluindo as domésticas, e doenças parasitárias (ver Capítulo 18), virais (ver Capítulo 19) e bacterianas (ver Capítulo 20). As doenças, de forma geral, representam riscos eminentes para a conservação dos mamíferos carnívoros das Américas, haja vista os relatos descritos para populações selvagens da África (ver Capítulo 21). Sem contar, ainda, com o potencial risco de transmissão de doenças para humanos. Caso alguma espécie selvagem seja suspeita de participação, como vetor ou hospedeiro intermediário, na transmissão de doenças ao seres humanos, podem ser caçadas indiscriminadamente, como recentemente ocorreu com os "civets" na China ou, mais remotamente, com as raposas, na Inglaterra. Em momentos atuais, é fundamental que se conheça o papel epidemiológico das espécies selvagens na transmissão de doenças, assim como o efeito das doenças sobre as populações selvagens. Porém, infelizmente, tais informações são praticamente desconhecidas para o grupo faunístico, aqui evidenciado.

Os carnívoros, há muito tempo, têm sido parte do ambiente e cultura folclórica ou religiosa. Estão em nosso imaginário como símbolos de beleza e força, considerados, muitas vezes, reis ou demônios. Quantos de nós já não ficamos hipnotizados pela grandeza destes animais? Quantos filmes ou desenhos já não destacaram o leão, o grande rei das selvas? Quantos livros de história já não destacaram a importância das onças nos rituais das civilizações pré-colombianas? Mas, e nos dias de hoje, como vemos estes animais?

Mais uma vez, podemos destacar a dualidade: reis ou demônios. Símbolos de força e beleza, ou a "praga", como costumeiramente ouvimos no Pantanal brasileiro. Aparentemente, a depredação de animais domésticos é uma experiência negativa para a maioria das pessoas que convivem mais de perto com estes animais (ver Capítulo 11). Mas inúmeros fatores podem influenciar a percepção da comunidade com relação aos carnívoros (ver Capítulo 11). Diante da histórica retaliação de proprietários rurais aos predadores, esforços têm sido concentrados na tentativa de elucidar os prejuízos econômicos causados por estes animais (ver Capítulos 10 e 13). Alternativas de manejo têm sido propostas para minimizar ataques de predadores (ver Capítulo 12), mas é fato que o conhecimento aprofundado da biologia da espécie predadora e o manejo de rebanho, adotados pelos proprietários rurais, são fundamentais para o estabelecimento de medidas efetivas de controle à predação.

O que se percebe é que, apesar dos esforços empreendidos, ainda apresentamos um grande déficit de conhecimento. Podemos encontrar centenas de artigos científicos sobre onças e lobos, mas concretamente o que sabemos acerca de tais animais? E os outros bichos? Diante disso, repetimos a pergunta, qual o impacto do déficit de conhecimento para a conservação das populações das espécies em questão? Será que teremos tempo para adquirirmos conhecimento "suficiente" para estabelecermos estratégias efetivas de conservação? Como direcionar nossas ações e escassos recursos? Como saberemos que estamos no caminho certo?

Talvez, levemos muito tempo para obtermos as respostas, das quais tanto necessitamos. Mas é da natureza humana não se conformar e nem se acomodar diante de questões não elucidadas. É essa característica inata, que nos faculta a condição de sermos autores das descobertas que modificam a vida em todos os seus aspectos, de forma positiva, em alguns momentos, e negativa, em outros, mas sempre nos conduzindo à realização de progressos.

Assim, é que nos vemos movidos pelo sentimento de que devemos dar continuidade ao que já foi começado (ver Capítulo 1), pois qualquer realização obtida, significará um passo que damos em direção aos objetivos que almejamos.

Marcus Luiz Barroso Barros
Presidente do Ibama

Parte I
Introdução

Capítulo 1
The history of carnivore research in Brazil

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Introduction

When I was asked to write a paper on the history of research on present terrestrial carnivores (Order Carnivora, Suborder Fissipedia) in the wild in Brazil, I tried to pass on the responsibility, mainly because I know many other people more qualified for this task, and more up to date on the literature, past and present. However, insistence was such that I reluctantly had to give in. Let the reader be advised, however, that I am more of a field researcher than an academic. Usually living away from big cities, the only times that I had regular access to good libraries was during my coursework at universities – and even then, I was not a compulsive user of libraries, as many good professionals I know. The only extensive literature searches I conducted, moreover, were those required for work on specific papers and reports, or connected to my thesis and dissertation. Even at this age of communication, in my present location (the São Francisco de Paula National Forest), I cannot rely on the advantages conferred by the use of the Internet, because of phone system limitations. In addition, readers should be aware is my tendency to concentrate on jaguar studies and other felids. Given my past and present work, mostly with cats, it is hard to overcome this trend. I will, however, do my best to include work on other carnivores.

With these restrictions in mind, I will try to somehow compensate with references to unpublished information issuing from the various projects I, students, and former trainees developed over the years. Although some of this information may have, by now, been formally published, or is in the process of being so, most is still in report, thesis and dissertation form, available only through university libraries or institutions. I believe, however, that collectively they add up to considerable information on the ecology and behavior of neotropical carnivores. I do hope that, in due time, they will contribute to the conservation of these species. In an attempt to provide a more complete picture of the current state of carnivore research in Brazil, I will also refer to studies conducted by other research groups that have come to attention. If it so happens that I have left out too many other studies, I would still be happy if this paper provokes other people to respond to it, disclosing more references.

It would be a long, and most certainly unfair, process to try to comment on all studies cited in the present paper. Moreover, it would make it excessively long for the purposes it serves. Therefore, I will limit myself to provide references, as complete as possible, to all (at least to the ones I know), in Appendix 1. I trust that a pressing interest and a little persistence from the part of interested parties, facilitated by modern communication means, will certainly lead them to access to the information available about the different species.

A historical personal perspective

To my knowledge, the so-called "modern" research on carnivores in Brazil started in the late seventies, with two studies that were conducted almost simultaneously. One was a study on the jaguar (*Panthera onca*) by Dr. George Schaller, in the Pantanal of Mato Grosso, and the other was a study on the maned wolf (*Chrysocyon brachyurus*), by James Dietz, in the Serra da Canastra National Park, in Minas Gerais state in southeastern Brazil (Dietz, 1984). Both studies used radio-telemetry, then at its early days, to monitor individual animals in the wild, and, thus, set the stage for a new era for modern field research on carnivores in Brazil. Given my direct involvement with the jaguar study, I will give a brief historical perspective of this project and of its different components. [Note: Recently, Sunquist (2002) published a very useful paper on the history of the research on jaguars throughout the species' range. Remember, however, that I have only included references to work conducted in Brazil.].

Prior to the above mentioned studies, most of the information on carnivores came from accounts of naturalists and early explorers, usually associated with museum collections for taxonomic studies. In the case of the jaguar, one of the only sources of published information on the jaguar in the wild was the book by Almeida (1976). As a trained guide for trophy hunters, Tony Almeida kept detailed records on measurements, sex, and stomach contents of animals shot by himself and his clients. He also provided some information on estimated home ranges of individual animals, based on tracks and other known signs from identified individuals. Working with a few ranch-owners in the Pantanal of Mato Grosso, he concentrated most of his efforts on animals that had become accustomed to preying on cattle, with benefits to all (human) parties involved. Using well-trained dogs, specialized on scenting and trailing cats, he was able to follow specific individuals, usually starting from a fresh kill, until the cat was treed and shot. During the pursuit, he recorded details of the behavior of the cat, including association with conspecifics, other kills found, and so forth.

In 1977, Dr. George Schaller started the first in-depth study of the jaguar in the wild, in the Acurizal ranch, in central-west Pantanal. The study was a joint program between the New York Zoological Society (today the Wildlife Conservation Society) and the Brazilian Institute for Forest Development-IBDF (today the Brazilian Institute for the Environment and Renewable Natural Resources – Ibama). The purpose of the study was to investigate the ecology of the jaguar as well as that of it's main prey, including the capybara (*Hydrochoerus hydrochaeris*), the marsh deer (*Blastocerus dichotomus*), and the caiman (*Caiman crocodilus yacare*). The Acurizal ranch was chosen as the study site under the agreement that the ranch would be purchased by IBDF and incorporated to the adjacent Caracará Biological Reserve, which would then be upgraded to a National Park. I first heard of the study from a friend that was working in Inpa, at the time, and I wrote to Dr. Schaller about the possibility of any kind of involvement with the project, even if as a helper. Following a very encouraging reply through correspondence from Dr. Schaller and a visit to the ranch in August 1977, I became the Brazilian counterpart of the project in January of 1978, hired by IBDF, with whom I work to this day (as Ibama).

After some 7 months of intensive trapping, we had only two animals fitted with transmitters, one adult female jaguar, captured in a neighboring ranch, and one male puma. To increase our efficiency in the capture of individuals for radio-collaring and expedite the telemetry component of the study, Schaller decided to take advantage of the expertise of an experienced hunter. After an ill-fated attempt with a local hunter, the project hired Tony Almeida and his associate Richard Mason to capture cats at the Acurizal ranch. After a total of about 21 days in two visits, in early 1978, we had recaptured and re-collared the male puma, whose previous collar had failed, and another female jaguar. Unfortunately, because of bad politics between the ranch owner and his manager, on one side, and the IBDF administration, in Brasilia, on the other, the purchase of the ranch didn't happen, and we were forced to leave the ranch, prematurely terminating the project there. After that discouraging episode, I remember Schaller mentioning more than once how difficult it was to conduct research in Brazil, not as much for the biological aspects of habitats and species, but because the political and administrative environment interfered directly in the field work. When I once asked him if he would write a book about his studies in Brazil (as he had done in all other projects he had conducted), he replied that he probably wouldn't, because it would be too sad. (Note: in 1995, this ranch was bought by The Nature Conservancy-TNC, under the management of the Fundação Ecotrópica, in Cuiabá, MT, as a private reserve; it is today a virtual extension to the adjacent Pantanal National Park). Between mid-1978 and early 1980, we switched our attention to studies on caiman and capybaras, in the region of Poconé, further north in the Pantanal.

In August 1980, after we had chosen and established a new study area for the jaguar project (see text below), at the Miranda ranch, Schaller left the Pantanal to start the project on the pandas (SCHALLER et al., 1985;

SCHALLER, 1993), appointing Howard Quigley to replace him in Brazil. Together, Howard and I worked for other 4 years at the Miranda ranch. The study in Miranda was concluded in early 1984 with a sample size of 7 jaguars captured a total of 12 times (another 5 pumas were captured 7 times). The results of this and of the previous studies in the project were published in various papers (SCHALLER; VASCONCELOS, 1978a,b; SCHALLER; CRAWSHAW, 1980, 1981, 1982; SCHALLER, 1983; SCHALLER et al., 1984; CRAWSHAW, 1979, 1981, 1987a,b, 1989, 1991, 1995; CRAWSHAW; QUIGLEY, 1983, 1989, 1991, 2002; QUIGLEY; CRAWSHAW, 1989, 1992, 2002).

After completing the required reports to IBDF on the Pantanal project, I left the country for graduate studies in the US, in 1985. I returned in early 1990, to begin a study on carnivores in Iguaçu National Park, as the subject of my doctoral dissertation. This Park was chosen because of its size (185,000 ha) and importance to conservation in southern Brazil, with occurrence of the full array of large mammals, including peccaries, tapir and top predators such as jaguar and puma. The critical condition of the Park as an island surrounded by agriculture on the Brazilian side, but retaining a connection with the last remaining large block of subtropical forest in the province of Missiones, in Argentina, made it an ideal study site. The original objective of the study was a comparison of the use of resources between a typical canid and a typical felid, using the crab-eating-fox (*Cerdocyon thous*) and the ocelot (*Leopardus pardalis*) as representatives of these families. As strange as it may seem, I had better success trapping jaguars than the foxes, and the study had to be changed into a comparison between the two different-sized felids (CRAWSHAW, 1995; CRAWSHAW et al., in press).

Although some students had visited the Pantanal project as trainees, it was in the Iguaçu project, with a wide exposure in the national press that training became an important component of the study. There was an almost continuous stream of requests from students and young professionals in the fields of Biology, Ecology, and Veterinary Medicine, to spend some time with the project. Many of these students excelled in their efforts and some continued to study carnivores as the subjects for graduate studies.

During the early 1990's, I was involved in two other studies funded by large companies in Brazil that resulted from different circumstances. One evolved from a survey of large felid populations to evaluate the impact of a large hydroelectric dam built on the Paraná river, between the states of Mato Grosso do Sul and São Paulo. Between 1992 and 1995, 6 jaguars and 3 pumas were captured, equipped with radio-collars, and monitored for a total of 27 months of information on home ranges and movement patterns (CRAWSHAW et al., 1995). In 1996, following changes at the top administrative levels of the company, the study was discontinued. After new political changes within the company, the project re-started in 1998, and continues to this day (SANA; CRAWSHAW, 2000; SANA et al., 2002). The other study mentioned started from a tragic incident, in which a child was killed by a puma in the residential area of a large mining company, in southern Pará state, in the Amazon. Following a meeting with the directors (local and national) of the company, it was decided that a study should be done, using radio-telemetry to monitor activity and movements of large felids captured in the vicinity of the residential and working areas. Another measure decided at the meeting was the construction of a 4.5 m-high wire-mesh fence around the village, with gates controlled 24 h that was built in a few months. Six jaguars and 2 pumas were captured and monitored between 1992 and 1995. Two of the jaguars (one was a black female) were translocated early in the study and their signals were lost subsequently, due to the lack of the aerial monitoring that had been agreed to by the company. Even though both of these studies produced important, new information on jaguar and puma, for various personal and professional reasons, the results have yet to be published.

In the different projects I've been involved with over the years, I have always tried to train as many students as possible in field techniques in the study of carnivores. As a result, many of them proceeded to conduct their own projects, usually also on carnivores. In taking these trainees, the most important consideration, to me, was that he/she was willing not only to endure the rigors and hardships of fieldwork, but to enjoy them. A few times I was criticized for training people whose background was not in Biology, Ecology, nor in Veterinary Medicine. Actually, I feel proud that some of these "un-appropriate" trainees found their niche in Conservation Biology, producing important contributions to the conservation of carnivores. One of my favorite examples is Jorge Schweizer, a Brazilian medical doctor of Swiss origin, who, through his acquaintance with the owners of the Miranda Ranch, was responsible for the establishment of the jaguar project in that ranch, in 1980. As a very inquisitive, intelligent, and methodical person, he was fascinated by the capabilities conferred by radio-telemetry to study the behavior of cryptic animals. Having been a former hunter of jaguars, and with a lot of outdoors experience, he offered to help with the monitoring of radio-collared animals, both in the Acurizal study, and later in Miranda, making available his personal Cessna airplane for aerial locations. During a period at the onset of the project in Miranda, while I was temporarily incapacitated by hepatitis, he stayed at the ranch and kept track of our study animals. In return for his invaluable help, he only asked us to teach him how to conduct field research on animal behavior. At that time (early 80's), he became interested in buying a small ranch in the Rio Negro area, in southern Pantanal, for the only reason that it had the last known group of giant-otters (*Pteronura brasiliensis*) in the region. The species had been virtually extinct from the Pantanal, first because of the high value of their skins in the pelt market, and later because of the alleged competition with fishermen. Because of their bold, noisy and diurnal social habits, the species was an easy target. Before leaving for China, Schaller donated some personal money to help Schweizer buy the land. From then on, Schweizer started a very dedicated effort not only to study that group of otters, but also visiting all the ranches along the river and talking to owners and ranch-hands about the importance of preserving that species. His involvement and enthusiasm was such that today the species has recovered, and is widespread, and not uncommonly seen, in the rivers of the region. As a result of his work, he produced a very informative book (SCHWEIZER, 1992).

One other example of well-worth investment in training was Sandra Cavalcanti, who as a recently graduated agronomist went to Iguaçu National Park in a field trip of her class, in November, 1990. After a talk I gave to the group of students about the project, then in its early stages, she came to me and asked if she could spend some time with the project, to learn about field techniques. As it turned out, she impressed me so much with her learning capacity and determination, that I invited her to work as a field assistant. Despite her completely different academic background, she soon became proficient in all aspects of the project, from trapping and sedation, to ground and aerial telemetry monitoring, and environmental talks at schools. She worked in the project between January 1991 and February 1992, when she received an irrefusable offer and moved to the US, subsequently enrolling at Utah State University for a MSc degree. Her thesis dealt with evaluation of non-lethal methods to decrease depredation of domestic sheep by coyotes in Utah (CAVALCANTI, 1997). She is now at the final stages of her PhD program at the same university, conducting a study that uses state-of-the-art GPS telemetry to investigate the impact of livestock depredation by jaguar in the Pantanal. With her now solid background both academic and in the field, I have no doubt she will continue to contribute significantly to the conservation of jaguar and other carnivores in her career.

I cited these examples to justify my personal position regarding academic training and involvement in Conservation, already expressed in earlier work (CRAWSHAW, 1992). Even though the ideal situation is clearly when a person can make use of formal academic knowledge and training to work in Conservation Biology, sometimes all that is required is one's commitment and diligent effort to effectively contribute to conservation of a particular population, species, or habitat. In this sense, there are many examples of former hunters, with no academic training, that became exemplary game wardens, throughout the world.

Summary statistics on research on carnivores in Brazil

As a summary of the accumulated research effort on carnivores in Brazil for the last two decades, I divided all the references cited in Appendix 1 according to species. I included in my survey unpublished reports that contain actual field data and approved project proposals submitted to universities as part of graduate studies, as I believe most of them will be followed to term (and hopefully to publication) and that they reflect on the total research effort on carnivores. Although I also included a few references to papers on genetics and veterinary aspects of carnivores, I did not make a specific effort to search for them as it would require access to specific journals with which I am not familiar. It should be noted, also, that the total number is somewhat inflated, since I included theses, dissertations, and reports that were later also published in journals. However, most of those are usually more complete than the published version of the data, so I believe the references to them may still be helpful. Since most universities now have a system where theses and dissertations are made available through the Internet in PDF format, they are also easily accessible.

I was able to find references to 175 papers dealing with carnivores in Brazil (Appendix 1). Of these, 111 (63.4%) were results of studies on single species, 21 (12.0%) were on two-species' associations, and 43 (24.5%) were at the order/family level (Figure 1). Of the single-species studies, three species together comprised 68.2% of the total: the maned wolf, with 28 papers (25.2%), the river otter, with 25 (22.5%), and the jaguar, with 23 (20.7%, Table 1). When separated by family (N= 148), however, the great majority of the studies were on felids, (79, 53.4%), followed by canids (38, 25.7%) and mustelids (31, 20.9%). Even though for most of the carnivore species, I could find references to less than 5 papers (Table 1), there are others for which I could not find any references at all. These were the three procyonids, the coati (*Nasua* sp.), the crab-eating raccoon (P*rocyon cancrivorus*), and the kinkajou (*Potos flavus*), five mustelids, the tayra (*Eira barbara*), the two species of grison (*Galictis vittata* and *G. cuja*), and the two species of hog-nosed skunks (*Conepatus chinga* and *C. semistriatus*).

For those for which I could identify the geographic region (97), there was a marked difference towards studies conducted in the southeast (35.0%), south (29.0%), and central-west (23.7%) regions, as opposed to those in the north (7.2%) and northeastern (5.1%) regions. This difference is certainly caused by the concentration of universities outside the Amazon region, with the many graduate (and under-graduate) studies associated to them.

Considering only the most common subjects of the studies, those on diet were, by far, the most numerous, with 38.3%, followed by ecology (25.3%), conservation (21.8%), and on livestock depredation (14.3%).

Conclusions/recommendations

One aspect to be praised, from my perspective, is the usually applied nature of the studies (even if short-term) in Brazil, emphasizing conservation needs for the different species of carnivores. In a developing country, where conservation is more frequently relegated to lower priority, this is a very important characteristic. And given the usually controversial aspects of conserving carnivores, due to potential, perceived, and actual conflicts with humans, the issue is even more critical. On the other hand, one obvious reality is the need to put pressure for the actual conclusion of studies, with the publication of results. One disturbing aspect of the references is that only 37 (21%) were published in international refereed journals. Maybe even more disturbing is the fact that only 6 (3.5%) were published in Brazilian journals. Only through making the information available through publication and dissemination of results, both at the scientific and popular level, can it be ultimately used for the benefit of conservation. As well put by Schaller (1993), "telling and re-telling is imperative". We, as scientists, "must at least record our experiences with the hope that our writings will encourage action to preserve species". It is logical to expect that professionals prefer to publish their papers in well-known international journals, which reflects better in their CVs and usually imply greater dissemination of the information. However, one handicap for Brazilians is the difficulty in writing good papers in English, the world language, in order to be accepted in well-established technical journals. Almost invariably, good papers written by Brazilians that get accepted in the better international journals are restricted to those by professionals that had the opportunity to study abroad and are proficient in English. The language barrier ends up discouraging many researchers, being the main reason for much unpublished information. From another perspective, however, maybe we Brazilians should not worry so much with publishing in English and in the best journals. The fact that there are now more options of good refereed journals available in Brazil and in Latin America should play an important role in this aspect. The recent example set by the publication of the WCS/ Universidad Autonóma de México jaguar volume (MEDELLIN et al., 2002) in Spanish can hopefully be seen as a trend in this direction.

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I also thank Cenap and the Associação Pró-Carnívoros for the opportunity to participate in the workshop that originated this volume. I feel an inner satisfaction and am proud to have been involved in the birth and development of these two organizations. I am sure the results of this workshop and associated book will have profound and lasting effects on the future of carnivores in Brazil. Thank you, Ronaldo, for the patience in waiting long past the deadline for this paper. And, mostly, I sincerely thank and praise the many former students and trainees for their continued interest and commitment to the cause of carnivore conservation. For the sake of those species, I wish them all extremely successful careers! I also thank everyone who sent references to be included in this paper. On the other hand, I apologize sincerely to the many people whose works were not included. Scott M. Lindbergh contributed with many helpful comments on an early version of the manuscript.

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Appendix 1[2]

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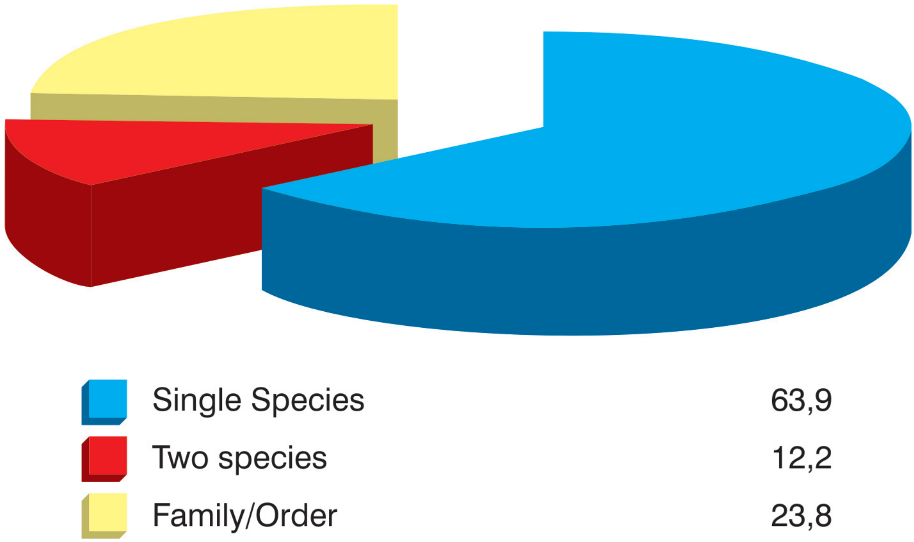
Table 1 – Summary statistics on the references to carnivore studies in Brazil (N = 175).

STUDIES ON SINGLE SPECIES	N	%
Maned wolf (*Chrysocyon brachyurus*)	28	24.5
River otter (*Lontra longicaudis*)	25	22.7
Jaguar (*Panthera onca*)	23	20.9
Puma (*Puma concolor*)	10	9.1
Crab-eating fox (*Dusicyon thous*)	6	5.4
Giant otter (*Pteronura brasiliensis*)	5	4.5
Ocelot (*Leopardus pardalis*)	3	2.7
Jaguarundi (*Herpailurus yagouaroundi*)	3	2.7
Margay (*Leopardus wiedii*)	2	1.8
Bush-dog (*Speothos venaticus*)	2	1.8
Little-spotted-cat (*Leopardus tigrinus*)	2	1.8
Hoary fox (*Dusicyon vetulus*)	1	0.9
Pampas cat (*Lynchailurus colocolo*)	1	0.9
TOTAL	**111**	**100.0**
STUDIES ON TWO-SPECIES ASSOCIATIONS		
Jaguar/Puma	17	81.0
Pampas fox (*Dusicyon gymnocercus*)/Crab-eating-fox	1	4.7
Short-eared-dog (*Atelocynus microtis*)/Bush-dog	1	4.7
River otter/Giant otter	1	4.7
Ocelot/Jaguar	1	4.7
TOTAL	**21**	**100.0**
STUDIES ON FAMILY/ORDER		
Carnivores	22	51.2
Felids	16	39.0
Canids	4	7.3
Small cats	1	2.4
TOTAL	**43**	**99.9**

Figure 1 – References on carnivore studies

(N = 175) grouped by subjects (in %). Single species studies = 111; studies on two species = 21; studies on the family or order = 43.


Single Species
63,9
Two species
12,2
Family/Order
23,8

Capítulo 2
Research in terrestrial carnivora from Brazil: current knowledge and priorities for the new millennium

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Introduction

The first records on the natural history of the Carnivora species found in Brazil came from the early explorers of the late 18th - mid-19th century, such as Rengger (1830), Wallace (1853), and von Hunbolt (1852), to name a few. However, this early anecdotal information provided by them and supplemented by others remained the sole source of knowledge up to the 1970's, when field research began in Brazil and elsewhere (e.g., SCHALLER, 1978; BRADY, 1979; SCHALLER; CRAWSHAW, 1980). The early information was largely on descriptive morphological characters and taxonomic status (e.g., WIED, 1826; THOMAS, 1903; POCOCK, 1917, 1941; CABRERA, 1957). The next step came with some data on status, distribution, and some natural history, at the country level, with works such as those of Osgood (1914) for Peru, Goodwin (1946) for Costa Rica, Leopold (1959) for Mexico, and Husson (1978) for Surinam. A remarkable and cornerstone work for South American species came with the compilation of the anecdotal information provided by Cabrera e Yeppes (1940 re-edited in 1960) in their natural history piece "Mamíferos sudamericanos: vida, costumbres y descripción." This work remained for years to come as "the" source of information for mammals, and carnivores of South America. In the 1970's an update of information could be found in encyclopedic works such as those of Grzimek (1975) and de la Fuente (1979). Updates on canids and felids worldwide that included Neotropical species also appeared in the 1970's (LANGGUTH, 1975; GUGGISBERG, 1975). Nevertheless, non anecdotal information on their ecology and conservation issues becomes largely available mostly only from the mid 1980's (e.g., SCHALLER; CRAWSHAW, 1980; THORNBACK; JENKINS, 1982; MELQUIST, 1984; BISBAL, 1986; RABINOWITZ;NOTTINGHAM, 1986; YAÑEZ et al., 1986; BROAD, 1987; LUDLOW; SUNQUIST, 1987; KONECNY, 1989). Unfortunately, for some species part of the knowledge on some aspects of their natural history still remains pretty much the same as that of the time of Cabrera e Yeppes work. In the upcoming pages I'll present the preliminary results of the state of knowledge, or lack thereof, of the 26 terrestrial carnivore species found in Brazil, and will try to come up with an assessment on research priorities important for their conservation.

Methods

I analyzed the compiled literature published on topics related to their ecology and natural history, including the gray. To give an idea on what has been going on in Carnivore research in Brazil, I evaluated the abstract books of the Brazilian Congress of Zoology from 1994 to 2002, and that of the First Brazilian Congress of Mammalogy (2001), as well as the main journals on the subject. I complemented this with graduate and undergraduate thesis and ongoing research I'm aware of on carnivore ecology. The same paper could have several entries for the same species, as well as for several species, depending on the subject/species dealt with.

I categorized research into four main categories related to ecology and conservation, each subdivided into three parts (sometimes four), according to their sample size. I also evaluated available information on life history, behavior, and biometry. For food habits I considered: anecdotal, small ($n < 10$ samples), medium ($n = 10$-30), large ($n > 30$); radio-telemetry (home range, activity, habitat use): anecdotal, small (< 5 radio-collared animals), good (> 5 radio-tagged individuals); distribution/records: incidental observations, some records, solid work on the subject; conservation: incidental comments, some emphasis, main focus. Life history: anecdotal, some, fair amount of information; behavior/social organization: anecdotal, small, good; biometry: small ($n < 10$), medium ($n = 11$-20), large ($n > 20$). Each part received a point from 1 to 3 for every research conducted on the matter, in order to evaluate priorities and level of knowledge for each species.

Results and discussion

Overview

I found a total of at least 319 entries (several multiple) regarding the ecology and conservation of Neotropical carnivore species found in Brazil. The Felidae has been the most studied group (52.4% of all entries), followed by Canidae (23.5%), Mustelidae (15%), and Procyonidae (9.1%). The family trend seems to be related to: 1) flagship species, 2) easiness of data collection (namely scats), which is also obviously related at the species level too. However, Kruskal-Wallis Analysis of Variance on Ranks marginally failed to show differences between groups ($H = 13.832$, d.f $= 7$, $P = 0.054$).

The vast majority of research conducted so far was on food habits (57.4%). This certainly relates to its low cost and usually a higher guarantee of a good data set compared to some other subjects, especially telemetry. However, a good amount of it consists of very limited data and/or incidental observations. Studies on home range-habitat, use-activity and on distribution and conservation were about on the same level (12-16%), although a more thorough search might boost up the latter two representations. One interesting aspect is that a good part of the studies presented at scientific meetings do not end up being published at all, other than on abstracts of proceedings.

At the species level, jaguar had, by far, the vast majority of records (19.7%), followed by puma (10.3%), crab-eating fox (7.5%), ocelot (6.6%) and maned wolf (6.3%). This unprecedented leadership of the jaguar was catapulted by the workshop held in Mexico with the subsequent publication of a book with 38 paper-chapters. This book alone represented 60.3% of the available knowledge on jaguar ecology-conservation. On the other extreme, the rare Amazon weasel had only one entry, and is indeed the least known species in the Neotropics. There was not much variation on species percentage total participation by number or weight of entries. Except for crab-eating fox, all other best studied species are or were present in endangered species lists and are also flagship or "cute" species. The vast majority of the most common species actually lack even the most basic information.

The species

- *Pseudalopex gynocercus*: although very common, little is known and mostly related to its diet. It needs all sorts of information, especially the extent of its distribution in Brazil.

- *Pseudalopex vetulus*: the species is currently under a thorough study covering many aspects of its natural history. Very few, but good studies on diet. It needs information on conservation issues, distribution and area requirements.

- *Cerdocyon thous*: diet well known, but mostly restricted to the Atlantic forest region.

- *Atelocynus microtis*: except for an ongoing study in the Peruvian Amazon, virtually unknown.

- *Speothos venaticus*: has been the subject of several captive studies but only two recent and broader works on ecology and conservation issues (including the first light into scat analysis). This canid needs basically all sort of information possible.

- *Chrysocyon brachyurus*: its diet has been well studied, and there is also some information on land tenure system, but needs considerable information especially in respect to conservation issues.

- *Procyon cancrivorus*: very few studies, basically restricted to its diet. It needs some more food habit studies and on all other subjects.

- *Nasua nasua*: some data on diet but none formally published yet. It needs all kinds of information.

- *Potos flavus*: has been the subject of few but interesting studies on land tenure system/social organization, but the diet studies are limited. Its distribution in Brazil would also prove interesting to be conducted.

- *Bassaricyon gabbi/beddardi*: only recently formally recorded in Brazil. It needs to verify its status and extent of distribution in Brazil, among all other things.
- *Mustela africana*: a complete unknown. It needs all sorts of basic information.
- *Galictis vittata*: only one limited study on diet and one on telemetry. Another virtually unknown. Its distribution pattern is badly needed to be understood alongside *G. cuja*.
- *Galictis cuja*: only very limited data on diet is available. It needs every kind of information, especially on its distribution pattern.
- *Eira barbara*: only one good study on diet and very few radiotracked individuals, even though the species is relatively common. Still lacks the most basic information.
- *Conepatus semistriatus*: very scanty information on diet and home range. It needs basically all kinds of information, even though it is quite common in several places.
- *Conepatus chinga*: the same as above.
- *Lontra longicaudis*: some robust studies on food habits, and isolated records, but still need a lot of research efforts, especially in the conservation front.
- *Pteronura brasiliensis*: has been the subject of few but good studies, but still lacks a lot of the basic information. Of special interest should be its status and current distribution, and other conservation issues.
- *Leopardus pardalis*: this species had been the subject of few but strong studies using telemetry, and a good amount of diet studies. However there is still a lot of information needed.
- *Leopardus wiedii*: basically, few isolated individuals have been radiotracked that provided very limited information; diet studies with good sample sizes were also very limited. It needs pretty much all sort of basic information.
- *Leopardus tigrinus*: the same as with the above species, with a special emphasis on its distribution within the Amazon basin.
- *Leopardus geoffroyi*: one robust radiotelemetry and diet study, all else is needed.
- *Leopardus colocolo*: available information is basically restricted to distribution. Very limited data on diet, as with the above small felids, needs everything.
- *Herpailurus yagouaroundi*: basically, almost on the same level as *L. wiedii* and *L. tigrinus*.
- *Puma concolor*: although well studied in North America, and even being the focus of some studies, especially onits diet, most still needs to be understood, especially its conservation needs.

● *Panthera onca*: even being the subject of most published information of carnivores found in Brazil, there is still very little information available on the jaguar, even on food habits, in certain areas (e.g., Cerrado and Amazon).

Priority should be given to the lesser known species that are also in any of the threat categories, or were considered as data deficient. Nevertheless, data deficient per se are basically all of the 26 species. If we consider only research conducted in Brazil, the vast majority were in the southeast especially in the Atlantic Forest biome. The Cerrado carnivores have also been the subject of some research, but the Caatinga and Amazon regions are almost a blank for carnivore research of any sort.

Regardless how many studies have been conducted, and the considerable progress in the last decade, very little is known on the ecology, conservation and natural history of all Neotropical species found in Brazil. Very few robust and thorough efforts have been made on very few species, even on diet. This means that efforts will be needed at all levels: species, community wide, distribution, conservation needs, area and habitat requirements, diseases, diet, social organization, and even basic biometry.

Parte II
Genética e sistemática de carnívoros

Capítulo 3
Definindo unidades evolutivamente significativas e unidades de manejo para a conservação de carnívoros neotropicais

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Introdução

A utilização de técnicas moleculares para o estudo de populações naturais tem se expandido de forma dramática nas últimas duas décadas, acompanhada também de uma aceleração vertiginosa no desenvolvimento de métodos laboratoriais e analíticos, empregados nessas abordagens. A aplicação desses métodos tornou-se parte integrante da biologia da conservação, atuando em conjunto com outras disciplinas de diversas maneiras, com o fim de elaborar estratégias adequadas e realistas para a preservação da biodiversidade. Novos campos científicos surgiram desses desenvolvimentos, como a Genética da Conservação e a Ecologia Molecular (SCHONEWALD-COX et al., 1983; AVISE; HAMRICK, 1996; SMITH; WAYNE, 1996; HOELZEL, 1998; FRANKHAM et al., 2002; DESALLE; AMATO, 2004; EIZIRIK [1996] para revisão de suas definições e ênfases), os quais hoje contam com vasta literatura e periódicos específicos (p.ex. *Molecular Ecology e Conservation Genetics*).

Um dos objetivos da Genética da Conservação é a definição de unidades demográficas (espécies e unidades infra-específicas) que se apresentem evolutivamente diferenciadas de outras unidades equivalentes, a ponto de apresentarem mérito próprio de conservação como uma entidade independente (ou semi-independente, ver a seguir). Diversos métodos moleculares têm sido desenvolvidos para este fim, e muitas vezes utilizados em conjunto com análises morfológicas, fisiológicas e/ou ecológicas para caracterizar a diferenciação evolutiva entre populações (RYDER, 1986; AVISE, 1994, 2000; MORITZ, 1994; AVISE; HAMRICK, 1996; EIZIRIK, 1996; CRANDALL et al., 2000; FRANKHAM et al., 2002; SCHLÖTTERER, 2004).

A definição destas unidades tem grande impacto sobre a elaboração de estratégias de conservação e manejo de espécies ameaçadas, pois influencia levantamentos de biodiversidade e endemismo, auxilia na identificação e priorização de áreas a serem protegidas, e também direciona ações em campo (p.ex. solturas e reintroduções) e cativeiro (p.ex., formação de plantéis reprodutivos, e pareamentos entre animais de diferentes regiões).

Neste capítulo discutiremos alguns dos conceitos envolvidos nesta definição (p.ex., espécie, subespécie, unidade evolutivamente significativa e unidade de manejo), algumas metodologias moleculares e analíticas disponíveis para este fim, a aplicação destas abordagens em estratégias conservacionistas, e o estado atual deste campo no que diz respeito aos carnívoros neotropicais.

A definição de espécies

A diversidade biológica está organizada em níveis hierárquicos de complexidade e inclusão, desde componentes moleculares de uma organela celular até comunidades ecológicas, ecossistemas e biomas. Existe também um componente temporal que relaciona de diversas maneiras cada um desses níveis, influenciando de forma dinâmica suas interações em escalas de tempo ecológicas e evolutivas.

Descrições evolutivas/sistemáticas da biodiversidade são usualmente subdivididas em níveis taxonômicos hierárquicos (p.ex. classes, ordens, famílias, etc.), que procuram refletir as relações filogenéticas entre grupos de organismos isolados em diferentes profundidades ao longo do tempo evolutivo. No contexto da Biologia da Conservação, considera-se como meta mínima identificar as espécies componentes de cada ecossistema ou bioma, e conservá-las em conjunto com os outros membros de suas comunidades, procurando manter a continuidade e sustentabilidade dos processos ecológicos naturais. Assim sendo, a identificação, delimitação e caracterização das espécies componentes de um grupo de organismos (um gênero ou família) seria um objetivo básico para a conservação de cada uma delas. Isto implica que espécies constituem "unidades" biológicas, cada uma caracterizada por diferenciação genética, ecológica, morfológica, fisiológica e/ou comportamental de outras espécies próximas, e apresentando também algum nível de isolamento reprodutivo em relação a estas (MAYR, 1942, 1976).

Na realidade, em muitos casos, a identificação conclusiva dos grupos de organismos que realmente constituem espécies independentes mostra-se complexa e difusa, pois existem diversos níveis de separação temporal e espacial entre espécies próximas, levando à ampla gama de combinações no que diz respeito a sua diferenciação nos itens mencionados anteriormente. Em muitos casos, é extremamente difícil avaliar a ocorrência de isolamento reprodutivo na natureza, por exemplo, quando as populações em questão encontram-se separadas geograficamente (por processos naturais ou derivados de fragmentação antropogênica). Em outros casos, este isolamento pode ser inferido (p.ex., a partir de análises estatísticas de características morfológicas consideradas como tendo base predominantemente genética), às vezes, parecendo incompleto, com zonas de contato (e possivelmente hibridação) intermediárias. Há ainda exemplos de espécies que parecem produzir híbridos viáveis e férteis em cativeiro, o que não reflete necessariamente algo que realmente ocorra na natureza, devido a nuances ecológicas e comportamentais.

Em grande parte dos casos, o conhecimento disponível é tão escasso que análises básicas de morfologia e ecologia (hábitos alimentares, habitats utilizados, e mesmo distribuição geográfica precisa) são ainda ausentes ou precárias. Nesses organismos, mesmo a definição de que grupos constituem espécies e onde exatamente elas ocorrem é incompleta ou pobre, e freqüentemente baseada em descrições superficiais com base em poucas características e indivíduos analisados. Se por um lado, esta carência de conhecimentos se aplica a grande parte dos carnívoros do planeta, a maioria destas espécies encontram-se descritas e delimitadas de forma relativamente estável, tendo sido alvo de alguns estudos morfológicos com este objetivo. Há, entretanto, exceções neste sentido, ou seja, grupos de carnívoros em que a definição e a delimitação de espécies permanecem imprecisas ou controvertidas (ver a seguir exemplos da região Neotropical).

Unidades infra-específicas

Abaixo do nível de espécie, a definição de unidades demográficas distintas pode ser ainda mais complexa. Historicamente, utilizou-se o conceito de subespécie, também com trinomial latino, com o objetivo de definir formas ou raças geográficas que se apresentem morfologicamente diferenciadas entre si (PAPAVERO, 1994; MAYR, 1976; O'BRIEN; MAYR, 1991). Em sua definição original, esse conceito refletia uma perspectiva tipológica da diferenciação entre organismos, ou seja, baseava-se nas diferenças observadas entre 'tipos' coletados em locais diferentes (muitas vezes limitando-se a números amostrais pequenos), sem levar em consideração aspectos como variação individual intra-populacional, gradientes ecológicos/geográficos de diferenciação, ou níveis variáveis de conectividade espaço-temporal entre populações. Com o desenvolvimento de campos como a genética de populações e a ecologia de populações, o conceito de subespécie tendeu a ser substituído ou modificado para incluir perspectivas mais dinâmicas e flexíveis que caracterizam, de forma mais realista, a diferenciação entre unidades demográficas naturais. Um campo novo, a filogeografia, se desenvolveu a partir dos anos 1980 e 1990, integrando esforços e metodologias cujo objetivo é caracterizar a estrutura genética e o espaço-temporal de populações naturais, usualmente empregando marcadores moleculares (AVISE et al., 1987; AVISE, 1994, 2000). Estudos realizados com estas metodologias, assim como análises estatísticas de caracteres morfológicos utilizando números amostrais mais expressivos, evidenciaram muitas vezes que subespécies clássicas propostas historicamente não correspondiam a unidades evolutivas reais, apresentando diferenciação genética significativa entre si (XIMENEZ, 1974; LARSON, 1997; EIZIRIK et al., 1998, 2001).

Se, por um lado, a complexa dinâmica espaço-temporal das populações naturais contraria o uso de unidades tipológicas e/ou estanques, por outro, a existência real de estruturação geográfica e genética em termos intra-específico (ou seja, diferenciação entre grupos populacionais locais ou regionais) tem sido amplamente demonstrada por estudos morfológicos, genéticos e ecológicos (AVISE, 1994; 2000; AVISE; HAMRICK, 1996; FRANKHAM et al., 2002). Assim sendo, permanece importante a definição de unidades infra-específicas, que reflitam a existência real de grupos demográficos diferenciados geneticamente. Além do interesse acadêmico, a identificação e a caracterização evolutiva e ecológica desses grupos tornam-se atualmente muito relevantes em termos práticos, no contexto da Biologia da Conservação, conforme mostrado a seguir. Muitos autores seguem utilizando o conceito de subespécie, porém incorporando aspectos mais realistas de dinâmica populacional histórica e filogeografia (O'BRIEN; MAYR, 1991; MITHTHAPALA et al., 1996; UPHYRKINA et al., 2001), enquanto outros têm utilizado conceitos similares desenvolvidos mais recentemente. Entre estes se destaca o conceito de Unidades Evolutivamente Significativas – UES -, similar em profundidade evolutiva à definição de subespécies, porém proposto no contexto de uma caracterização mais moderna da estruturação histórica das populações naturais (RYDER, 1986; MORITZ, 1994; EIZIRIK, 1996; CRANDALL et al., 2000; FRASER; BERNATCHEZ, 2001). Esse conceito tem por objetivo definir unidades demográficas infra-específicas que se apresentem diferenciadas geneticamente (implicando isolamento histórico) de outras unidades semelhantes contidas na mesma espécie (Figura 1). Assim como no caso das subespécies, diferentes UES ocupam áreas geográficas distintas. Ao apresentarem diferenciação filogeográfica significativa entre si, as UES representariam uma porção muito importante da diversidade genética contida em cada espécie, tendo o potencial de seguirem no processo de divergência até a formação de novas espécies.

Um conceito associado ao de UES é o de Unidades de Manejo - UM (ver Figura 1), proposto como sendo um subgrupo dentro de uma UES que se apresente como uma população local (ou conjunto de populações próximas) separada demograficamente de outros grupos semelhantes, em uma escala de tempo ecológica (MORITZ, 1994; EIZIRIK, 1996). As UMs não apresentariam diferenciação genética tão profunda entre si quanto as UES distintas, por terem uma origem histórica mais recente e/ou por manterem algum nível de conexão por fluxo gênico. Entretanto, em uma escala de tempo curta (uma ou poucas gerações), o contato entre estes grupos por migração ou re-colonização seria escasso, tornando-os entidades ecológicas relativamente independentes.

Relevância da definição de unidades infra-específicas para a conservação

O objetivo das estratégias para a conservação biológica não deve ser apenas preservar as entidades (p.ex., espécies) presentes atualmente em um ecossistema, mas também a continuidade dos processos ecológicos e evolutivos que ali atuaram e seguem atuando. Estes processos incluem flutuações no tamanho populacional e nas áreas de distribuição, adaptação contínua aos ambientes e aos outros membros da comunidade (competidores, presas, patógenos), e variação, ao longo do tempo, na estruturação geográfica (padrões de subdivisão demográfica e genética) de cada espécie. Como visto acima, populações localizadas em diferentes regiões geográficas podem tornar-se geneticamente diferenciadas, devido a fatores como (i) barreiras ao fluxo gênico (rios, montanhas, habitats inadequados); (ii) isolamento por distância (maior probabilidade de cruzamento entre indivíduos de regiões próximas, devido a limites naturais na capacidade de dispersão da espécie); e (iii) adaptação a ambientes locais (por ação da seleção natural, no caso de diferenças ecológicas, mesmo que sutis, entre as áreas).

Levantamentos de biodiversidade usualmente avaliam a riqueza de espécies ou endemismo destas em uma determinada região, comparando-a com outras, mas não necessariamente consideram a riqueza evolutiva existente em níveis infra-específicos. Populações geneticamente diferenciadas (p.ex., UES) representam uma parcela muito importante da biodiversidade, e necessitam de esforços de pesquisa e medidas de conservação apropriados. Compreender os padrões de estruturação geográfica e diferenciação histórica entre populações é fundamental para que se conserve os processos evolutivos que os formaram, e se permita o surgimento de novos processos no futuro.

No contexto de ações de conservação e manejo, ignorar a existência de diferenciação genética entre regiões geográficas pode (i) obliterar/obscurecer/alterar processos evolutivos que têm se desenvolvido por milhares ou milhões de anos; (ii) levar à perda de componentes importantes da biodiversidade; e (iii) causar “depressão por exocruzamento” quando são miscigenadas (p.ex., em cativeiro ou por solturas de fauna) populações que estejam adaptadas a ambientes distintos (FRANKHAM, 2002). A extinção de uma das UES de uma espécie representaria uma significativa perda de diversidade genética e evolutiva, tendo em vista que este grupo de populações geográficas não estaria satisfatoriamente representado por outras unidades demográficas remanescentes da mesma espécie. Assim sendo, é necessário implementar unidades de conservação (áreas protegidas) representando de forma viável cada uma das UES de cada espécie. Da mesma forma, planos de manejo em cativeiro devem levar em conta as principais subdivisões demográficas de cada espécie (UES, particularmente), procurando manter os níveis de conectividade entre elas similares aos estimados para as populações naturais. Em alguns casos isto implicará um fluxo gênico inexistente ou muito baixo entre diferentes UES, ou seja um manejo em cativeiro que considere estes grupos como entidades completamente distintas.

Enquanto a extinção de uma UES representa uma perda importante para a biodiversidade, o mesmo não se aplica a uma UM. Esses grupos não se encontram tão diferenciados geneticamente entre si (Figura 1), e, portanto, pode-se dizer que cada um deles tem um potencial razoável de representar os demais em termos genéticos. Por outro lado, a baixa conectividade demográfica entre eles significa que ameaças antropogênicas (particularmente no caso de um aumento de mortalidade) a um destes grupos não seriam compensadas de forma significativa, em curto prazo, por migração vinda dos grupos adjacentes. A extinção de um destes grupos só seria revertida por re-colonização natural após um tempo considerável (possivelmente décadas ou séculos), ainda assim podendo implicar significativa perda de diversidade genética durante o processo de re-colonização (FRANKHAM et al., 2002). A situação atual de fragmentação de habitats naturais exacerba este fenômeno, tornando virtualmente impossível, em muitos casos, a re-colonização natural de áreas onde carnívoros foram extintos. Neste contexto, cada população natural isolada poderia ser vista como uma UM. No que diz respeito a ações de manejo, a baixa diferenciação genética entre estes grupos tem implicações positivas: (i) estratégias visando a sua conservação em campo e propagação em cativeiro podem ser coordenadas ou integradas; (ii) áreas atualmente isoladas podem ser re-conectadas demograficamente através de corredores de habitat mantido ou restaurado, translocações ou técnicas de reprodução assistida; (iii) em casos de extinções locais, estas áreas podem ser re-colonizadas por ação humana, através de reintrodução a partir de UMs próximas (a escolha da UM-fonte pode ser definida por critérios genético-evolutivos).

Requerimentos para a definição de unidades operacionais para a conservação

Por unidades operacionais entenda-se UES e UMs, ou definições equivalentes. Para que se possa identificar e delimitar estas unidades, de forma que reflita adequadamente os padrões e processos históricos, há a necessidade de diversos tipos de informação. Inicialmente, é importante que se tenha uma delimitação suficientemente sólida da espécie em termos evolutivos, em relação a outras próximas (ver discussão anterior). É também importante delimitar e caracterizar a distribuição geográfica da espécie (histórica e atual), buscando o maior detalhamento possível em relação a habitats (ou biomas) onde ocorre, e possíveis descontinuidades que possam representar barreiras à movimentação de indivíduos. A seguir deve-se caracterizar a variabilidade inter-individual observada ao longo da distribuição, a qual deve incluir o maior número possível de aspectos (genético-moleculares, morfológicos, fisiológicos, ecológicos, comportamentais). Procura-se caracterizar a distribuição geográfica dessa variabilidade, avaliando que componentes variam primariamente dentro *versus* entre populações ou regiões, e identificando possíveis descontinuidades espaciais. É possível que padrões diferentes sejam inferidos com aspectos distintos (p.ex., análises moleculares *versus* morfológicas), o que pode refletir influências contrastantes de uma ou mais forças evolutivas (p.ex. mutação, deriva, seleção natural) sobre esses caracteres. Nesses casos é importante interpretar os dados da forma mais abrangente possível, buscando uma compreensão geral da história evolutiva da espécie.

A partir dos dados obtidos, pode-se realizar diversas análises que visam a inferir sobre a história demográfica e estruturação genética das populações investigadas. No momento, as análises históricas mais aprofundadas estão restritas, em grande parte, a dados gerados com marcadores moleculares (descritos a seguir), tendo em vista seu maior poder de resolução e facilidade inferencial. Isto se deve ao fato de que é possível caracterizar de forma bastante detalhada as propriedades evolutivas destes marcadores, e criar modelos que relacionam os seus padrões atuais de variabilidade a processos evolutivos passados ou recorrentes. Isso permite que diferentes cenários de subdivisão histórica sejam avaliados e comparados de forma quantitativa com outros processos, como efeitos de flutuações no tamanho populacional ou seleção natural atuando sobre partes do genoma.

Aplicando-se diferentes abordagens, pode-se obter uma perspectiva geral dos padrões de subdivisão espaço-temporal de cada espécie, e associá-los à história de biomas e faunas regionais. Freqüentemente padrões de subdivisão coincidentes são observados em várias espécies com distribuições similares (AVISE, 2000), o que pode facilitar o delineamento de estratégias de conservação regionais. De acordo com o padrão de subdivisão observado, incluindo a profundidade da diferenciação genética entre regiões, pode-se propor unidades como UES ou UMs que correspondam a segmentos populacionais geográficos. Estas devem servir de base para o delineamento de estratégias de conservação e manejo que procurem refletir e preservar os processos evolutivos em andamento.

Mesmo que uma caracterização filogeográfica detalhada (como descrito na seção a seguir) seja inviável no curto ou médio prazos (p.ex. devido à ausência de marcadores moleculares adequados para uma dada espécie, limitação amostral ou carência de informações ecológicas, comportamentais, etc.), pode-se construir progressivamente o conhecimento acerca de cada espécie e população, adicionando-se novas informações à medida que se tornam disponíveis. Desta forma vai-se refinando a inferência sobre os processos históricos, e ajustando as propostas de unidades operacionais para conservação. É claro que neste ínterim será necessário tomar decisões quanto às alternativas de manejo, o que deve ser feito para minimizar os riscos tendo em vista o conhecimento disponível naquele momento (EIZIRIK, 1996; FRANKHAM et al., 2002).

Técnicas moleculares e análises filogeográficas

A definição de unidades demográficas diferenciadas geneticamente envolve a investigação da história populacional da espécie em questão ou de um grupo de espécies próximas, o que seria recomendável tanto em termos comparativos quanto para a confirmação do *status* de diferenciação evolutiva entre as espécies. O campo da filogeografia (AVISE et al., 1987; AVISE, 2000) engloba o estudo evolutivo de linhagens genealógicas e seus padrões de variabilidade espaço-temporal, particularmente em investigações intra-específicas. Baseia-se em conceitos da filogenia e genética de populações, empregando predominantemente marcadores moleculares.

Diversas técnicas moleculares foram utilizadas nas últimas décadas para estudos populacionais, incluindo a análise de polimorfismos protéicos e isoenzimas, RFLPs (*Restriction Fragment Length Polymorphism*) do DNA mitocondrial (mtDNA), minissatélites (locos repetitivos empregados originalmente em *DNA fingerprinting*), RAPD (*Random Amplified Polymorphic DNA*), entre outras (ver descrições e comentários em AVISE, 1994; AVISE; HAMRICK, 1996; HILLIS ET AL., 1996; SMITH; WAYNE, 1996; HOELZEL, 1998; FRANKHAM et al., 2002; HEY; MACHADO, 2003; SCHLÖTTERER, 2004). Nos últimos anos houve uma crescente utilização de seqüências de segmentos do mtDNA e de variação de tamanho em locos de microssatélites (seqüências repetitivas curtas; também conhecidos como STR [*short tandem repeats*]) em estudos desta natureza, resultando em uma ampla literatura, inclusive sobre várias espécies de carnívoros (JOHNSON et al., 2001). Grande parte dos estudos atuais utiliza estes dois tipos de marcadores, que apresentam características complementares para fins de estudos de diferenciação populacional (FRANKHAM et al., 2002; SCHLÖTTERER, 2004).

O DNA mitocondrial é de fácil caracterização (devido ao conhecimento já disponível sobre sua estrutura); apresenta taxas de mutação relativamente altas (exibindo portanto maior variabilidade em termos de seqüência do que segmentos nucleares equivalentes), ausência essencialmente completa de recombinação, e um tamanho efetivo de população quatro vezes menor do que locos autossômicos, portanto evidenciando processos de diferenciação populacional mais rapidamente do que marcadores nucleares. Por outro lado, a herança essencialmente matrilinear do mtDNA (em vários grupos de organismos, incluindo animais, apenas fêmeas o transmitem à prole) implica que apenas parte da história demográfica da espécie é retratada por este marcador. Por exemplo, em casos em que o fluxo gênico entre regiões é mediado principalmente pela dispersão de machos (como em muitos carnívoros), o mtDNA tende a apresentar maior diferenciação geográfica do que marcadores nucleares e, em conseqüência, não representa de forma adequada a estrutura genética real do organismo.

Para superar essa limitação, é importante combinar análises do mtDNA com marcadores nucleares, e entre estes os microssatélites têm sido amplamente utilizados. Esses locos repetitivos apresentam altas taxas de mutação (principalmente adição ou deleção de repetições na série), levando a níveis acentuados de variabilidade no que tange ao tamanho do segmento envolvido. Essa variação pode ser genotipada facilmente por PCR (*Polymerase Chain Reaction* – SAIKI et al., 1985), permitindo diversos tipos de inferências quando vários locos são analisados (GOLDSTEIN; SCHLÖTTERER, 1999; SCHLÖTTERER, 2004).

Enquanto o estado atual deste campo enfatiza o uso de seqüências do mtDNA e locos de microssatélite nucleares (FRANKHAM et al., 2002), há, no momento, crescente interesse na incorporação adicional de seqüências de genes nucleares (HARE, 2001), tanto autossômicos quanto localizados nos cromossomos sexuais. O uso de tais segmentos, em combinação com marcadores usuais, permite maior versatilidade e poder inferenciais, já que contêm maior informação histórica do que microssatélites, mas ao mesmo tempo viabilizam a comparação de múltiplos locos em várias regiões do genoma, os quais podem apresentar propriedades evolutivas diferentes (p. ex. tamanho efetivo menor em segmentos do mtDNA ou cromossomo Y comparados a autossomos). Tais características permitem uma investigação mais detalhada de processos demográficos históricos, como flutuação no tamanho populacional e diferenciação influenciada por deriva genética e/ou seleção natural em locos específicos (ZHANG; HEWITT, 2003). A análise de múltiplos locos, incluindo representação de herança biparental, é necessária para que possa ser realizada uma inferência abrangente sobre processos atuando sobre o genoma (p.ex., deriva genética e sua relação com flutuações e subdivisões demográficas ou fluxo gênico entre populações), e seja obtido m parâmetro de fundo com o qual se pode testar a ocorrência de seleção natural sobre locos de interesse (p.ex., genes candidatos a influenciar fenótipos adaptativos).

Em alguns casos, o uso de marcadores moleculares convencionais pode não detectar padrões relevantes de diferenciação populacional (Figura 2). Isto ocorreria no caso de populações recentemente isoladas e/ou moderadamente conectadas por fluxo gênico, mas que se encontrem em processo de adaptação a ambientes distintos. Nesse caso o genoma como um todo se manteria muito similar, enquanto apenas alguns genes (atuantes na adaptação ao ambiente) sofreriam diferenciação significativa. No momento, pode-se investigar esse tipo de diferenciação através de estudos indiretos baseados em aspectos fenotípicos variáveis (morfológicos, ecológicos, comportamentais), buscando avaliar sua associação com diferentes ambientes ou áreas. No caso de carnívoros, há diversas dificuldades para estudos detalhados desta natureza, como carência de material em coleções científicas (para estudos morfológicos), e limitações amostrais para estudos ecológicos e comportamentais em campo. Entretanto, é provável que no futuro essas limitações sejam superadas (MORATO et al., 2004), viabilizando o aprofundamento destes estudos. Uma estratégia conservadora é a definição de unidades operacionais que levem em conta os diferentes ambientes em que a espécie ocorre, mesmo que estes grupos não apresentem uma diferenciação genética detectável com os marcadores disponíveis no momento. Esse tipo de estratégia foi recomendado para a onça-pintada, por exemplo, tendo em vista a baixa diferenciação genética estimada entre áreas próximas (com altos níveis de conectividade histórica), em conjunto com algumas barreiras ao fluxo gênico (p. ex. rio Amazonas) e a ocorrência em diferentes biomas, possibilitando algum nível de adaptação local (EIZIRIK et al., 2001).

No longo prazo, o ideal é poder analisar, em conjunto com marcadores moleculares 'neutros' (que representam a história demográfica da espécie e seus impactos gerais no genoma), genes que estejam diretamente envolvidos em variação fenotípica e adaptação a diferentes ambientes. Alguns estudos neste sentido já foram realizados, incluindo análises de variação na expressão gênica entre populações (como os de OLEKSIAK et al., 2002) e investigações diretas de genes envolvidos em polimorfismos fenotípicos naturais com potencial impacto adaptativo (EIZIRIK et al., 2003). A expansão destas análises, em conjunto com estudos ecológicos, morfológicos, etc., permitiriam a compreensão global dos processos evolutivos atuando sobre a espécie, e o delineamento de estratégias adequadas para sua conservação no longo prazo.

Estado atual da definição de unidades evolutivas em carnívoros neotropicais

A região zoogeográfica neotropical (estendendo-se do México ao sul da América do Sul) abriga cerca de 47 espécies de carnívoros, as quais são tradicionalmente classificados em seis famílias distintas: Canidae, Felidae, Mustelidae, Otariidae, Procyonidae e Ursidae (WOZENCRAFT, 1993; NOWAK, 1999). Estudos mais recentes sugerem que os cangambás, zorrilhos e jaratatacas não se agrupam evolutivamente com os demais mustelídeos, e que devem ser colocados em uma sétima família independente, Mephitidae (DRAGOO; HONEYCUTT, 1997; EIZIRIK et al., dados não publicados). À exceção de Otariidae (leões e lobos-marinhos), as demais famílias compreendem carnívoros terrestres, e serão o foco desta revisão (enfatizando as 26 espécies que ocorrem no Brasil).

Na maior parte dos casos, a definição dessas espécies e sua delimitação em relação a espécies próximas não é controvertida (Tabela 1), tendo sido evidenciadas com base em aspectos morfológicos, ecológicos ou genéticos (p. ex. EMMONS, 1990; EISENBERG; REDFORD, 1999; NOWAK, 1999; JOHNSON et al., 1998, 1999). Entretanto, alguns casos permanecem relativamente indefinidos, como os furões (*Galictis* sp.) e jaratatacas (*Conepatus* sp.), em que há dúvidas sobre a distinção entre espécies e sua delimitação evolutiva e biogeográfica (resultado de discussões durante o I Workshop sobre Pesquisa e Conservação de Carnívoros Neotropicais – MORATO et al., 2004). O mesmo pode ser dito de espécies pouco conhecidas dos gêneros *Procyon* (essencialmente formas insulares próximas da América Central) e *Bassaricyon*. Um estudo recente sobre espécies de *Conepatus* na América do Norte (DRAGOO et al., 2003) reorganizou estes táxons com base em análises morfológicas e moleculares, reduzindo o número de espécies válidas. Estudos similares são necessários para as demais espécies desse gênero, bem como dos outros mencionados acima (Tabela 1).

Quanto às subdivisões infra-específicas e inferências sobre história demográfica, diversos estudos foram realizados nos últimos anos com espécies de carnívoros, utilizando diferentes classes de marcadores moleculares (JOHNSON et al., 2001). No entanto, poucas delas abordaram especificamente espécies neotropicais (Tabela 1). Estudos moleculares publicados até o momento, sobre diferenciação filogeográfica entre populações e/ou espécies próximas de carnívoros neotropicais, incluem investigações de um grupo de canídeos (YAHNKE et al., 1996; VILÀ et al., 2004) e algumas espécies de felídeos (EIZIRIK et al., 1998, 2001; JOHNSON et al., 1998, 1999; CULVER et al., 2000). Algumas destas análises estão sendo continuadas com expansão da amostragem e uso de novos marcadores (como discutido acima), e há novos estudos desta natureza em andamento para espécies adicionais deste grupo (Tabela 1). Contudo, é essencial expandir esses esforços, em um futuro próximo, visando a obter subsídios confiáveis para estratégias de conservação dessas espécies (MORATO et al., 2004). A seguir, serão sintetizados os principais resultados e inferências obtidos dos estudos realizados até o momento com carnívoros neotropicais.

Os estudos sobre canídeos enfatizaram as espécies do gênero *Pseudalopex* habitando o Chile e a Argentina (*P. culpaeus*, *P. griseus* e *P. fulvipes*). A principal questão abordada era testar a diferenciação histórica de *P. fulvipes* (até recentemente encontrada apenas na ilha de Chiloé, na costa do Chile) em relação às outras espécies, o que poderia ter importantes implicações para sua conservação. Grande parte dos autores considerava *P. fulvipes* apenas uma subespécie insular de *P. griseus* (p.ex. WOZENCRAFT, 1993; NOWAK, 1999); entretanto, análises do DNA mitocondrial sugeriram que se tratava de uma espécie distinta, divergente de *P. culpaeus* e *P. griseus* (YAHNKE et al., 1996). Estes resultados, aliados à identificação de ao menos uma população continental desta espécie (MEDEL et al., 1990 [dados de campo]; VILÁ et al., 2004 [dados moleculares]), sugerem que as populações atuais são remanescentes de uma distribuição historicamente mais abrangente na região, restrita a habitats florestais primários, hoje extremamente fragmentados. Isto contribuiu para ressaltar a prioridade de conservação destas áreas e sua fauna associada.

No caso dos estudos com felídeos, foram elucidados alguns aspectos sobre a delimitação de espécies e suas relações filogenéticas, subdivisões filogeográficas intra-específicas, e padrões comparativos de demografia histórica. De forma geral, estes estudos revelaram alguns padrões filogeográficos coincidentes entre várias espécies (p. ex. o rio Amazonas como barreira demográfica histórica), indicando a ocorrência de processos históricos comuns afetando os felídeos neotropicais. Outros aspectos acerca da história demográfica inferida para estas espécies são marcadamente distintos, como a diferença em profundidade filogenética observada em *Leopardus pardalis* e *L. wiedi* (EIZIRIK et al., 1998) em comparação a *Puma concolor* (CULVER et al., 2000) e *Panthera onca* (EIZIRIK et al., 2001), sugerindo idades diferentes para a origem e expansão destas populações na região Neotropical. Em vários casos (p. ex. *L. pardalis*, *L. wiedi*, *P. onca*, *P. concolor*) foi possível observar um padrão de expansão demográfica no sentido sul-norte, em que as populações da América Central e do Norte parecem derivar de ancestrais provenientes da América do Sul (EIZIRIK et al., 1998, 2001; CULVER et al., 2000). Outra observação interessante foi a de que *Leopardus tigrinus* apresenta dois grupos filogeográficos (América Central e Sudeste brasileiro) altamente divergentes entre si, evidenciando um longo período de isolamento histórico (JOHNSON et al., 1999). Esta inferência a partir do mtDNA tem sido corroborada pela análise de múltiplos segmentos nucleares (EIZIRIK et al., em preparação).

Além disto, identificou-se a ocorrência de híbridos naturais entre *L. tigrinus* e *L. colocolo* no Brasil central (Johnson et al., 1999), o que tem sido corroborado por análises adicionais de novas amostras (dados não publicados), e estendido à inferência de provável hibridação também entre *L. tigrinus* e *L. geoffroyi*, no Sul do Brasil (TRIGO, 2003; EIZIRIK; BONATTO et al., em preparação; TRIGO et al., dados não publicados). Estes achados salientam a necessidade de investigações mais aprofundadas a fim de identificar as causas ecológicas e implicações evolutivas desse fenômeno nessas espécies, e devem ser incorporados na construção de estratégias adequadas de manejo.

Um resultado relevante desses estudos foi a conclusão de que as subespécies tradicionalmente reconhecidas geralmente não refletem a estrutura filogeográfica observada nestas espécies. Isto tem importantes implicações para a definição de estratégias de conservação, tendo em vista que avaliações de ameaça e planos de manejo em grande escala têm sido realizados com base em subespécies tradicionais (p. ex. CBSG, 1995, IBAMA, 2003). Uma reavaliação destas unidades populacionais para fins de manejo já é possível para algumas espécies, com base em resultados filogeográficos aqui mencionados. Isto tem sido realizado nos últimos anos, com recomendações embasadas nessas análises sendo repassadas a planos de manejo em campo e cativeiro. Mesmo para estas espécies, análises mais aprofundadas, utilizando diferentes abordagens, são necessárias para obter-se um conhecimento mais detalhado de seus padrões regionais de subdivisão histórica. Para a maioria dos carnívoros neotropicais, entretanto, os estudos filogeográficos básicos ainda não foram realizados (Tabela 1), o que se apresenta como prioridade de pesquisa para os próximos anos (MORATO et al., 2004). Espera-se que a expansão e o aprofundamento dessas análises, e sua integração com outras áreas de pesquisa, viabilizem a conservação efetiva e de longo prazo destas espécies e de seus habitats.

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Tabela 1 – Estado atual do conhecimento filogenético e filogeográfico dos carnívoros terrestres neotropicais

Família	Definição e delimitação de espécies	Definição de unidades infra-específicas
Canidae	- Bem definidas em sua maioria; - Diversificação recente do gênero *Pseudalopex* (possibilidade de diferenciação incompleta e/ou hibridação entre algumas destas espécies); há necessidade de mais estudos.	- Estudos em andamento com *Chrysocyon brachyurus*, *Cerdocyon thous*, *Pseudalopex gymnocercus* e *P. vetulus* (PRATES Jr. et al., em prep.; TCHAICKA et al., em prep.; MALDONADO; GONZALEZ, com. pes.).
Felidae	- Bem definidas em sua maioria; - Separação recente entre *Leopardus guigna* e *L. geoffroyi*, potencialmente envolvendo diferenciação incompleta e/ou hibridação; - Ocorrência de hibridação entre *Leopardus tigrinus* e *L. colocolo*; - Provável ocorrência de hibridação entre *Leopardus tigrinus* e *L. geoffroyi*.	- Estudos filogeográficos gerais já publicados sobre *P. onca*, *P. concolor*, *L. pardalis*, *L. wiedi*, *L. tigrinus*, *L. colocolo*, *L. geoffroyi*; - Estudos mais detalhados em andamento sobre *P. onca*, *L. pardalis*, *L. wiedi*, *L. tigrinus*, *L. colocolo*, *L. geoffroyi* e *P. yagouaroundi* (EIZIRIK et al., em prep.; JOHSONet al., em prep.; TRIGO et al., em prep.).
Mustelidae	- Bem definidas em sua maioria; - Dúvidas acerca da diferenciação evolutiva e limites geográficos entre *Galictis cuja* e *G. vittata*.	- Estudos gerais em andamento sobre *Lontra longicaudis*, *Pteronura brasiliensis* e *Galictis* spp. (TRINCA et al., em prep.; SANTOS, com. pes.)
Mephitidae	- Dúvidas acerca da diferenciação evolutiva e limites geográficos entre *Conepatus chinga*, *C. semistriatus* e *C. humboldti*.	- Não há estudos iniciados.
Procyonidae	- Bem definidas em sua maioria; - Dúvidas acerca da diferenciação evolutiva entre algumas espécies congenéricas (*Bassaricyon* spp., *Procyon* spp. [espécies insulares]).	- Não há estudos iniciados.
Ursidae	Uma única espécie (*T. ornatus*).	- Estudos de genética de populações inciados (M. RUIZ-GARCIA, com pess.; WAITS, com. pes).

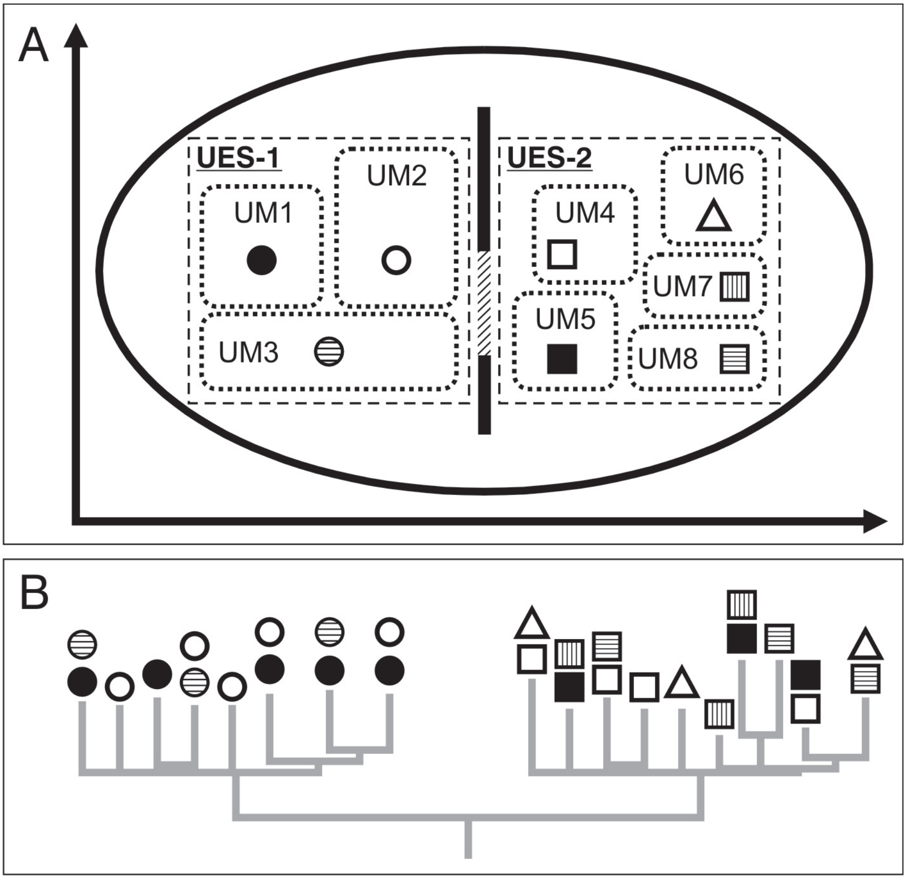

Figura 1 – [A] Esquema demonstrando a relação entre diferentes níveis de diferenciação genética, envolvidos na definição de unidades demográficas para fins de conservação. Os eixos indicam duas dimensões espaciais. A espécie A está subdividida em duas Unidades Evolutivamente Significativas (UES-1, UES-2), separadas por uma barreira histórica (barra vertical escura) a qual apresenta alguma permeabilidade ao fluxo gênico, ao longo do tempo evolutivo, em sua porção central (área hachureada). Cada UES está subdividida em diferentes Unidades de Manejo (UMs), as quais estão identificadas por símbolos distintos. [B] Filogenia molecular de linhagens genealógicas (p.ex., de DNA mitocondrial) evidenciando uma separação profunda entre as duas UES, contrastada ao compartilhamento de seqüências ao nível das UMs (símbolos indicam a presença de uma dada seqüência em cada UM). Observa-se que as UES apresentam diferenciação genética bastante significativa entre si, e devem ser manejadas de forma independente na maior parte dos casos. As UMS apresentam níveis menores, e diversos, de separação demográfica entre si, e podem ser manejadas de forma independente ou coordenada, dependendo da situação (ver texto).

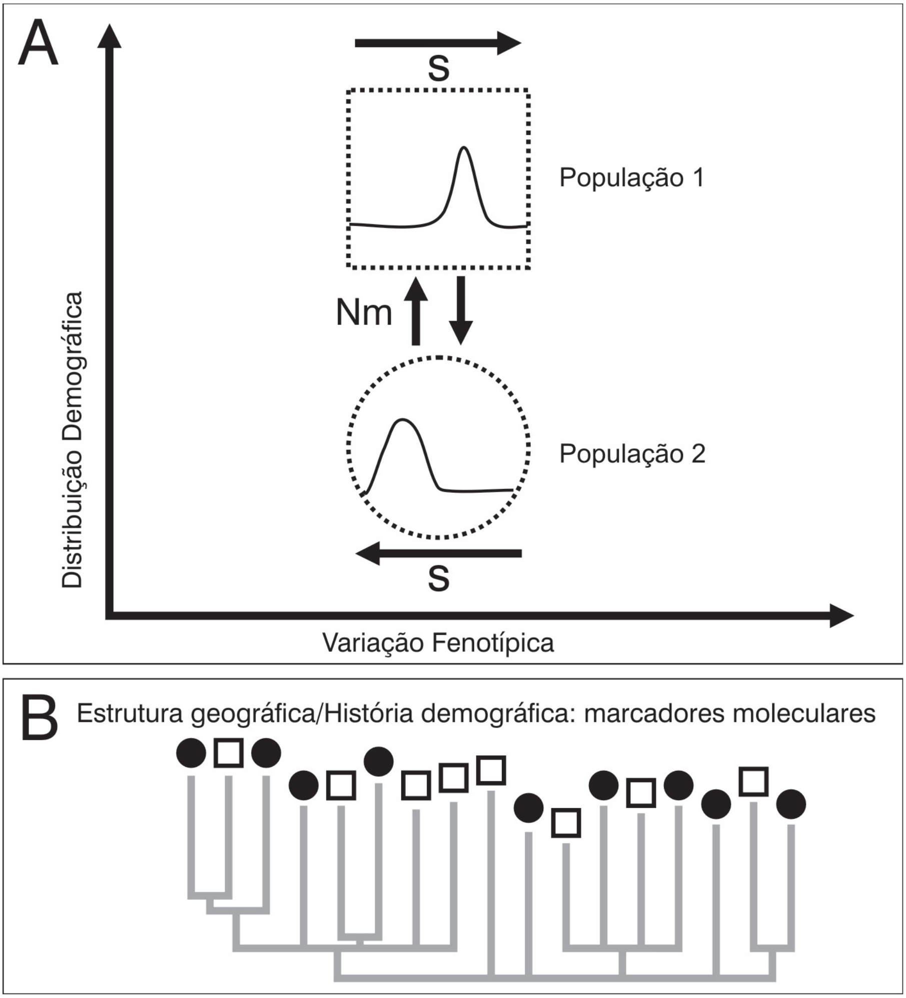

Figura 2 – Esquema exemplificando uma relação possível entre padrões de diversidade genética identificados com marcadores moleculares 'neutros' (genômicos) *versus* diferenciação fenotípica entre populações naturais, influenciada por um antagonismo entre seleção natural (diferenciando-as) e fluxo gênico (homogeneizando-as). Em [A] observa-se duas populações (representadas por um quadrado [1] e um círculo [2]), as quais apresentam algum nível de conexão por fluxo gênico [Nm], o que impede uma diferenciação significativa de todo o seu genoma. Isto se reflete na ausência de diferenciação clara entre elas quando analisadas com marcadores moleculares convencionais [B]: observa-se uma mescla de círculos e quadrados (representando indivíduos das duas populações) na árvore gerada com estas análises. Ao mesmo tempo, diferenças ambientais entre as duas áreas podem levar a regimes diferentes de seleção natural [s] sobre alguma característica fenotípica (representada pela distribuição de um caráter [p.ex. comprimento, ou intensidade de coloração] nas duas populações), de modo que pode haver diferenciação genética entre elas, no que se refere a alguns genes específicos, relacionados a esta adaptação local. Este tipo de estruturação é mais sutil do que o evidenciado na Figura 1, mas também deve ser considerado durante o delineamento de estratégias de conservação.

Capítulo 4
Molecular ecology and carnivore conservation: the application of molecular techniques for inferring identity, kinship, and social structure in the neotropics

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Introduction

Advances and developments in molecular genetics impact many aspects of our world, entering our lives through currently unimaginable, but fundamental changes in our approaches to health care, agriculture, engineering, and the environment. The integration of molecular genetic tools into ecological studies and conservation has been especially rapid, providing scientists with tools for exploring evolutionary processes in robust, repeatable, and testable ways using approaches that were previously impossible (JOHNSON et al., 2001). Ecological studies have and will continue to change because these tools and analytical techniques have increased the scientific rigor of the discipline, providing means to more confidently characterize past and current events and to simulate and project trends into the future. The role of molecular genetics will increase greatly in the realm of conservation because the approaches will facilitate the estimation of important life history and fitness variables and directly address concerns about loss of genetic variation, inbreeding, fragmentation of habitats and identification of conservation units (O'BRIEN, 1994). This will be especially true for carnivores, for which even the most basic natural history data can be difficult to collect.

The goals of this chapter are to review how molecular techniques and/or genetic approaches have influenced and contributed to Neotropical carnivore conservation and to discuss future applications of these techniques. In particular we will review some of the major issues and methods and will provide details on several case studies which demonstrate a direct link between molecular genetics and the study of the ecology, natural history, identity, and the characterization of past and more recent demographic patterns of natural populations. These examples will include the use of molecular genetic techniques to describe behavior and social structure in a variety of species, including coati, pumas, bears, wolves, and foxes.

Study design considerations

As with most scientific ventures, the ultimate success of molecular ecology studies depends on the appropriate choice of methods and analyses relative to the objectives and questions of interest. This includes the judicious choice of molecular genetic markers and techniques for each species and population and an adequate spatial and temporal sampling of individuals. The most commonly used genetic techniques in molecular ecology include mitochondrial DNA sequence variation, microsatellite size variation, multilocus fingerprinting, SNPs (single nucleotide polymorphisms), variation in non-neutral markers (e.g. MHC, other functional genes), and sex-linked markers. The technical aspects of these methods have been well described elsewhere (AVISE, 1994; SMITH; WAYNE, 1996; HOELZEL, 1998; FRANKHAM et al., 2002) and therefore will not be addressed in this chapter. Instead, we will focus on topics that should be considered while determining if these tools might be useful for specific research questions. These questions fall into three main categories: 1) the type and quantity of sample available, 2) the availability of established markers that are suitable for the species or population of interest, and 3) the amount of accompanying information that is or will be available for the population or individuals.

Samples and sampling design

When exploring the feasibility of utilizing molecular genetic techniques, one of the first considerations is the quantity and quality of samples that are available or targeted for collection in the study. Freshly collected and well-preserved samples yielding large amounts of high-quality DNA, such as blood, skin biopsies, or tissue provide the most useful and robust material for most studies. These types of samples provide the researcher with the maximum amount of flexibility both in terms of applying different genetic techniques, but also in terms supplying extra archival material for addressing future questions that might not be the primary focus of the study. In most cases the collection of these samples is complicated by the need to handle individuals, and thus requires some practical experience in trapping and anesthesia. However, these samples should be collected habitually whenever animals are handled for other purposes, such as the fitting of radio-telemetry collars, or when live animals or carcasses of known origin are encountered during the study. When utilized wisely, the additional information that can be garnered from the collection of these high quality samples readily compensates for the added effort.

Samples yielding low quantities of DNA or only degraded DNA, such as hair samples and fecal samples, pose additional technical challenges and greatly limit the number of techniques that can be used and the number of genetic experiments that can be run (TABERLET et al., 1999). The advantage of these samples is that they can generally be collected non-invasively, are easier to collect, and may be the only source of genetic data for elusive species that are difficult to trap in large numbers. Special considerations relating to the use of these samples will be discussed below.

The design of a study and the formalization of objectives require a realistic assessment of not only what kinds of samples can be collected, but also of how many samples can be collected from which animals in the population and over what time period. The answer to this question will determine, in large part, the type of behavioral, ecological and evolutionary questions that can be addressed. Unlike broad evolutionary or phylogeographic studies (see e.g. Chapter 3 [EIZIRIK et al.,]), molecular ecology studies often require sampling schemes that are as thorough as possible. The breadth and power of inferences increases as a larger proportion of the population is sampled.

Availability of appropriate markers

Samples in hand are useless without adequate molecular genetic markers. With carnivores this is a lesser problem than with many other taxa because genome projects for the domestic dog and domestic cat have facilitated and motivated the development of molecular tools, providing a wide range of options for most studies. Several factors are involved in defining whether a specific marker is “appropriate”, including the type of information it provides, technical issues such as the compatibility of a method with the type of available biological samples, the availability of technical support, and the cost of the assays. However, for most research objectives the initial issue is whether genetic markers have been developed that are variable among individuals in the population(s) of interest. Generally this implies markers with relatively rapid mutation rates, such as microsatellites, or the ability to collect large amounts of data using markers that are less variable. Therefore, it is prudent to have a prior assessment of the extent of molecular genetic diversity in the population, as well as the distribution and frequency of this variation. This is especially important if the effective population size is suspected to be small and/or if inbreeding is suspected to be high. The effective population size can be seen as the average number of individuals contributing equally to the next generation and is often much smaller than the actual census size (FRANKHAM, 1995). It is influenced mainly by demographic and behavioral factors such as population fluctuations over time, unequal sex ratios, and variable rates of reproductive success (unequal family sizes).

Auxiliary data collection

The usefulness of biological samples and molecular genetic data will be limited by the amount of information that is known about the individuals and population. Detailed information on population size, life history parameters, spatial and temporal patterns, behavior, physical and physiological parameters and natural history information will greatly affect the inferences that can be made from the genetic data. The more information that is available for each sample, the more powerful the genetic data becomes. These types of data can be collected most efficiently when health, morphological, and physiological data on each animal and when intense radio telemetry efforts are providing detailed estimates of movements, sex- and age-specific mortality rates, and life history data. Radio-telemetry also facilitates periodic visual contact of individuals, which provides important information on habitat utilization, social interactions, foraging behavior, and other activities.

Noninvasive sampling

Samples

The collection of hair and feces has become an important source of DNA samples for carnivores (KOHN; WAYNE, 1997; TABERLET et al, 1999). For example, hair samples have been collected on barbed wire or specially designed collection pads to identify and count particular carnivore species (KOHN et al., 1995; TABERLEt et al., 1997; WOODS et al., 1999; MCDANIEL et al., 2000; POOLE et al., 2001; MOWAT; PAETKAU, 2003). Fecal DNA analysis has also been used to detect and count mustelids (DALLAS et al., 2003; FRANTZ et al., 2003), felids (ERNEST et al., 2000; PALOMARES et al., 2002), canids (PAXINOS et al., 1997; KOHN et al., 199; LUCHINNI et al., 2002; ADAMS et al., 2003; CREEL et al., 2003; VALIERE et al., 2003), seals (REED et al., 1997) and ursids (KOHN et al., 1995; TABERLET et al., 1997). In addition to obtaining critical information about presence and number of different carnivore species, these samples can be used to evaluate genetic diversity, phylogenetic relationships, population structure, kinship, and gene flow (KOHN et al., 1995; TABERLET et al., 1997; LUCHINNI et al., 2002; FRANTZ et al., 2003). Morphologically-based criteria for species identification of scats can be evaluated and updated using fecal DNA analysis (FARRELL et al 2000, DAVISON et al., 2002). Carnivores that predate livestock (FARRELL et al., 2000) or endangered species (ERNEST et al., 2002; BANKS et al., 2003a) can also be identified using fecal DNA analysis.

Special considerations

The DNA obtained from non-invasive genetic sampling is generally low in quantity and quality (TABERLET et al., 1999). As a result, non-invasive genetic sampling studies must overcome three main problems – high potential for contamination, low DNA amplification success rates, and high microsatellite genotyping error rates. Contamination problems can be minimized by establishing facilities dedicated to the extraction and PCR set up of non-invasive DNA sources (TABERLET et al., 1996, 1997, 1999). Researchers have suggested a number of different ways to maximize success rates and DNA quality from hair and fecal samples including designing PCR primers that target short DNA fragments (< 250 base pairs) (REED et al., 1997; FRANTZ et al., 2003, MURPHY et al., 2003), optimizing sample storage methods (WASSER et al., 1997; FRANZTEN et al., 1999; FRANTZ et al., 2003; MURPHY et al., 2003; ROON et al., 2003), optimizing

DNA extraction methods (REED et al., 1997, FRANTZ et al., 2003), focusing collection in the dry season (FARRELL et al., 2000; MURPHY et al., 2003), and minimizing the amount of time samples remain in the field (BANKS et al., 2003; FRANTZ et al., 2003).

To obtain optimal quality and quantity of DNA from non-invasive sources, hair and fecal samples should be as fresh as possible. DNA degrades more rapidly in wet conditions so optimal success rates will be obtained in the dry season. Hair samples should be preserved in the field by placing each sample in a small paper envelope and placing the envelopes in a ziplock bag with a cup full of desiccant (such as silica beads). For long-term storage of hair samples, freezing at – 20°C can help delay DNA degradation, but freeze-thawing damages DNA and should be avoided (ROON et al., 2003). Even when stored in the freezer, DNA amplification rates for microsatellite loci drop 15 – 20% between 6 months and one year of storage (ROON et al., 2003) so DNA should be extracted as soon as possible. Extraction of DNA from hair samples utilizes the cells that are attached to the root of the hair and special care should be taken to preserve the cells on the root. DNA extraction is generally performed using two methods: chelex protocols (WALSH et al., 1991; WOODS et al., 1999, Palomares et al 2003), or commercially available silica-binding extraction kits (e.g. from Qiagen) (POOLE et al., 2001, RIDDLE et al., 2003). Some researchers have reported DNA degradation in chelex DNA extracts after 1 year or more of freezer storage, and most carnivore projects currently use the Qiagen extraction kits.

The three main storage methods used for fecal samples are ethanol preservation, silica desiccation and freezing. Most recent studies agree that preservation in 75 – 100% ethanol is the optimal storage method (DALLAS et al., 2003; Frantz et al., 2003; LUCCHENI et al., 2002; MURPHY et al., 2003; EGGERT et al., 2003) and experiments have shown that DNA will not degrade during six months of storage at room temperature (MURPHY et al., 2003). Ethanol can be difficult to transport and silica desiccation can provide an alternative storage method (WASSER et al., 1997). The optimal DNA extraction method appears to vary by laboratory, species and storage method (KOHN et al., 1995; REED et al., 1997; PAXINOS et al., 1997; Frantz et al., 2003). Fecal DNA extracts can contain high concentrations of PCR inhibitors, thus extraction methods must be optimized to maximize DNA yield and minimize inhibitors. Currently, researchers report optimal results with a diatomaceous earth/guanidine-thiocyanate method (FRANTZ et al., 2003, KOHN et al., 1995, LUCCHINI et al., 2002), and a commercially available Qiagen silica-binding stool extraction kit (FARRELL et al., 2000, CREEL et al., 2003).

The moderate to high rates of microsatellite genotyping errors is another serious challenge for researchers using microsatellite loci to obtain genetic data for hair or fecal samples (TABERLET et al., 1996, GAGNEUX et al., 1997, GOOSSENS et al., 1998, TABERLET et al., 1999). To obtain accurate microsatellite data researchers must utilize protocols that quantify and remove genotyping errors (TABERLET et al., 1996, MORIN et al., 2000, MILLER et al., 2002, FRANTZ et al., 2003). Currently, the optimal methods for obtaining accurate genotypes are the maximum-likelihood approach (MILLER et al., 2002) and the comparative multiple-tubes approach (TABERLET et al., 1996, FRANTZ et al., 2003). All of these protocols require multiple amplifications of each microsatellite locus and thus greatly increase the time and cost of collecting genetic data. If genetic markers used lack the variability (power) to distinguish individuals, then two distinct individuals in a population may carry the same genotype by chance. This can cause an inaccurate increase in the number of recaptures and a decrease in the minimum count of individuals in a population. The likelihood of this occurrence, or probability of identity ($P_{(ID)}$), is dependent on allelic diversity, number of loci analyzed, and the percentage and degree of related individuals in a population (WAITS et al., 2001). By using a sufficient number of highly polymorphic loci and a conservative threshold, such as $P_{(ID)}$ (sibs) < 0.05, underestimates of population size will be minimized (WOODS et al., 1999, WAITS et al., 2001; PAETKAU, 2003).

Applications

The potential uses of molecular genetic approaches to the study and conservation of carnivores are numerous, varied, and increasing rapidly as technical and theoretical methodologies are developed (JOHNSON et al., 2001). An exhaustive and detailed description of these is beyond the scope of this chapter, but here we will provide a short summary of some of the most common of these applications.

Assessment of population history

Conservation genetics has historically been most concerned about the measurement and maintenance of genetic variation, and the impact that parameters such as population size, gene flow, selection, mating systems, and genetic drift can have on this variation. Molecular markers enable us to directly measure or estimate not only specific levels of existing genetic diversity, but to also to estimate related population parameters, to assess past demographic history and to predict likely levels of future genetic variation. For example, molecular markers can provide insights into the effective population size (which is usually much smaller than the actual census size; FRANKHAM, 1995), whether the population has been growing or declining and whether there is any evidence of historic bottlenecks, isolation, barriers to gene flow or natural selection (FRANKHAM et al., 2002).

Inbreeding and relatedness estimation

Population size, specifically in small or shrinking populations, is of special concern for managers, as genetic variation is lost and populations become at greater risk of extinction. Inevitably, small populations suffer from inbreeding as eventually all animals are related to each other. The maintenance of genetic variation is important for numerous reasons, including avoidance of inbreeding depression, or the relationship between genetic variation and parameters of reproductive fitness that has been documented in numerous species (LACY, 1997). Inbred animals almost invariably have poorer attributes relative to more outbred individuals (CRNOKRAK; ROFF 1999). One of the best examples for a Neotropical species is the decreased survival demonstrated by inbred golden lion tamarins in their natural habitats in Brazil relative to non-inbred individuals (DIETZ et al., 2000). More fundamentally, genetic variation is linked to evolutionary potential, and the ability to adapt to changing environments and conditions.

One of the major consequences of inbreeding is the increased probability that an individual is homozygous at a locus, and is thus more susceptible to deleterious or lethal traits that are often recessive, and thus require two copies of the same allele to be manifested in an individual. Levels of inbreeding can be estimated in several ways, including comparing changes in heterozygosity over time, utilizing knowledge of the founding individuals or through analysis of a known pedigree. When little is known about the population, or of the relationships among specific individuals, levels of inbreeding between specific animals can be estimated in a couple of ways. One approach often used with established captive breeding programs is to assume that individuals that have more genomic uniqueness, or share less genetic variation, are also less closely related. It is also possible to establish calibration curves by comparing the degree of genetic relatedness (genetic similarity) among animals of known relationships (e.g. mother/offspring, sib-pairs, etc.) and to use this curve to estimate the degree of relatedness with pairs of animals of unknown relationships.

Parentage assessment

The direct assessment of parentage is closely related to the estimation of inbreeding, the genetic management of threatened species and the determination of effective population size. Perhaps more than any of the other applications of genetic methods described here, parentage analyses require highly variable and robust markers (to ensure accurate results) and a very thorough sampling of the population of interest (to have a complete assessment of the population). Parentage determination is also of fundamental interest to molecular ecology, providing insights on social behavior that otherwise would not be apparent or might be misinterpreted based only on direct observations.

Identification of populations

Molecular genetic techniques are especially suited for objectively defining populations and identifying population structure that might otherwise not be apparent. This information is useful for establishing the number of populations, determining how they are unique and to what extent, and how they are interconnected (or isolated). Once populations have been defined, similar techniques can be used to assign individuals to populations, to identify migrants, and determine the existence of hybrids. Similarly, these techniques can be applied to forensic questions, determining the provenance of animals or parts of animals.

In the development of the recovery plan for the Mexican wolf, which is extinct in the wild, molecular genetic analyses of allozymes, mtDNA, DNA fingerprints, and microsatellite data were used to establish genetic criteria for the definition of a Mexican wolf (GARCIA-MORENO et al., 1996). These data were then used to distinguish "pure" Mexican wolves from animals that had a mixed heritage with domestic dogs, coyotes, or different gray wolf populations (HEDRICK et al., 1997). This process was instrumental in identifying additional founders and genetic variation to be introduced in the remaining population of wolves.

Application in neotropical species

Molecular ecology and conservation genetic approaches have yet to be widely applied to studies of Neotropical carnivores. Due to the type of biological samples available for analysis and the general scarcity of in-depth field studies of carnivore populations, most of the effort has gone towards using molecular genetic techniques to describe patterns of subspecies- and species-level biogeographical variation in the context of elucidating past evolutionary history and using objective criteria to define subspecies, evolutionarily significant units, and management units (see chapter x [EIZIRIK et al.,]). However, some studies focusing on species with Neotropical and nearby Nearctic distribution provide useful examples of the techniques and approaches being presented here.

Social Organization and relatedness in Procyonids

Molecular genetics were first used in tropical species to learn more about the natural history and social behaviour of social carnivores. Gompper et al., 1997, 1998 used multilocus fingerprints to estimate genetic relationships among coati (*Nasua narica*) in Panama to determine how relatedness among individuals influences social structure and dispersal patterns. The study confirmed that coati bands are largely composed of related females. Upon independence, adult males leave their natal group, but not the general area, and except for a short time period maintain a relatively solitary lifestyle. Thereby, males in the same area with overlapping home ranges tend to be more closely related than more distantly located males, and members of bands are more closely related to each other than to other bands.

In a later study in Panama, (KAYS et al., 2000) used a similar approach to study social groups of kinkajou (*Potos flavus*). Instead of multilocus fingerprints, they used microsatellite size variation (a more flexible and more commonly used technique) to assess the degree of relatedness among individuals in four social groups and to determine the parents of numerous offspring in these groups.

The red wolf

The red wolf (*Canis rufus*) of the southeastern United States was listed as an endangered species in 1973 due to extensive declines across its historic range. When the recovery program was initiated, only a small number of animals remained in a region on the border of Texas and Louisiana (PARADISO; NOWAK, 1972). To preserve the red wolf gene pool and avoid hybridization with coyotes (*Canis latrans*), over 400 animals were captured and 43 were brought into captivity (USFWS, 1989). As a result, the red wolf was declared extinct in the wild in 1980 (USFWS, 1989). Through breeding experiments and extensive morphological and behavioral evaluations, seventeen wolves were chosen as founders of the captive population (PHILIPS et al., 1995), only fourteen of which bred and produced offspring (USFWS, 1989). In 1987 a reintroduction effort was initiated in North Carolina using animals from the captive breeding program (USFWS, 1989). Currently, 80 – 100 animals range over the 6000 km^2 recovery area, but the long-term genetic integrity of the population is threatened by hybridization with coyotes (Kelly et al., 1999). To characterize the red wolf and coyote gene pools, mitochondrial DNA sequence data and 18 loci of microsatellite data were collected from the 14 genetic founders (using bone samples), and over 80 coyotes from North Carolina and Virginia. Hybrids, back-cross animals, coyotes and red wolves can be discriminated using a maximum-likelihood genetic assignment test (MILLER et al., in press). To effectively screen the recovery area for the presence of coyotes and hybrids, fecal DNA sampling techniques have been developed and optimized (ADAMS et al., 2003). From 2000 – 2001, over 700 samples were collected along dirt roads in the recovery area and several hybrids and coyotes were identified.

Andean bear

The Andean bear (*Tremarctos ornatus*) is considered an umbrella species for paramo and forest habitats in South America. However, there is limited information about the biology and ecology of this species due to difficulties in trapping and observing it in the wild. In 1999, EcoCiencia initiated a pilot study of the Ecuadorian Andean bear population using non-invasive genetic sampling. The goals of this project were to 1) evaluate the feasibility of using non-invasive sampling methods, 2) to optimize molecular tools for this species, and 3) collect baseline data on numbers of individuals and levels of genetic diversity in 72,000 ha Oyacachi River basin of the Cayambe-Coca Ecological Reserve (VITERI; WAITS 2002). Thirteen different microsatellite loci developed for brown (*Ursus arctos*) and black (*U. americanus*) bears were tested using captive and wild Andean bear samples, and 10 polymorphic loci were optimized for Andean bears. Hair and fecal samples of Andean bears were collected by local people from January to December 2000 along 53 transects of 1.6 km. Twenty-two hair samples and 95 fecal samples were obtained over this one-year period. Success rates for individual identification were fairly low for fecal samples (28%) but higher for hair samples (90%). Ten individuals were identified, and genetic diversity levels were moderate (VITERI; WAITS, 2002). Based on these results, researchers have gained valuable information on population size, genetic diversity, and movement patterns. This research project has been expanded and is currently ongoing.

Florida panther (puma)

One study in particular involving a Neotropical species perhaps best exemplifies the benefits of utilizing molecular genetic tools when studying wildlife ecology and when designing and implementing conservation plans (HEDRICK, 1995). It is the ongoing story of the Florida panther, the only remaining puma population in North America east of the Mississippi, which was listed as state and federally endangered in 1973. The Florida panther (*Felis concolor coryii*) was first described as one of 30 subspecies of puma, but is now considered by most to be an isolated sub-population within a fairly homogenous group (subspecies) of North American pumas (CULVER et al., 2000). Florida panther numbers diminished due to a combination of population, human, and habitat factors. The population, which is confined to approximately 8,800 km^2, a very small portion of southern Florida, has been studied extensively since the early 1980's, with a large proportion of the 30-70 known adults followed by radio telemetry during this time period. Concern over the fate of the population increased as signs of inbreeding and loss of genetic diversity were reported in the early 1990's. These included very low levels of genetic variation, high levels of sperm abnormalities, an increased incidence of heart defects, and increased fixation of physical traits such as "cow lick" patterns in the hair along their backs and kinked tails relative to other puma populations and felids in general (O'BRIEN et al., 1990, ROELKE et al., 1993). Faced with the compounding effects of reduced genetic variation, likely inbreeding depression, low numbers, and evidence of compromised health, there was general agreement that these interrelated factors would inevitably result in eventual extinction if the genetic diversity and health of the Florida panther population were not restored. This led to a plan for the genetic restoration and management of Florida panther in 1994 and to the release in 1995 of eight Texas females in southern Florida.

A molecular genetic analysis based on 23 microsatellite loci of over 250 panthers collected in southern Florida over 30 years enabled researchers to determine parentage of around 200 animals and to construct a detailed pedigree. Some of these confirmed suspected relationships that had been assigned based on field evidence. Animals for which parentage could not be determined could be assigned ancestry (genetic heritage) based on their genetic makeup. The pedigree analysis and characterization of genetic variation confirmed the incidence of numerous inbreeding loops and the gradual loss of molecular genetic variation in the 1980's and early 1990's, the prevalence of deleterious traits such as cardiac defects and cryptorchidsm (the descent of either one or neither testicle) in certain genetic lineages. Following the release of the Texas females, there was an increase in genetic variation, an apparent increase in population size and a decreased incidence of deleterious physiological traits in F1 crosses and backcrosses between pure Florida panthers and Texas females. From a behavioral perspective, it was also documented that panther males were often mating at a younger age than was thought possible, that resident males were not always the successful sires, and that dispersal and gene flow continues among different areas of Florida.

Five of the eight Texas females produced young, and the likely representation of Texas cougar genes in the southern Florida population is probably close to the original genetic restoration program goal of 20%. However, most of the Texas genes are derived from only two of the eight introduced females, which reduced the genetic diversity inserted into the populations. Although the introgression of Texas genes into the population appears to have had a beneficial impact on the population, the results may be a relatively short-term benefit, especially if the population remains small. To avoid the return of significant inbreeding and loss of genetic diversity, further releases of non-local cats may have to be considered as part of ongoing management of the Florida panther population (LAND; LACY, 2000).

Recommendations

The Neotropics is a region in which the tools of ecological and conservation genetics hold incredible promise for uncovering many of the patterns and processes related with the area's carnivores. Most of the details concerning the spread of carnivores during and after the formation of the land bridge between North and South American during the Pleistocene remain almost entirely unknown. The correlations between habitat distributions, the Andean mountains, and the major river systems (e.g. Orinoco, Amazon, Rio Negro, and Paraná) and carnivore evolution would be of great interest and utility. Most of the important life history parameters of these species are also poorly understood. Molecular tools might be particularly useful in large homogenous and difficult to access areas such as the Amazon rain forest. Molecular genetic data would provide the basis of a better understanding of the social ecology of Neotropical species and ideally would lead to more scientifically based management programs and more reasoned approaches to the protection of populations, species and habitats.

Several concrete steps would facilitate and promote increased use of these techniques. Initially, political and economic support for capacity building, both in terms of greater training of personnel and the establishment of laboratories equipped with appropriate technologies are crucial. This may perhaps not be as difficult to achieve as other expensive conservation initiatives. Significant advances in training and technology building can accompany efforts to improve human medicine and agricultural output as many of the approaches, methods, and techniques used in conservation genetics are similar to those being applied in human genetics labs and in those focusing on economically important agricultural species. As access to molecular genetic technologies increases and as more opportunities are available for their use, the integration of genetics into conservation and wildlife management will become increasingly common.

Increased availability of species-specific molecular genetic markers designed for Neotropical species and populations would also increase the use of the techniques discussed in this chapter. Once markers have been designed and tested, their application to other studies becomes more rapid, cheaper, and more productive as results of data collection can be analyzed in the context of other related studies and data sets. These studies, in turn, form the basis for more-integrated, long-term or comparative studies that begin to provide the most interesting insights.

The integration of molecular genetics into carnivore studies in the Neotropics could actually happen relatively quickly. Rapid progress in efforts to map and sequence the domestic dog and domestic cat genomes are already providing more precise and sophisticated methods and tools to study related species (O'BRIEN, 1995). If these advances are applied in well-designed studies to answer important questions relating to focal species in key locations, the results could be impressive. Studies of high profile, but isolated populations of jaguars or giant river otters, or of more common and broadly distributed species such as the ocelots, margays, and coatis, or of widespread but rarer species such as the bush dog would provide further incentive and motivation to extend efforts to other species, from carnivores to other mammals, vertebrates, and non-vertebrates. Knowledge and the utility of this knowledge are a self-feeding process, which once started becomes increasingly self-sustaining. Like other forms of technologies, once the genie of molecular genetics has been let out of the bottle, it is difficult to imagine working without the benefit of these improved approaches. The management of wild populations taking advantage of genetic information is still relatively new, but can help ensure the long-term survival of threatened or endangered species. This process would benefit not only the species we are studying and that we are interested in conserving, but ultimately would improve our understanding of the processes that have shaped our biological world. As we continue to alter our world and as global changes occur at an increasing pace, our ability to improve our understanding of molecular genetic processes may be crucial to the future quality of life of humankind, as well as the maintenance of our surrounding biodiversity.

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Capítulo 5
Population viability analysis and conservation planning

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Introduction

Unfortunately, wildlife conservation is often crises management. Changing environments, usually human-induced, produce tremendous pressures on populations and species, often to the point of significantly enhancing their probability of extinction. The major culprits are pollution, over-harvesting, habitat loss (and fragmentation), and introduction of exotic species. These factors drive once large, widely distributed species to become small fragmented populations. These small populations are now not only at risk of extinction due to those deterministic threats that originally caused the population decline, but also to threats caused simply because they are small. These "small population" threats include problems associated with inbreeding, chance fluctuations in vital rates (demographic variation) and fluctuations in population size due to environmental variation and catastrophes.

Wildlife managers and conservation biologists must evaluate the relative severity of the various threats facing the populations they manage and recommend conservation actions to mitigate the most serious threats. With this, conservation biologists have joined many others in the fields of environmental management in the process of risk assessment: quantitatively determining the risks and their impacts on populations and ecological communities.

The process of formal risk assessment has developed rapidly over the last few years (STERN; FINEBERG, 1996). Risk assessment involves four steps, which when applied to species survival and conservation include:

<u>Risk identification</u>: the process of identifying all possible factors that could be associated with the survival of a population and identifying the possible sources of risks associated with those factors;

<u>Risk Assessment</u>: an objective estimate (quantitative or qualitative) of both the probability of that factor occurring and the severity of its effect, usually with the intent of prioritizing or identifying the most significant factors;

<u>Risk Management</u>: identifying and implementing measures to reduce the risk of the most serious factors; and

Risk communication: the process by which the risk assessment and management is communicated to decision makers and stakeholders.

At its simplest level, risk assessment can be defined as:

$R = \{s_i, 1_i, x_i\}$

Where determining risk (R) requires identifying: 1) What can go wrong (s_i); 2) How likely is it to happen (l_i); and 3) if it does happen, what are the consequences (x_i) (Kaplan 1981), (MORLEY, 1993). This fundamentally involves compiling a list of the hazards and estimating likelihoods and consequences for each. These estimates can be qualitative or highly quantitative depending on the amount of information available and the objective of the risk assessment.

A major concern of risk assessment is that often risk hazards affecting population survival are extremely complex, poorly understood and involve significant uncertainty. Furthermore, risk factors will often interact with other risks as well as characteristics of the ecology, population and environment. Often, without a quantitative risk assessment these complex and interacting risks are evaluated intuitively and subjectively based on the evaluator's individual experiences and training (e.g. a geneticist might give more weight to genetic threats than might an ecologist). This can be problematic as: 1) intuition will differ depending on who is making the evaluation; 2) intuition is often incorrect, particularly in highly complex situations.

The problem with intuition

One of the principal benefits of quantitative risk assessment approaches is that they are able to test our intuitive hypothesizes about the degree of risk in complex situations. It is well documented that most humans are notoriously poor at intuitively arriving at correct answers when faced with complicated probability assessments (PIATTELLI-PALMARINI, 1994). Often our intuitive perception of risks or the likelihood of events are not realistic.

An interesting illustration of how difficult it sometimes is to think through a simple probability problem can be found with the "Three Door", or "Monty Hall Problem" (PIATTELLI-PALMARINI, 1994). The problem gets its name from the popular TV game show, "Let's Make A Deal," but is not strictly based on the game rules. A contestant is given the opportunity to select one of three closed doors. Behind one of the doors is a highly valued prize, behind the other two doors are booby prizes. The contestant announces his selection, but the door stays closed. Monty Hall, the host who knows which door holds the prize, will then open one of the two remaining doors that does not have the prize (he will always pick a door without the prize). He then asks the contestant if the contestant would like to switch his selection to the other unopened door, or stay with his original door. Here is the question: What should the contestant do? Switch, stay with the original door, or doesn't it matter? Consider it for a moment and make a decision.

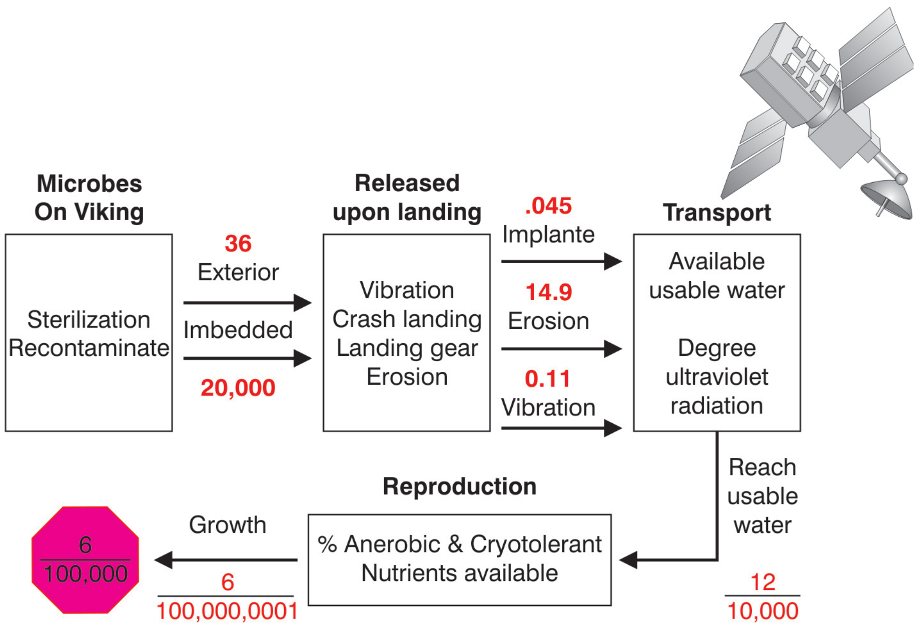
The answer is surprisingly counter-intuitive and clearly illustrates the sometimes flawed intuition with which we commonly approach even simple questions of probability and likelihood. Quantitative risk assessment tools help avoid these counter-intuitive problems by helping us systematically step through a problem and carefully identifying the three components of risk assessment: 1) what is the risk factor; 2) how likely is it; and 3) what are the consequences? This process helps in avoiding such mistakes as high risk situations being labeled as low risk and visa versa, as is shown in the case study below.

Case study: NASA risk assessment and mars exploration

This case study is provided by North (1995), and represents an interesting example of the use of a more quantitative approach to assessing risks. While it involves interplanetary exploration, it is not dissimilar to evaluating decisions that might be faced in conservation biology: the risks of translocating diseased animals between populations.

In the early 1970s the United States and Soviet Union were interested in sending an unmanned space probe to the planet Mars. The United States initiated the Viking program with the intent of landing the Viking I unmanned module on our sister planet on the date of our bicentennial, July 4, 1976. One concern was the possibility of contaminating Mars with microbes carried by the spacecraft from Earth. At that time, scientists believed that Mars might have microhabitats with enough water and nutrients to allow microbial reproduction. Some scientists were concerned that the likelihood of contamination was quite high and that the Viking program was too risky to proceed. The decision was made to proceed with the mission only if it could be done in such a way that the risk of a microbe replicating on Mars was less than 1/10,000. A detailed risk assessment was conducted.

The approach taken was to assess the probability of a specific sequence of events taking place. The first step was to estimate how many microbes might survive the sterilization process, recontaminate the craft and survive the voyage (Figure 1). The estimate was 20,036, with the majority, 20,000, imbedded in plastic insulators inside the craft. Next was a consideration of how many microbes might be released: 1) due to vibrations as the vessel landed; 2) if the vessel crash landed; 3) from the landing gear implanting microbes into the soil; and 4) due to Martian dust storms eroding the plastics and releasing imbedded microbes into the atmosphere. The risk assessors estimated that on average 15.055 microbes could be released. Next considerations of transport: what was the probability of a released VTO (viable terrestrial organism) reaching a hospitable environment taking into consideration such factors as ultraviolet radiation and availability of usable water. This was estimated as 1.2 in 10,000. Lastly they considered the probability of a microbe actually reproducing, given that it reached a hospitable habitat. This was considered to be 6 in 1,000,000. Overall, taking these (and other) frequencies and probabilities into consideration, it was estimated that the likelihood of a VTO reproducing on Mars was about 6 out of 100,000 (NORTH, 1995), which was lower than the maximum tolerance of 1 out of 10,000. The mission proceeded as planned.



North et al. 1975

Figure 1 – Risk assessment of VTO contamination

The scientists involved in this risk assessment found the process of determining the risk to be just as useful as the numerical results (i.e. estimating the probability of VTO contamination). By systematically identifying the points of concern, working through each step, and visually illustrating the problem (Figure 1), the scientists were able to clearly articulate the problems involved, identify the uncertainties, and communicate their reasoning to a wider audience. The process also helped scientists and managers understand exactly how the information they provided was being used in the analysis. The process further showed that the scientists' initial intuitive impressions of the risk were overestimated; the more quantitative analysis convinced the skeptics that in fact the risks were acceptable. (The decisive point was that to reach water, the microbes would have to be small enough to sustain themselves in the atmosphere over long distances; yet small size increased vulnerability to ultraviolet radiation). Overall the process of systematically and quantitatively assessing the risks not only led to an estimate of the risk but also showed that initial subjective, intuitive impressions of the risk were incorrect (NORTH, 1995).

Quantitative risk assessment in conservation biology: population viability analysis (PVA)

The conservation community's preferred method of quantitative risk assessment is Population Viability Analysis (PVA) or Population and Habitat Viability Analysis (PHVA), a systematic and quantitative evaluation of the factors affecting the probability of extinction and, often, loss of genetic variation in a population (BOYCE, 1992; BEISSINGER, MCCULLOUGH, 2002). This approach considers the effects of multiple factors (life-history, reproductive and survival rates, geographic distribution, susceptibility to environmental variation, genetic factors such as inbreeding depression, likelihood of catastrophes, severity of catastrophes, etc.) on long-term population survival and the probability of the population going extinct.

A number of PVA computer models are available for simulating the deterministic and stochastic (random) properties of populations and the factors that affect them. One model, VORTEX (LACY, 1993), is used by the Conservation Breeding Specialist Group (CBSG) as a tool to help develop conservation plans for endangered and threatened wildlife (ELLIS; SEAL, 1995). This model uses data on the mean and variance of the population's life-history characteristics (as well as data on factors that affect these rates) to repeatedly simulate the growth of a population over a given time frame. Extinction probabilities are determined by the number of times the population goes extinct relative to the number of simulations run. Genetic diversity is also modeled to determine the impact of population structure on retention of genetic diversity in the population.

PVA example: viable populations of golden lion tamarins

The golden lion tamarin (GLT; *Leontopithecus rosalia*), an endangered Brazilian primate, has been the subject of an extensive international conservation program since in early 1970s (KLEIMAN; MALLINSON, 1998). This program has twice used Population Viability Analysis in workshops facilitated by the CBSG to help direct conservation and research efforts (BALLOU; LACY; ELLIS; 1996; SEAL; BALLOU; PADUA, 1990). Data used in the 1997 PHVA are derived from 13 years of field data collected on wild tamarins (BAKER; DIETZ, 1996; DIETZ et al., 2000). As the GLT population is highly fragmented in the wild, one fundamental question asked by conservation biologists relates to the degree of vulnerability of populations relative to the size of the population. PVA, using the Vortex software, was used to determine the correlation between size and extinction risk present graphic of population size, and probability of extinction.

Uses of PVA

PVA models, like other risk assessment approaches, provide an opportunity to systematically, quantitatively and objectively evaluate the effects of a particular threat to a population. Lack of data, incomplete understanding of the threats involved, and the complexity of the problem as a whole often hamper such analyses. Because of this, risk assessments can not be expected to provide exact predictions of extinction probability. Nevertheless, relative comparisons can be made (e.g., recognizing that the risk of a hepatitis epidemic of 1% only marginally increases extinction risk relative to the baseline scenario). Conservation biologists can evaluate the relative effects of different risk factors, different scenarios, and different management actions to help formulate details of management actions. As in the case of the Viking I explorer above, simply going through the process of assessing the risks using population viability models leads to a heightened understanding of the problem as a whole. The CBSG, US Fish and Wildlife Service and others frequently take advantage of this attribute of PVA modeling as a facilitation tool when developing conservation programs (ELLIS; SEAL, 1995).

Do PVA's work?

If PVAs are going to be used extensively in conservation planning, then it is important to know if they work: do they provide a reliable and usable estimate of the probability of extinction (or other diagnostic of population viability, e.g. future population size; retention of genetic diversity)? There is considerable literature of this subject (AKÇAKAYA; SJÖGREN-GULVE, 2000; BEISSINGER; MCCULLOUGH, 2002; BOYCE, 1992; BROOK et al., 2000; ELLNER et al., 2002; REED et al., 2002; SHAFFER et al., 2002).

On the one hand, Beissinger e Westphal (1998) and others are seriously apprehensive about the use of PVAs for conservation planning because they are often unreliable due to poor data quality (e.g., too much uncertainty in estimating parameter rates and their variances), and lack of information on dispersal (e.g., distances, ages, mortality, and movement patterns, which are critical for evaluating extinction in fragmented populations). There is also concern about the accuracy of incomplete models: those that do not include the full extent of environmental and catastrophic variation affecting populations over the long term or those that ignore density dependence (COULSON et al., 2001).

On the other hand, there are those who recognize that PVAs will almost always be limited by poor data, assumptions about unknown parameters and difficulties in estimating parameters due to lack of long-term studies, yet still find significant value in using PVAs for conservation planning (BROOK et al., 2002; BROOK et al., 2000; BURGMAN; POSSINGHAM, 2000; LACY, 1992; REED et al., 2002; SHAFFER et al., 2002). Here the value comes in many forms, including:

● Providing a forum for thinking through a problem. As in the example above of the Mars exploration, the risk assessment process in itself provides a structure for organizing information needed to solve conservation programs and identifying critical data or information that may be missing. Stepping through a demonstration of PVA demonstrates the type of data needed for thinking about population viability, even if those data are not available.

● A facilitation tool. When appropriate, the CBSG uses the PVA models to focus discussion on the biological issues of a conservation program. This is particularly useful in a workshop where participants likely hold widely divergent views of the conservation problems involved and where facilitated discussion is a critical factor in success of the workshop.

● An education tool. Many of those involved in conservation planning (e.g., administrators, educators, policy makers) have little or no training or experience in population biology and are thus unable to evaluate the effect of specific factors on population processes. Using PVAs to illustrate the effect of specific threats (e.g., increased poaching, declining habitat) on probability of extinction clearly demonstrates the population biology processes involved.

Brook et al. (2002) also argue that even when data are sparse, use of PVA is still often more appropriate than vaguely defined alternatives that may be less able to deal with uncertainty and more susceptible to subjective intuitive reasoning. PVA provide a transparent way to: specifically identify which data are being used to make decisions, how the data are being used, and allow for testing of alternative assumptions.

What we have learned from PVA analyses

The field of PVA is now in its 25th year (one of the earliest PVAs was conducted by Mark Shaffer in 1978 on grizzly bears in Yellowstone National Park - (SHAFFER, 1978) and PVAs have been extensively used for a variety of purposes in many different species, including plants and invertebrates. Furthermore, more recently PVAs have been used as a research tool to explore the general issues of population vulnerability and conservation planning. What has this accumulated history taught us about population viability?

<u>The need for long-term datasets</u>: One of the most important factors is that accurate PVAs require extensive data from long-term field studies (SHAFFER et al., 2002). Long-term studies are needed to detect rare fluctuations in environmental parameters and population size that have significant effects on viability over the long term (PIMM; REDFEARN, 1988). In a study of 102 species, Reed et al. (2003) found that the population size needed to survive with 99% probability for 40 generations increased by 67% with a doubling of study length. Presumably, this occurred because data used to parametrize PVA models that were based on longer studies had higher catastrophes and parameter variances. Populations needed to be larger to survive. Data based on short studies underestimated extinction probabilities.

The need for redundancy: In their review of PVAs, Shaffer et al. (2002) also conclude that given the size and distribution of most populations of conservation interest, redundant populations are needed. Single solitary populations are often too vulnerable to be of long-term conservation value. Multiple populations in habitats that experience independent environmental variation and independent catastrophes are significantly less vulnerable to extinction.

The need for large populations: Many studies directly or indirectly demonstrate that the larger a population is, the less likely it will go extinct (BERGER, 1990; SHAFFER, 1981; GRIFFITH et al., 1989; PIMM et al., 1988; GOODMAN, 1987; SOULE, 1987). But how large? SOULE, (1987) suggest "several thousand" were needed. Reed et al. (2003) in the of 102 species study previously mentioned found that median number of adults needed to ensure 99% chance of surviving for 40 generations was about 5800-7000.

Default data on catastrophes: Rare but severe catastrophes have significant but usually underestimated impacts on population viability (BEISSINGER, WESTPHAL, 1998). Many PVAs ignore catastrophes because their frequency and severity are difficult to predict and measure. While this is true of many parameters, some parameters can be estimated from other species, or default values based on average estimates across many species (e.g. lethal equivalent estimates for modeling inbreeding depression (RALLS et al., 1988). We may now have default values to use in cases where species specific values are unavailable: In a recent review of the frequency and severity of catastrophes across 88 species, Reed et al. (in press) found that the probability of a severe population die-off (50% or greater reduction in population size) was 14% per generation. Future PVAs may be able to use this rate to examine "average" effects of catastrophes where none are available.

Most important data: What data are the most critical for evaluating a population's vulnerability? The amount of data needed for a complete accurate PVA is beyond the resources of all by a very few long-term field studies. With limited time and resources yet faced with the need to quickly evaluate a species or population's vulnerability, which factors best predict a species probability of extinction? Again, multiple species analyses help provide an answer. Examining 16 factors commonly used to predict extinction risk, O'Grady et al. (Submitted) found that population size and trend in population size alone explained 40% of the variation in time to extinction. Adding information on degree of fragmentation, fecundity and taxonomic status explained almost 70% of the variation in time to extinction. Clearly population size, trends in size and degree of fragmentation over time are critical parameters for evaluating extinction risk (GOODMAN, 1987; BEISSINGER; WESTPHAL, 1998).

Conclusion

Population viability analysis has become an important tool for conservation planning. While there are significant concerns about the reliability of PVAs for estimating accurate extinction probabilities for species with limited data (which covers almost all species of conservation concern), PVAs are nevertheless recognized as a valuable tool for conservation planning when used as a tool to facilitate discussions, educate non-biologists and determining what is and is not known about a specific population's status. Burgman and Possingham (2000) correctly state that "traditional views about population models and decision-making must be suspended and reviewed" when evaluating the conservation use of PVAs.

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Parte III
Conservação *in situ*

Capítulo 6
Unidades de conservação e seu papel na conservação de carnívoros brasileiros

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Introdução

A destruição e a degradação dos ambientes naturais em todo o mundo induzem a que milhares de espécies estejam em processo de extinção. Alguns ambientes estão sob sério risco, com a maior parte de sua cobertura original já destruída (MYERS et al., 2000), e a maior parte da biodiversidade pode não ser capaz de sobreviver sem efetiva proteção (BRUNER et al., 2001). A criação de áreas protegidas ou unidades de conservação (UC), como são chamadas no Brasil, atendem à necessidade de se conservar amostras intactas de ambientes naturais que, de outra forma, estariam sujeitos à degradação ambiental e perda de espécies. Entretanto, a eficácia destas áreas para o alcance dos objetivos de conservação da biodiversidade tem sido questionada (WWF, 1999; BRUNER et al., 2001). Este capítulo tem o objetivo de discutir o papel de UCs para a conservação de Carnívoros no Brasil. Para avaliar o sistema atual de unidades de conservação compilamos informações disponíveis sobre as unidades de conservação federais de proteção integral, que constam do site na Internet do Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – Ibama (www.ibama.gov.br). Para as UCs estaduais, utilizamos a compilação do GeoBrasil (2002). Dados sobre a ocorrência de espécies nas UCs foram compilados de planos de manejo, relatórios e outras publicações.

As UCs brasileiras

O Sistema Nacional de Unidades de Conservação (Lei nº. 9.985, de 18 de julho de 2000), institui doze categorias de UCs, divididas em dois grupos com características específicas:

– UCs de Proteção Integral, que têm como objetivo básico a preservação, permitem a utilização apenas indireta dos recursos naturais e englobam os parques (PN – parques nacionais; PE – parques estaduais), estações ecológicas (Esec), reservas biológicas (Rebio), monumentos naturais (MN) e refúgio de vida silvestre (RVS); e

– UCs de uso sustentável, que visam compatibilizar a conservação da natureza com o uso sustentável dos recursos naturais e englobam áreas de proteção ambiental, áreas de relevante interesse ecológico, florestas nacionais, reservas extrativistas, reservas de fauna, reservas de desenvolvimento sustentável e reservas particulares do patrimônio natural. Esta última categoria, apesar de classificada formalmente entre as de uso sustentável, funciona na prática como de proteção integral, já que o único artigo da lei que previa seu uso direto foi vetado.

Entendemos que a conservação de carnívoros está relacionada com o manejo de toda a paisagem, isto é, deve envolver as áreas protegidas como parte dessa paisagem. Porém, como o foco deste trabalho são as áreas protegidas, analisaremos apenas as UCs de proteção integral e as reservas particulares do patrimônio natural (RPPN).

As UCs de proteção integral e as RPPNs cobrem 3,58% do território nacional (Tabela 1), valor bem aquém do mínimo sugerido de 10%, tanto no geral quanto para os biomas separadamente, à exceção de ambientes costeiros. Na floresta Amazônica e Mata Atlântica está o maior número de UCs federais de domínio público, enquanto que a maioria das RPPNs encontra-se na Mata Atlântica e no Cerrado (Tabela 2). A proporção de área protegida por reservas particulares em relação às UCs federais de domínio público é baixa, exceto para o Pantanal, onde 58% da área protegida constituem RPPNs. Na Amazônia há maior área protegida, seguida por Cerrado e ecótono Cerrado-Amazônia, e Mata Atlântica (Tabela 1, Figura 1). As UCs amazônicas são, em média, muito maiores que as de qualquer outro ambiente (Figura 2).

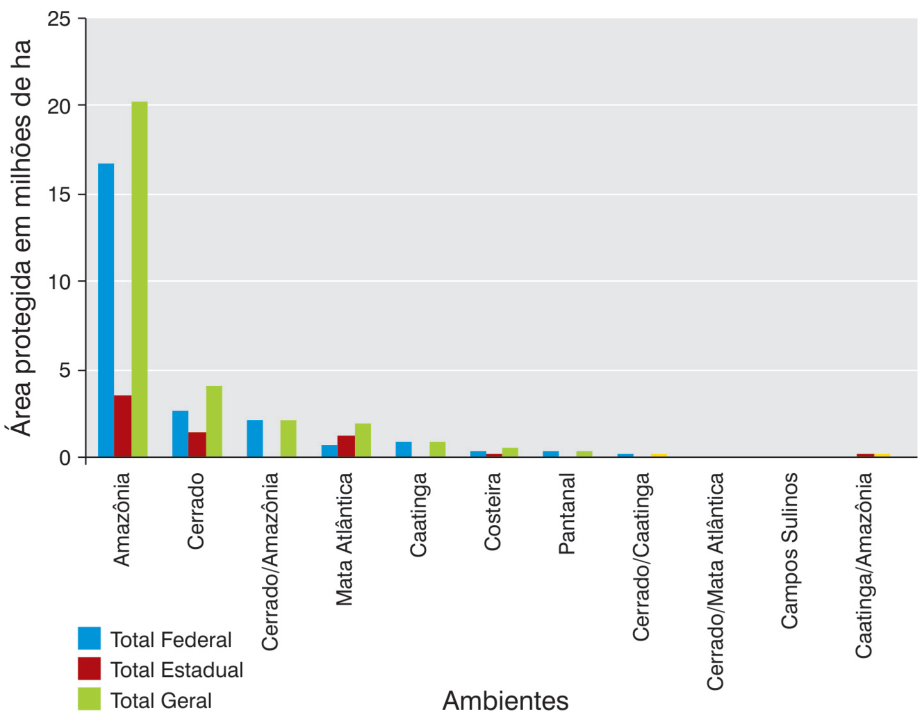
Tabela 1 – Área ocupada por unidades de conservação e proporção de áreas conservadas em cada bioma brasileiro.

Áreas	Área ocupada ha	UCs federais ha	% federal	UCs estaduais ha	% estadual	Total ha	% total
Amazônia	368.896.022,37	16.756.456,30	4,54	3.485.406,08	0,94	20.241.862,38	5,49
C/A	41.700.717,92	2.151.215,00	5,16	4.124,74	0,01	2.155.339,74	5,17
C/Ca	11.510.813,00	216.500,00	1,88	3.946,12	0,03	220.446,12	1,92
Caatinga	73.683.115,53	793.172,76	1,08	2.886,16	0,00	796.058,92	1,08
Cerrado	196.776.092,28	2.598.290,33	1,32	1.401.123,38	0,71	3.999.413,71	2,03
Campos Sulinos	17.137.704,54	63.168,27	0,37	0,00	0,00	63.168,27	0,37
Ca/A	14.458.259,63		0,00	233.833,73	1,62	233.833,73	1,62
Costeira	5.056.768,47	367.074,72	7,26	136.893,05	2,71	503.967,77	9,97
Mata Atlântica	110.626.617,41	651.979,46	0,59	1.237.021,15	1,12	1.889.000,61	1,71
Pantanal	13.684.530,26	352.455,01	2,58	224,41	0,00	352.679,42	2,58
C/MA	0	78.875,00		0		78.875,00	
TOTAL	**853.530.641,41**	**24.029.186,85**	**2,82**	**6.505.458,82**	**0,76**	**30.534.645,67**	**3,58**

Tabela 2 – Unidades de conservação federais no Brasil.

Unidades	UCs federais (ha)	RPPNs federais (ha)	Total (ha)	Nº UCs fede-rais	Nº RPPNs fede-rais	Total	Média UCs federais (ha)	Média RPPNs (ha)
Amazônia	16.736.354,75	20101,55	16.756.456,30	28	37	65	597726,96	543,29
C/A	2.151.215,00		2.151.215,00	5		5	430243,00	
C/Ca	216.500,00		216.500,00	3		3	72166,67	
Caatinga	729.113,20	64059,56	793.172,76	8	30	38	91139,15	2135,32
Cerrado	2.537.644,55	60645,78	2.598.290,33	13	98	111	195203,43	618,83
Campos sulinos	59.822,63	3345,64	63.168,27	4	11	15	14955,66	304,15
Costeira	363.499,39	3575,33	367.074,72	14	5	19	25964,24	715,07
Mata Atlântica	604.200,33	47779,13	651.979,46	27	174	201	22377,79	274,59
Pantanal	146.200,00	206255,01	352.455,01	2	12	14	73100,00	17187,92
P/C/MA	78.875,00		78.875,00	1		1	78875,00	
TOTAL	**23.623.424,85**	**405.762,00**	**24.029.186,85**	**105**	**367**	**472**		

Figura 1 – Área, em unidades de conservação, em cada bioma brasileiro.



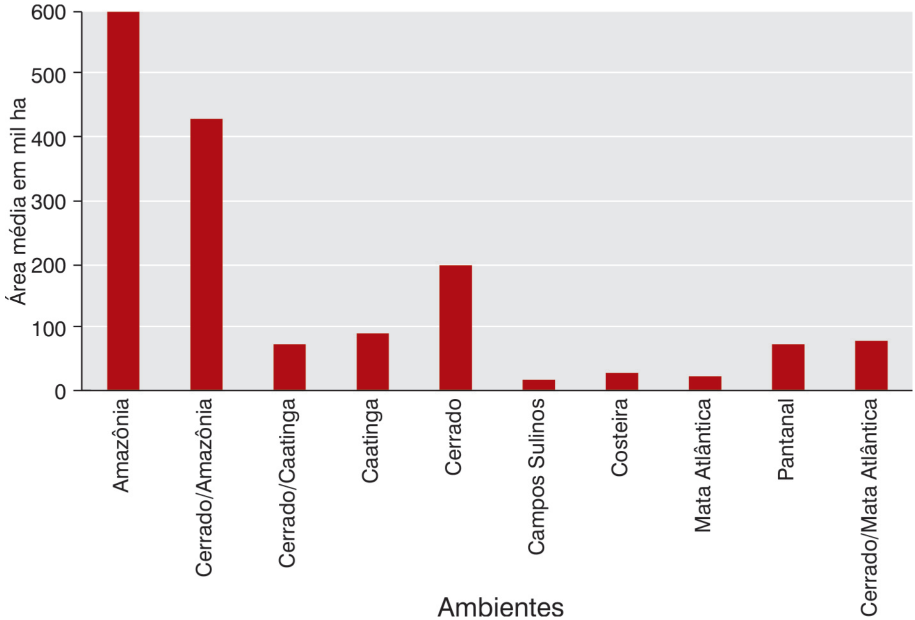


Figura 2 – Tamanho médio das unidades de conservação em cada bioma brasileiro.

O tamanho das UCs traz conseqüências diretas para a conservação de animais que requerem grandes áreas, como os carnívoros. A Figura 3 apresenta a distribuição de tamanhos de UCs nos cinco ambientes com maior número de unidades. A quase totalidade (96%) das UCs amazônicas possui mais de 50 mil ha e 82% mais de 100 mil. Há ainda 3 UCs com mais de 1 milhão de ha, o que não ocorre em nenhum outro ambiente. Na região do Cerrado a maior parte das UCs está na faixa entre 100 e 500 mil ha, porém, nesta faixa, nenhuma delas ultrapassa 250 mil ha. Há ainda duas UCs com mais de 500 mil ha. Quando analisamos os outros ambientes, a distribuição de tamanhos das unidades é bem distinto, e a maior parte delas possui tamanho pequeno. Na Mata Atlântica, a maior parte (44%) das UCs encontra-se na faixa entre 10 e 50 mil ha e 92% têm menos de 50 mil ha. Na Caatinga, 87,5 % das UCs têm menos de 100 mil e, na zona Costeira, 86% têm menos de 50 mil ha.

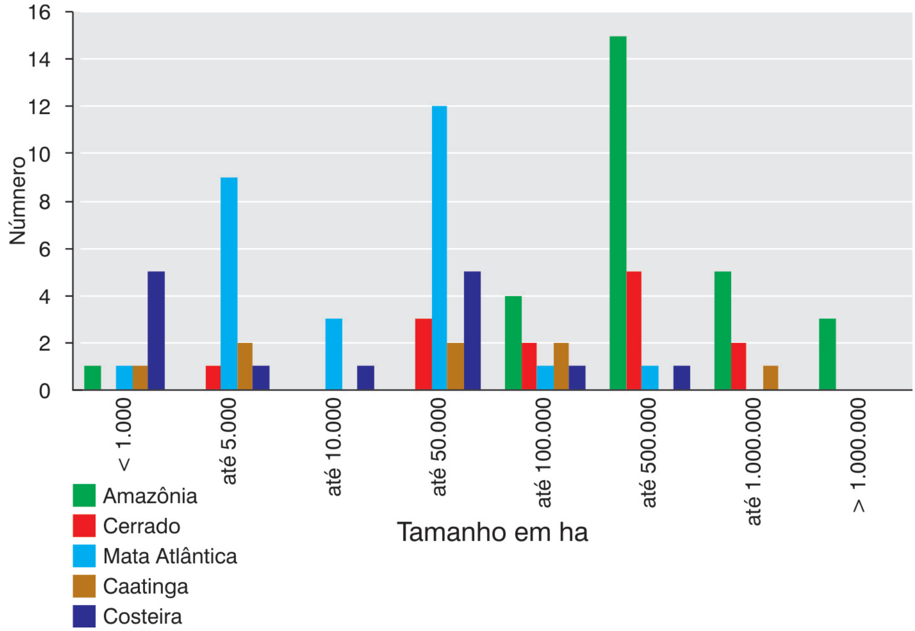


Figura 3 – Distribuição de tamanho das UCs, por bioma.

Carnívoros em UCs

As UCs têm a finalidade de proteger toda a biodiversidade regional, porém são particularmente importantes para a conservação de algumas espécies que, em algumas paisagens, dificilmente encontram possibilidade de sobreviver sem essas áreas conservadas. A lista oficial brasileira das espécies da fauna ameaçadas de extinção (MMA, 2003) reconhece que 10 táxons de mamíferos da ordem Carnivora estão em alguma categoria de ameaça (todas na categoria "vulnerável", seguindo critérios da IUCN), incluindo duas subespécies de *Puma concolor* (onça-parda/vermelha) e uma de *Leopardus pardalis* (jaguatirica/maracajá-verdadeiro). Três espécies estão na categoria "quase ameaçada" e outras três foram consideradas com "dados insuficientes" portanto, não puderam ser analisadas por falta de dados. Entretanto, é quase certo que pelo menos duas destas devam ser enquadradas em algum nível de ameaça quando houver dados disponíveis.

A informação sobre a fauna de grande parte das UCs é escassa, dificultando uma análise precisa sobre a ocorrência de espécies de carnívoros. Conseguimos informação, ainda que parcial, sobre a ocorrência das 14 espécies de carnívoros ameaçados, quase ameaçados ou com dados insuficientes, em 51 UCs. Das UCs avaliadas na região do Cerrado (n = 29), *Chrysocyon brachyurus* (lobo-guará) esteve presente em todas, enquanto *P. concolor* (79%), *Lontra longicaudis* (lontra - 72%) e *L. pardalis* (69%) também foram bem representadas. *Leopardus tigrinus* (gato-do-mato-pequeno) e

Leopardus wiedii (gato-maracajá) estiveram presentes em apenas 17% das áreas e *Panthera onca* (onça-pintada) em 14%. Os carnívoros desse grupo menos representados em UCs do Cerrado foram *Speothos venaticus* (cachorro-do-mato-vinagre), *Leopardus colocolo* (gato-palheiro, ambos registrados em 10% das UCs) e *Pteronura brasiliensis* (ariranha - 3% das UCs). O naturalmente raro *S. venaticus* esteve presente apenas no Parque Nacional das Emas, Parque Estadual de Mirador e no Parque Nacional do Araguaia, este último já na região de ecótono com a Floresta Amazônica. Também está presente no recentemente criado Parque Nacional das Nascentes do Parnaíba (Oliveira, no prelo a). Já *P. brasiliensis* foi registrada apenas no Parque Nacional do Araguaia. Na região da Mata Atlântica, *P. concolor* e *L. pardalis* foram encontradas em 69% das 13 UCs inventariadas, *L. longicaudis* em 53%, enquanto *P. onca* e *L. tigrinus* estiveram presentes em 46% das UCs. *L. wiedii* foi registrado em 38% das UCs, *C. brachyurus* (23% cada), enquanto *P. brasiliensis* e *S. venaticus* (8%) foram as espécies registradas em menor número de UCs. As duas últimas espécies estiveram presentes apenas no PN Iguaçu, mas *P. brasiliensis*, possivelmente, também pode ser encontrada nas Reservas Biológicas de Sooretama e Poço das Antas, de acordo com os planos de manejo dessas UCs. No entanto, é improvável a sua ocorrência nessas duas Rebios, o que reduziria a ocorrência da espécie a apenas uma das áreas analisadas. *C. brachyurus* foi pouco representado em UCs de Mata Atlântica (apenas nos PNs Itatiaia, Serra da Bocaina e Caparaó), porém esse não é seu ambiente normal de ocorrência, tratando-se assim de ocorrências fortuitas, provavelmente devido à expansão de distribuição em função de desmatamento de florestas. Na região amazônica obtivemos informação de apenas sete UCs. Em todas elas foram encontradas *S. venaticus* e *P. brasiliensis*, enquanto 86% registraram a presença de *P. onca, P. concolor, L. longicaudis, L. pardalis* e *L. wiedii.* Apesar de não estar tradicionalmente associado a este bioma, *L. tigrinus* foi registrado em quatro UCs (57%) e os raros *Atelocynus microtis* (cachorro-do-mato-de-orelha-curta), e *Mustela africana* (doninha-amazônica) em 43% das UCs amostradas. *Bassaricyon gabbii*/*beddardi* (gogó-de-sola), cuja distribuição no Brasil não é bem conhecida, esteve presente em 28% das áreas. Nos demais biomas não foram obtidos dados suficientes para essa análise.

Apesar de os dados apresentados acima estarem sujeitos a fortes vieses, uma vez que as espécies de grande porte são registradas mais facilmente que as menores (por serem mais conspícuas e despertarem mais o interesse das pessoas da região) e pela tendência de as presenças serem mais relatadas que as ausências, os resultados indicam que, tanto na Mata Atlântica quanto no Cerrado, as espécies que menos estão representadas em UCs são *P. brasiliensis* e *S. venaticus*, estando as duas, portanto, sob maior risco. Outra influência na análise é que a maioria das UCs de pequeno tamanho não foram contempladas na análise. Mas isto só reforça o risco que as duas espécies mais raramente registradas na nossa análise estão sofrendo, uma vez que elas estão ausentes na maioria das pequenas UCs isoladas. Mas o fato de algumas espécies estarem bem representadas em nossa análise para o Cerrado e Mata Atlântica, não significa que estão fora de risco, uma vez que a densidade populacional é, por vezes, muito baixa e poucas UCs mantêm populações viáveis de espécies de grande porte ou predadoras de topo.

As espécies de ampla distribuição geográfica nos biomas brasileiros e que não são consideradas ameaçadas de extinção tendem estar bem representadas e com populações teoricamente robustas, pelo menos numa boa parcela das UCs de maior tamanho. Isso deve ser verdadeiro principalmente para as áreas amazônicas, apesar de ainda estarem mal amostradas quanto à sua composição faunística. Nessa categoria estariam *Cerdocyon thous* (cachorro-do-mato/raposa), *Nasua nasua* (quati), *Procyon cancrivorus* (mão-pelada/guaxinim), *Eira barbara* (irara/papa-mel), *Potos flavus* (jupará – especiamente na Amazônia) e, numa escala bem mais reduzida, talvez *Herpailurus yagouaroundi* (jaguarundi/gato-mourisco) e as populações amazônicas de *L. pardalis. Pseudalopex gymnocercus* (graxaim-do-campo), de distribuição restrita no Brasil, estaria muito mal representado em UCs, ocorrendo em apenas duas áreas, mas de qualquer maneira, não aparenta estar inerentemente ameaçado fora das áreas protegidas do Sul do país. As espécies não ameaçadas de áreas abertas, como *Conepatus semistriatus* e *C. chinga* (jaritataca/cangambá, zorrilho) e *Pseudalopex vetulus* (raposa-do-campo), também devem apresentar situação, de certo modo, confortável. Já a situação dos furões (*Galictis vittata* e *G. cuja*), seria incerta, mais por carência de informações, do que, necessariamente, por não serem encontrados em áreas protegidas de tamanho suficiente para manter populações geneticamente viáveis em longo prazo. Das espécies proximamente ameaçadas, *L. longicaudis* e *P. concolor* estariam bem representadas nas UCs, mas não *Leopardus geoffroyi* (gato-do-mato-grande), confirmado em apenas uma UC. Daquelas consideradas com dados insuficientes, *B. gabbi*/*beddardi* deve apresentar populações saudáveis em pelo menos algumas das UCs da Amazônia, enquanto *A. microtis* seria encontrado numa boa parcela de UCs (LEITE-PITMAN; WILLIAMS, no prelo), entretanto, é possível que, por ser naturalmente rara, não disponha de população robusta talvez nem mesmo nas maiores UCs da região. A situação de *M. africana* deve ser semelhante, com o agravante de aparentar ser ainda bem mais rara que a anterior.

Dos táxons ameaçados, *C. brachyurus* (lobo-guará) está bem representado em UCs. *Panthera onca* (onça-pintada) também deve apresentar situação confortável para as UCs da Amazônia, mas críticas para os outros biomas, em especial a caatinga (Oliveira, 2002). Já as populações ameaçadas de *Puma concolor* (*P. c. greeni* e *P. c. capricornensis*) muito certamente não devem ter populações geneticamente viáveis no longo prazo se ficarem restritas às UCs isoladas. *L. wiedii* (gato-marcajá/gato-peludo) quase certamente apresenta populações razoáveis, pelo menos nas áreas amazônicas. Entretanto, o mesmo não pode ser dito de *L. tigrinus*, a qual aparenta ser muito rara nesse bioma, que detém as maiores unidades e é o porto seguro para a maioria das espécies do país. Para essa espécie, as UCs do Cerrado, da Mata Atlântica, e as maiores da Caatinga devem ser mais importantes para garantir sua sobrevivência no longo prazo (OLIVEIRA, no prelo b). Conforme citado, a situação é mais preocupante para *S. venaticus* e *P. brasiliensis*, especialmente fora da bacia amazônica.

Efetividade das UCs

O Brasil possui atualmente 105 UCs federais de proteção integral (49 PN, 24 Rebio, 29 Esec, 1 RVS e 2 Reservas Ecológicas, categoria que não consta da lei do Sistema Nacional de Unidades de Conservação e terá que ser reclassificada). O número de reservas e áreas protegidas vem crescendo muito nos últimos anos, o que representa um avanço conservacionista no Brasil. Porém, temos que analisar o quanto essas unidades são efetivas para o cumprimento de suas funções. O plano de manejo de uma UC é o documento que vai direcionar as atividades de administração, fiscalização, visitação, pesquisa, etc. dentro de cada unidade e, portanto, é muito importante para que estas atividades sejam gerenciadas da melhor forma possível. A elaboração de um plano de manejo é requisito obrigatório por lei e deve ser revisado a cada cinco anos, para adequar a unidade às mudanças ocorridas no entorno, às informações adquiridas desde a última revisão e às experiências adquiridas no manejo da UC. Apenas 37 (35%) das UCs de proteção integral federais possuem um instrumento de planejamento da área e destas apenas 16 (15,2 %) têm esses documentos revisados nos últimos cinco anos. Isso tem implicação direta no grau de implementação dessas UCs. Segundo dados do WWF (1999), a maior parte (54,6%) das UCs brasileiras estava em situação precária em relação ao grau de implementação, 37% estavam minimamente implementadas, 8,4% razoavelmente implementadas e nenhuma plenamente implementada. Ainda segundo esse documento, apenas as razoavelmente implementadas estariam aptas a enfrentar satisfatoriamente as crescentes pressões enfrentadas por áreas naturais. Algumas dessas Unidades não possuem nem um chefe, por vezes nenhum funcionário, não tem terras devidamente demarcadas e existem basicamente nos decretos que as criaram (WWF, 1999).

Um outro ponto de extrema relevância seria a efetividade das UCs para conservação, no longo prazo, de algumas espécies notadamente as raras e/ou aquelas predadoras de topo, i.e., as que necessitariam de maiores áreas. Isto em função do reduzido tamanho da maioria das UCs, e, num cenário de isolamento, sem conectividade com outras áreas e sem manejo. Se considerarmos uma população efetiva de 500 animais para conservação no longo prazo, como aquela capaz de manter >90% da heterozigosidade (FRANKLIN, 1980) e levando-se em conta que os felinos têm baixa relação do tamanho efetivo da população com o tamanho observado (Ne = 0,4N), isso implicaria um total de 1.250 indivíduos adultos na população (OLIVEIRA, 1994). Considerando-se a densidade de um predador de topo como *P. onca* (onça-pintada – média = 0,029 indivíduos/km^2: 0,013 – 0,067/km^2) (OLIVEIRA, 2002), seriam necessárias UCs com tamanho médio de 4.310.300 ha para manter populações viáveis. Por outro lado, tomando-se a estimativa média (mais abrangente e precisa) apresentada por Reed et al. (2003) de 7.316 indivíduos adultos como a necessária para manter populações mínimas viáveis com 99% de chance de sobrevivência por 40 gerações, a área requerida para *P. onca* seria de exorbitantes 25.227.600 ha. Isto quer dizer que, tomando a densidade média, nenhuma das UCs do Brasil poderia manter populações viáveis no longo prazo, e apenas as três maiores unidades, os Parques Nacionais Montanhas do Tumucumaque, Jaú e Pico da Neblina, estariam mais próximas dessa condição no primeiro cenário, e nenhuma na segunda estimativa. Assim, apesar de as UCs amazônicas serem as maiores, mesmo essas seriam incapazes, isoladamente, de manter populações viáveis desse felino.

Teoricamente, protegendo-se populações dos grandes predadores de topo, protegeríamos as das demais espécies também. Mas quão eficientes seriam as UCs para aquelas espécies raras, de distribuição fragmentada e com baixíssimas densidades, como *S. venaticus, A. microtis* e *M. africana*? Tamanho corporal e posição trófica não devem ser usados para prever os requerimentos de área de uma espécie. Isso porque áreas capazes de manter os predadores de topo como *P. onca* podem não ser suficientes para manter populações viáveis de seus competidores menores (WOODROFE, 2001). Estes últimos necessitariam utilizar áreas maiores para adquirir recursos necessários e, assim, apresentar densidades menores. Exemplo típico dessa situação nas planícies africanas seriam *Panthera leo* (leão) e *Lycaon pictus* (cão-selvagem-africano), tendo *P. onca*/*P. concolor*/*L. pardalis* e *S. venaticus* situação um pouco equivalente nos neotrópicos. Se assim for, aparentemente nenhuma UC brasileira isoladamente teria condições de manter populações isoladas geneticamente viáveis no longo prazo deste canídeo (Oliveira, no prelo *a*). O mesmo deve ser verdadeiro também nos casos em que o potencial de competição ou predação não seja significativo.

Apesar de ocorrer em todas as UCs da região do Cerrado, a situação de *C. brachyurus* (lobo-guará) não pode ser encarada como fora de risco, pois o tamanho efetivo das populações muitas vezes é insuficiente para manter populações viáveis no longo prazo. Rodrigues (2002) estimou que cinco casais reprodutivos desse canídeo utilizam os 10.000 ha da Esec Águas Emendadas. Assim, proporcionalmente, áreas mínimas de 500.000 ha ou 731.600 ha (critérios de FRANKLIN, 1980; REED et al., 2003, respectivamente) seriam necessárias para manter populações viáveis desta espécie. Apenas três das UCs federais na área de ocorrência da espécie têm essas características: o PN Araguaia, o PN Nascentes do Rio Parnaíba e a Esec Serra Geral do Tocantins, estes dois últimos formando um só complexo, além do PE Mirador (MA), e assim mesmo apenas pelo critério original populações mínimas viáveis de Franklin (1980). No entanto, apesar de abranger uma área grande, esta última UC tem uma população pequena de lobos-guarás (OLIVEIRA, 1996), e pode não ter alta significância para proteção da espécie. Assim, são conhecidas com segurança, apenas duas áreas com possível capacidade de manter, isoladamente, populações viáveis de lobos no longo prazo.

Desta forma, fica ressaltada a urgência em manter a conectividade entre UCs para preservar o potencial evolutivo/adaptativo das espécies. Por exemplo, o recém-criado PN das Nascentes do Rio Paranaíba, com 729.813,55 ha, está conectado com outras duas unidades: a Estação Ecológica Serra Geral do Tocantins e o Parque Estadual do Jalapão. Formam assim uma unidade com impressionantes 1.500.000 ha, de longe a maior área de Cerrado preservado do Brasil. A Rebio do Gurupi (342.600 ha) e as Áreas Indígenas adjacentes formariam uma unidade com cerca de 1.634.000 ha. Os PNs Montanhas do Tumucumaque, Pico da Neblina e Jaú estão conectados a diversas áreas conservadas (proteção integral e uso sustentável), resultando, cada um dos conjuntos, em áreas de mais de cinco milhões de ha. A necessidade de conectividades é especialmente importante para as áreas de Mata Atlântica (Leite et al., 2002) e Caatinga, de pequeno tamanho e, na maioria, isoladas. Na região da Mata Atlântica, apenas o complexo de UCs que se estende pelo litoral de São Paulo, desde o PN da Serra da Bocaina, abrangendo o PE da Serra do Mar e Esec da Juréia, até o complexo de Parques Estaduais no sul deste estado e avançando ainda pelo Paraná (PN Superagüi, PN Saint-Hilaire/Lange), possui condições adequadas de conectividade. Da mesma forma, na Caatinga é vital a conexão entre as duas maiores UCs: os PN Serra da Capivara e Serra das Confusões.

Perspectivas e recomendações

Até 1998, as UCs de proteção integral (na época denominadas como de uso indireto), representavam 2,61% do território nacional (MMA, 1999). Desta época em diante, houve um grande esforço para aumentar o número de UCs e a área protegida por elas. Atualmente, as UCs de proteção integral e RPPNs cobrem 3,58% do território Nacional (Tabela 1). Esse aumento na proporção de áreas protegidas, apesar de significativo, ainda está bem aquém do mínimo de 10% sugerido, tanto no geral quanto para os biomas separadamente, à exceção de ambientes costeiros (Tabela 1). A proporção de proteção entre os diferentes ambientes também é desigual, com regiões como a Caatinga e a Mata Atlântica muito pouco representadas. Parte disso deve-se ao fato que, na Mata Atlântica, por exemplo, muito pouco resta de vegetação original e praticamente não existe possibilidade de aumentar significativamente a área protegida em UCs. Porém no Cerrado, Pantanal e Caatinga ainda há essa perspectiva. Dessa forma, a primeira recomendação que pode ser feita é a criação de novas áreas protegidas. O tamanho dessas áreas protegidas, em sua maioria, também não é suficiente para manter, isoladamente, populações de carnívoros no longo prazo. Neste sentido, esforços devem ser empreendidos em duas direções: a) dar preferência, quando possível, à criação de UCs de grande porte, que possibilitem por si só a manutenção de populações viáveis, b) priorizar criação de UCs que tenham possibilidade de conexão com áreas protegidas já existentes.

Algumas espécies de carnívoros estão sub-representadas no atual sistema de unidades de conservação e um fator na escolha de novas áreas protegidas pode ser a representação dessas espécies. Porém, o grau de conhecimento da fauna da maioria do país é ainda incipiente e para esta recomendação ser atendida é necessário que sejam intensificados os esforços de pesquisa e levantamento em regiões prioritárias. Essas áreas prioritárias foram na maioria identificadas em vários workshops realizados para cada bioma específico (MMA, 2002). Entretanto, existem outras áreas quase totalmente desconhecidas que não foram contempladas e podem ser acrescentadas. Esta necessidade de conhecimento é especialmente grave nas Unidades de Conservação. A maior parte delas não conta com um levantamento de fauna mínimo para termos um panorama mais compreensivo do real padrão de ocorrência de espécies nas UCs e boa parte das ausências registradas devem-se à falta de bons inventários. Estes inventários serviriam também para embasar os Planos de Manejo das UCs. Atualmente, apenas 15% das UCs contam com este instrumento de gestão atualizado. Atualizar os planos de manejo de UCs que já o possuem e elaborá-los para as unidades que não os possuem será primordial para melhor proteção dos seus recursos naturais, incluindo os carnívoros, seus habitat e recursos alimentares.

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Capítulo 7
Metapopulações, ecologia de paisagens e a conservação dos carnívoros brasileiros

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Introdução

O termo fragmentação de hábitat tem sido usado na literatura científica para definir "a modificação ou remoção de grandes áreas de vegetação natural que resulta na criação de um mosaico de ambientes fragmentados e isolados" (KATTAN et al., 1994), ou o "processo de subdivisão de habitats contínuos em pequenas porções" (ANDRÉN, 1994, WIENS, 1994), ou "perturbações externas que alteram grandes áreas contínuas, criando vários fragmentos isolados ou debilmente conectados, os quais ficam inseridos em um mosaico formado por outros tipos de ambientes", (WIENS, 1989) ou simplesmente a "quebra de uma continuidade" (LORD; NORTON, 1990).

A fragmentação de hábitat pode ocorrer naturalmente pela ação do fogo, vendavais, pela movimentação de dunas, por mudanças em cursos d'água e inundações, ou ser provocada pela ocupação humana (WIENS, 1989). Ambientes fragmentados, geralmente, caracterizam-se por possuir uma grande heterogeneidade espacial dentro da escala da paisagem (FORMAN; GODRON, 1986). Isto significa que a paisagem resultante da ação de perturbações (humanas ou naturais) configura-se em um mosaico de ambientes que apresenta baixa similaridade com o ambiente pré-perturbação. À medida que diminuem as perturbações, há retomada das condições próximas às originais e diminuição da heterogeneidade espacial.

As conseqüências da perda de hábitat disponível para as espécies estão entre as maiores preocupações atuais da Biologia da Conservação, Ecologia e Biogeografia. Em geral, a redução do habitat ou o aumento na degradação ambiental levam à diminuição e até mesmo causam a extinção local de espécies associadas aos ambientes afetados. Nas áreas de alta diversidade biológica, como é o caso das florestas tropicais (MYERS, 1986), qualquer supressão de hábitat pode implicar a perda de várias espécies. Atualmente, as taxas de conversão de ambientes encontram-se em ritmo muito acelerado. Em algumas regiões, como a Amazônia, as estimativas dos desmatamentos chegam a 21.000 km^2 anuais (Instituto de Pesquisas Espaciais, 1980 apud SKOLE; TUCKER, 1997), e a perda de florestas tropicais em âmbito mundial varia entre 92.000 km^2 (MYERS, 1986) e 165.000 km^2/ano (SKOLE; TUCKER,1997). Ecossistemas inteiros, como a Mata Atlântica e o Cerrado no Brasil, as florestas tropicais do sudeste de Madagascar, do sudeste asiático e da região do Congo estão em má situação, pois grande parte da cobertura original já foi removida. Na Mata Atlântica brasileira, formação florestal que ocupava originalmente cerca de 1,1 milhão de km^2, as estimativas indicam que cerca de 95% já tenha sido destruído (MITTERMEIER et al., 1992, Fundação SOS Mata Atlântica & Instituto Nacional de Pesquisas Espaciais, 1993). Acreditando-se que as Florestas Tropoiciais sustentem cerca de 50% de todas as espécies viventes na Terra (MYERS, 1986; SKOLE; TUCKER, 1997), não é de estranhar a enorme preocupação dos conservacionistas. A expansão agropecuária, exploração madeireira, mineração e construção de grandes barragens estão entre as principais causas de destruição ambiental no mundo. No caso do Cerrado, estima-se que essas atividades já tenham provocado a perda de 80% da cobertura original do bioma (MYERS et al., 2000).

Duas das principais conseqüências imediatas do processo de ocupação humana são a perda de habitat disponível para as espécies e a fragmentação dos ecossistemas. O resultado geral é traduzido na diminuição ou eliminação de populações locais, o que também se traduz como perda da variabilidade genética. Para as espécies que possuem pequena área de distribuição ou que se encontram ligadas a determinados tipos de hábitat ou ainda para aquelas que possuem uma distribuição do tipo agregada (que aparentemente é o caso da maioria das espécies [HANSKI, 1994]), a fragmentação é mais grave (SIMBERLOFF, 1994). Outra conseqüência imediata da fragmentação é a criação de várias manchas de ambientes, em geral pequenas e isoladas, ou que apresentam uma tênue conexão a outras áreas similares. Como resultado, os padrões de movimentação das espécies são alterados e, com a perda da qualidade de hábitat pelos efeitos da fragmentação, a área de vida das espécies também é alterada (OPDAM et al., 1994).

Nas últimas décadas, o crescente interesse pela conservação das espécies em ambientes fragmentados pela ocupação humana levou à procura de novas abordagens para o delineamento de ações globais de conservação de ecossistemas. Desde o surgimento da teoria da biogeografia insular (MAC ARTHUR; WILSON, 1967), logo se percebeu que seus princípios poderiam ser aplicados aos ambientes fragmentados, pois as áreas remanescentes (manchas isoladas em ambientes inóspitos para as espécies) poderiam ser comparadas às ilhas oceânicas (SIMBERLOFF; ABELE, 1976; FONSECA, 1981).

A teoria da biogeografia insular

Em 1967, os ecólogos Robert MacArthur e Edward Wilson desenvolveram uma teoria denominada Teoria da Biogeografia de Ilhas, embora algumas das idéias propostas por eles já tivessem sido descritas por outros autores (WILKINSON, 1993, para críticas). Essa teoria determina que a diversidade de formas de vida encontrada nas ilhas oceânicas depende de uma relação entre a imigração e a extinção de espécies. Quando ambas as taxas são iguais, a diversidade insular atinge um estado de equilíbrio. Esse processo é dinâmico e depende de fatores como o tamanho da ilha, o nível de isolamento (proximidade do continente) e o seu tempo de isolamento (Figura 1).

O tamanho da área relaciona-se diretamente com a taxa de extinção, pois ilhas maiores suportam maiores populações de espécies e, por conseguinte, as chances de extinção das populações são menores. Um dos argumentos é o de que quanto maior for a área da ilha, maior será o número de habitats disponíveis, oferecendo maior diversidade de nichos a serem explorados. Além do tamanho da ilha, o tempo de isolamento também influencia na diversidade de espécies encontradas. Em ilhas que estavam conectadas ao continente e que ficaram isoladas, observa-se um período de relaxamento, que é definido como sendo o tempo necessário para que a fauna primária relaxe para 36,8% da diversidade inicial. Após 2,3 unidades de tempo, o relaxamento total terá sido alcançado (Mac ARTHUR; WILSON, 1967). O tempo de relaxamento depende basicamente de dois fatores: da imigração e da taxa de extinção. Como visto anteriormente, a taxa de extinção dependerá do número de indivíduos das populações originais e este, segundo a teoria da Biogeografia Insular, depende do tamanho da área considerada. Já a taxa de imigração (chegada de novas espécies à ilha) depende de uma série de fatores: 1) da distância da fonte colonizadora (do continente); 2) da capacidade de dispersão das espécies; 3) das possíveis barreiras ou empecilhos à dispersão das espécies; 4) da distância entre ilhas.

Em situações em que a ilha esteja próxima ao continente, as espécies do continente são capazes de chegar à ilha e não existem barreiras efetivas à essa movimentação. A imigração será importante fator para a composição final das espécies presentes na ilha. Proporcionalmente, a taxa de extinção também será elevada, pois um maior número de espécies estará sujeito ao processo de competição. Se considerarmos diferentes ilhas localizadas a diferentes distâncias da fonte de colonização, será esperado que, nas ilhas mais próximas ao continente, as taxas de imigração sejam mais altas do que naquelas mais afastadas. Uma vez que as comunidades das ilhas vão se tornando mais equilibradas, as taxas de imigração vão diminuindo, pois, com a estabilização, novas espécies encontrarão mais dificuldade para se estabelecerem nas comunidades que tendem à saturação.

O paradigma do *SLOSS*

Alguns anos após a sua apresentação, o modelo de biogeografia de ilhas já encontrava aplicações práticas. DIAMOND (1975) propôs que reservas (unidades de conservação) seriam ilhas funcionais num mar de áreas alteradas pela ação humana e propôs seis princípios derivados de seus estudos sobre os padrões de distribuição de pássaros na Nova Guiné: 1) Reservas maiores preservam mais que pequenas; 2) Uma única área maior é preferível a várias pequenas totalizando a mesma área; 3) Se forem pequenas, as áreas protegidas devem estar agrupadas/próximas; 4) O arranjo das áreas em bloco é melhor que o linear; 5) A conexão por corredores ajuda a dispersão dos organismos; 6) A forma circular reduz efeitos de borda.

Mas também houve críticas à aplicação direta do modelo de biogeografia de ilhas à definição de áreas para conservação. Cole, 1981 apud Simberloff; Abele, 1982, demonstrou que, baseado na fórmula proposta por MacArthur e Wilson (1967) para explicar a relação entre a área e o número de espécies presentes, em algumas situações (o autor usou aquelas que possuíam distribuição do tipo série geométrica em seus estudos), o número de espécies presentes em duas pequenas ilhas foi maior do que em uma ilha grande. Simberloff e Abele (1982) salientam o fato de que a teoria é neutra no que diz respeito ao desenho de reservas necessárias para a conservação das espécies. Segundo esses autores, dependendo da habilidade de colonização das espécies, poderia ser criada uma única reserva de tamanho grande ou várias reservas pequenas, dicotomia que gerou o acrônimo SLOSS (do inglês *single large or several small*).

A controvérsia encontra defensores de ambos os lados. A existência de várias reservas pequenas implica maior dificuldade de fiscalização e da necessidade de estabelecer um esquema tal que permita o intercâmbio de indivíduos entre essas áreas. Por outro lado, a criação de uma única reserva grande pode ser inviável em várias regiões onde não existem mais áreas com tamanho suficiente para o estabelecimento da reserva ou, ainda, em que os custos envolvidos sejam muito elevados. A discussão do SLOSS foi abandonada porque em pouquíssimas situações os manejadores do ambiente e tomadores de decisões poderiam enfrentar este dilema (SAUNDERS et al., 1991), e também porque houve um grande avanço nos estudos da ecologia de áreas fragmentadas. Em realidade, considerando o mundo dinâmico e cada vez mais alterado pelas pressões humanas, uma atualização da abordagem de poucas áreas grandes ou muitas áreas pequenas deveria ser lida como SLASS (*single large AND several small*). Ou seja, um esquema de áreas protegidas deve considerar todas as porções de ecossistemas nativos disponíveis.

Estudos mais recentes mostram que há uma série de alterações ecológicas na dinâmica do ambiente dos fragmentos que podem ser mais importantes que o tamanho da área ou o seu nível de isolamento, para a manutenção das espécies. As características da matriz na qual se insere um determinado fragmento podem afetar bastante uma determinada área (WIENS, 1994). Por outro lado, sabe-se que espécies que ocupam o topo da cadeia alimentar necessitam de grandes áreas para assegurar sua sobrevivência.

Abordagem ecológica da fragmentação de habitat

Com o desenvolvimento de estudos de campo, várias conseqüências da fragmentação foram registradas. Lovejoy et al. (1986), trabalhando em áreas fragmentadas na Amazônia, determinaram que áreas florestais recém-fragmentadas apresentam mudanças nas características microclimáticas da borda da área, fenômeno denominado efeito de borda. Segundo esses autores, esse efeito surge quando uma parte do interior da floresta fica exposta, passando a sofrer as alterações climáticas típicas das bordas: aumento na incidência de ventos e da insolação, diminuição da umidade relativa do ar e aumento da temperatura. As alterações climáticas são sentidas a várias dezenas de metros no interior da mata fragmentada, aspecto que, segundo Lovejoy et al. (1986) provoca a diminuição da área útil para a conservação no interior do fragmento. O conjunto das mudanças nas características físicas dos fragmentos isolados é bem discutido por Saunders et al. (1991). Alterações microclimáticas – são aquelas que afetam os fragmentos e envolvem os seguintes tópicos:

- Fluxos de radiação – a criação de áreas abertas no entorno de uma área de vegetação nativa provoca um aumento na radiação solar, aspecto que acarreta maior elevação da temperatura durante o dia, especialmente na vegetação da borda dos ambientes fragmentados. Um aspecto interessante é que talvez o efeito de borda não seja significativo em ecossistemas com vegetação mais aberta ou esparsa, como o Cerrado.

- Ação do vento - além de ser um elemento de alteração do microclima, o vento pode provocar na borda das áreas recém-abertas, a queda de árvores por ação direta. O carreamento de sementes de espécies da matriz circundante para o fragmento é um outro aspecto da ação do vento.

- Fluxo hídrico – com a eliminação da vegetação nativa, os fluxos hídricos (precipitação e evapotranspiração) ficam bastante alterados. Sem a vegetação nativa, os riscos de erosão e lixiviação dos solos aumentam.

- Isolamento – o isolamento de áreas (criação de ambientes alterados entre os ambientes nativos) traz como conseqüências os seguintes aspectos:

- Tempo decorrido desde o isolamento – com base nas predições da Teoria da Biogeografia Insular, áreas recém-isoladas pela fragmentação apresentam um aumento inicial no número de espécies (maior afluxo de espécies vindas das áreas destruídas), mas com o passar do tempo, há um decréscimo natural.

- Distância entre remanescentes – a distância entre os fragmentos é um obstáculo a ser vencido pelas espécies. A capacidade de dispersão e as características de cada espécie influenciam grandemente as suas chances de sobreviverem em áreas alteradas.

● Conectividade – a conectividade (grau de contato que um fragmento possui com o outro) é associado ao efeito da distância entre áreas. As conexões podem ser de várias formas, dependendo do tipo de matriz circundante. Em qualquer situação, acredita-se que os corredores sejam importantes fatores de manutenção local de populações de espécies.

● Alterações na paisagem circundante – as alterações externas aos fragmentos tanto podem agir prejudicando (criação de ambientes inóspitos para espécies florestais, por exemplo) quanto beneficiando algumas espécies (as típicas de áreas abertas, por exemplo). Entretanto, a existência de um mosaico de ambientes como matriz básica de uma paisagem pode ser um fator atenuador dos efeitos da fragmentação.

Características físicas

● Tamanho do remanescente – o tamanho é uma importante característica física do fragmento. Quanto maior for o fragmento, menores serão os efeitos negativos da fragmentação, por exemplo, o efeito de borda e a relação espécie-área (princípio biogeográfico).

● Forma – a forma do fragmento é importante à medida que ela é responsável pelo contato do fragmento com o meio externo. Maior irregularidade do fragmento significa maiores influências da matriz circundante sobre o fragmento.

● Posição na paisagem – possui importantes influências de todos os aspectos discutidos acima. As características do fragmento na fase "pré-fragmentação" (tipo de solo, declividade, tipo de vegetação) determinarão as características do mesmo após o processo de fragmentação.

Alternativa para a teoria insular: o conceito de metapopulação

O termo metapopulação é usado para definir um conjunto de subpopulações que se interagem localmente por meio de uma dinâmica de colonização e recolonização de áreas fragmentadas (LEVINS, 1969, 1970 apud ANDRÉN, 1994). O conceito de dinâmica de metapopulação segue basicamente o esquema de imigração e extinção da Teoria de Equilíbrio da Biogeografia de Ilhas. Além disso, ambas consideram que as ilhas/fragmentos estão inseridas em uma matriz inóspita para a maioria das espécies, ressaltando o efeito do isolamento das ilhas/fragmentos (Figura 2). Alguns pesquisadores (p.ex. WIENS, 1994) sugerem que o modelo metapopulacional possui algumas características que se aplicam à Biologia da Conservação, especialmente no que diz respeito à viabilidade populacional de espécies que apresentam subpopulações isoladas.

Entretanto, uma vez que o modelo básico de metapopulação assume que as dinâmicas das subpopulações são independentes e não-sincronizadas com as demais, sua aplicação na conservação de áreas fragmentadas ainda deve ser vista com certa ressalva (WIENS, 1994). Tanto a Teoria da Biogeografia de Ilhas quanto a Teoria das Metapopulações não consideram as características da matriz de paisagem na qual os fragmentos encontram-se inseridos. Tais características têm papel importante na dinâmica dos fragmentos de hábitat considerados em uma determinada região e na dinâmica das populações locais, uma vez que as espécies capazes de se deslocarem entre os fragmentos apresentam maior chance de sobrevivência, do que aquelas de baixa mobilidade (STACEY; TAPER, 1992; FAHRIG; MERRIAM, 1994).

A aplicação dos princípios da ecologia de paisagens

A Ecologia de Paisagens é uma ciência recente que está voltada para os estudos de paisagens alteradas ou nativas. Os estudos vão desde a descrição das paisagens em termos da presença e proporcionalidade de seus três elementos básicos (matiz, corredor e manchas) (FORMAN; GODRON, 1986), até estudos de dinâmica de fragmentos, ecologia de espécie (dispersão, recolonização de ambientes, extinções locais) e relações entre os elementos da paisagem. O estudo das características das paisagens é de grande importância para o entendimento da distribuição local de espécies. Os vários arranjos espaciais dos fragmentos de hábitat influenciam grandemente tanto a abundância e distribuição das espécies (PULLIAM et al*.,* 1992) quanto a sua dinâmica local (ANDRÉN, 1994; HANSKI, 1994; WIENS, 1994) ou sua reprodução (OPDAM et al*.,* 1994; MARINI et al*.,* 1995).

Nos estudos da ecologia de paisagens, os fragmentos não são tratados de maneira isolada como é proposto pela Teoria da Biogeografia de Ilhas. Enquanto as ilhas de MacArthur e Wilson são essencialmente estáticas (mudam somente quando o nível do mar varia), os fragmentos de ambientes terrestres apresentam uma alta taxa de modificação (FORMAN; GODRON, 1986). Áreas fragmentadas podem dar origem a formações maiores e contínuas, caso ações de manejo sejam promovidas ou as ações que causam degradação cessem. Os fragmentos terrestres estão sempre inseridos em uma matriz de paisagem que se apresenta na forma de um mosaico (FORMAN; GODRON, 1986; ANDRÉN, 1994; WIENS, 1994). Essa heterogeneidade espacial é responsável por uma gama de oportunidades de estabelecimento ou deslocamento de indivíduos ao longo da paisagem (FORMAN; GODRON, 1986). Além disso, os efeitos externos, oriundos da matriz de paisagem sobre os fragmentos de ambientes, também variam. Dentro de paisagens heterogêneas, a taxa de imigração de indivíduos aparenta ser alta (FORMAN; GODRON, 1986). Isto significa que há maior fluxo de indivíduos que conseguem chegar aos fragmentos vizinhos dentro do período de tempo necessário para que eles completem seu ciclo de vida. Assim, quando uma subpopulação se extingue em um fragmento este é rapidamente recolonizado, aspecto que minimiza o efeito da insularização

(FORMAN; GODRON, 1986), embora as habilidades de colonização e ocupação de áreas variem de espécie para espécie (WIENS, 1989).

Talvez essas características das paisagens heterogêneas sejam um fator atenuador das chances de extinção das espécies que sobrevivem nos ambientes alterados. Para exemplificar, Simberloff (1994) cita o caso das florestas temperadas do noroeste dos Estados Unidos. Após 300 anos de exploração, esse ecossistema ficou reduzido a 1% de sua área original. A despeito disto, apenas três das 70 espécies de aves (4%) se extinguiram. Mesmo assim, duas delas devido principalmente às atividades de caça. A Mata Atlântica brasileira é outro exemplo de processo semelhante. Desde a chegada dos portugueses em 1500, esse ecossistema foi bastante alterado, a ponto de restarem somente cerca de 5 a 8% da área original (MITTERMEIER et al., 1992; FUNDAÇÃO SOS MATA ATLÂNTICA; INPE, 1993). Da avifauna original, apenas uma espécie é considerada extinta na natureza (IUCN, 2003; IBAMA, 2003), o mutum-do-nordeste, *Mitu mitu*. No início dos anos 1980, alguns ecólogos como Darlington (1957 apud FONSECA, 1981) calcularam que, quando mais de 90% de um ecossistema é destruído, a metade das espécies presentes entra em processo de extinção. Entretanto, não é esse o quadro observado nas duas regiões citadas acima. Apesar de extremamente ameaçadas, é possível ainda realizar ações em prol da conservação dessas áreas. Simberloff (1994) cita o fato de que várias áreas de matas do noroeste dos EUA se encontram em regeneração, formando um mosaico de ambientes de diferentes idades. Na Mata Atlântica a mesma situação é observada. Embora existam algumas regiões com uma devastação total, em outras há ainda boas fontes de colonização que podem servir de base para a recuperação ambiental.

Perspectivas para a conservação e futuras pesquisas

Um dos grandes problemas para os estudos de conservação em regiões fragmentadas é que existem várias escalas de trabalho (LORD; NORTON, 1990, HANSKI, 1994) que vão do nível regional ao populacional (SAUNDERS et al., 1993). Além disso, os enfoques variam muito podendo ser tanto sob a ótica da Biogeografia, Ecologia, Ecologia de Paisagens, Genética, etc. Nos estudos de caráter biogeográfico, a maior preocupação é com a conservação de espécies ou conjunto de espécies afetadas pela fragmentação. As propostas de manejo visam ao desenho de esquemas de unidades de conservação, ao estabelecimento dessas e à escolha de áreas prioritárias para conservação. Nos estudos de cunho ecológico, a maior preocupação é com as alterações nas comunidades de espécies (relações de competição, predação), nas características dos fragmentos. Sob o enfoque da ecologia de paisagens, as características da arquitetura dos fragmentos (forma, tamanho, espaçamento), da matriz onde os fragmentos se encontram inseridos (tipo de matriz dominante, dinâmica de uso) e das características que permitem a movimentação de indivíduos entre áreas (p.ex., presença de corredores) são alguns dos aspectos abordados. Finalmente, os estudos que se orientam pelos aspectos genéticos buscam, entre outras coisas, informações a respeito da viabilidade das populações ou subpopulações isoladas e da perda de variabilidade genética.

Os modernos estudos com fragmentação de habitat tendem a combinar vários dos aspectos já citados. Por exemplo, o desenvolvimento de modelos espacialmente explícitos (PULLIAM et al., 1992; DUNNING JÚNIOR et al., 1995, LIU ET AL., s/d) visam conciliar os tradicionais modelos de viabilidade populacional com as características da paisagem onde as espécies em estudo se encontram. Tais modelos, como o desenvolvido para análises de viabilidade da coruja-pintada ("spotted owl - *Strix ocidentalis*) (LAHAYE et al., 1994), têm grande aplicação nos planos de ação para a conservação de espécies, pois os resultados obtidos são mais realistas. Em vez de avaliar toda a população, os modelos mais realistas trabalham com o conceito de metapopulação e da viabilidade das subpopulações, surgindo o termo "metapopulação mínima viável", ou seja, o número mínimo de subpopulações interativas necessárias para a persistência no longo prazo de uma metapopulação (HANSKI et al., 1996). Modelos espacialmente realísticos (HANSKI, 1994) são modelos de simulação onde os parâmetros ecológicos das espécies estão amarrados a uma estrutura espacial real (incorpora-se à posição real das subpopulações na paisagem) bem como das características dos fragmentos de ambiente existentes.

Estudos sobre a fragmentação de habitat requerem a obtenção de dados sobre as características da paisagem de interesse bem como dados básicos sobre as espécies presentes localmente (preferência de hábitat, capacidade de dispersão, dados demográficos) para que os modelos de simulação indiquem as alternativas mais viáveis para o manejo e conservação da biodiversidade. A capacidade de predição dos modelos dependerá da qualidade dos dados obtidos e da integração de vários aspectos da fragmentação de hábitat.

Metapopulações e a conservação dos carnívoros

O quadro teórico-conceitual sobre a fragmentação dos habitats naturais evoluiu muito desde a formulação da Teoria de Biogeografia de Ilhas. Mas, do ponto de vista prático, a principal ação conservacionista ainda é o estabelecimento de unidades de conservação, em que frações significativas da biodiversidade em todos os níveis, genético, populacional e ecossistêmico, podem ser preservadas. As UCs tendem a se tornar ilhas do ambiente natural original inseridas numa matriz mais ou menos hostil e que restringe a movimentação dos organismos e do fluxo gênico (Figura 3) entre os fragmentos.

Carnívoros, sendo organismos do topo de cadeias tróficas e com alta demanda energética, vivem em áreas relativamente grandes, densidades populacionais baixas e tendem a ser fortemente dependentes de ambientes de boa qualidade e, portanto, num quadro de fragmentação dos seus habitats são fortemente impactados.

Mesmo em unidades de conservação, a limitação espacial impõe limites aos seus números populacionais. Estudos recentes sobre carnívoros têm indicado que algumas espécies de maior porte vivem em áreas consideravelmente grandes: 30 km^2 para suçuaranas e 160 km^2 para onças-pintadas na região do Cerrado (Silveira 2004) e áreas entre 22km^2 e 115km^2 para lobos-guará, também no Cerrado (DIETZ, 1984; CARVALHO; VASCONCELLOS, 1995; SILVEIRA, 1999; RODRIGUES, 2002). Isso resulta em números populacionais também pequenos. Estimativas para o Parque Nacional das Emas, GO, com seus 132.000 ha, indicam a presença de não mais que 40 indivíduos de suçuarana e de cerca de 15 indivíduos de onça-pintada (Silveira 2004) o que, evidentemente, não configura populações capazes de se manterem por muito tempo nas condições que atualmente prevalecem na região. Unidades de conservação menores devem manter contingentes ainda menores de carnívoros. De fato, o estudo de Rodrigues (2002) sobre o lobo-guará, o carnívoro de maior porte ainda existente nos aproximadamente 10.000 ha da Estação Ecológica de Águas Emendadas, DF, indica a ocorrência de não muito mais do que uma dezena de indivíduos dessa espécie nessa unidade de conservação.

Torna-se evidente que a conservação de carnívoros de maior porte demanda unidades de conservação muito maiores do que as que temos atualmente. Apenas na Amazônia existem UCs maiores que 1 milhão de hectares que poderiam conservar populações geneticamente saudáveis na maioria das espécies do topo de cadeias tróficas no longo prazo. Mesmo assim, isso depende fortemente do andamento do processo de colonização e conversão de novas áreas na Amazônia.

O conceito de efeito de borda, apresentado em outra seção deste capítulo, pode ser entendido de forma mais ampla, como o conjunto de alterações causadas no interior de um fragmento de qualquer tamanho, por influência da matriz na qual se encontra o fragmento. Assim, o impacto de cães domésticos sobre as populações de outros carnívoros e da fauna silvestre em geral, pode ser considerado como um efeito de borda, o qual pode se estender por mais de 1 km para dentro das bordas da UC (LACERDA 2002; LACERDA; TOMÁS; MARINHO-FILHO em preparação).

Além disso, há riscos adicionais. A presença de populações humanas e movimento de pessoas e produtos no entorno das UCs representa a possibilidade de atropelamentos da fauna silvestre que se desloca entre as áreas protegidas e outras manchas de hábitat natural, atravessando estradas, propriedades e até áreas urbanizadas.

A ignorância e a má informação priorizam a eliminação em vez do manejo dos predadores que, forçados pela depleção dos recursos, atacam os animais de criação. A caça dos predadores de maior porte também representa uma demonstração de potência do caçador e a busca de troféus de caça ainda causa significativo impacto sobre as populações de carnívoros.

Num quadro de progressiva e acelerada redução das áreas contínuas das formações nativas pela expansão da malha urbana, rural, viária e de infra-estrutura que um país como o Brasil demanda, inclusive para o atendimento básico de grandes parcelas carentes da sua população, o cenário previsível nos próximos anos não é nada favorável à conservação dos carnívoros, particularmente daqueles de maior porte. Entretanto, a teoria de metapopulações e a ecologia de paisagens oferecem um instrumental que permite melhor compreensão e abordagem dos processos associados à insularização das áreas nativas e a construção de cenários que permitam uma melhor tomada de decisões e definição de estratégias de conservação.

A alimentação dos modelos preditivos, sejam eles análises de viabilidade populacional, sejam modelos espacialmente implícitos ou explícitos, depende de informações de dados de boa qualidade sobre a ecologia e a história natural e de vida das espécies. Demanda, ainda, para o aperfeiçoamento de sua aplicação imensa quantidade de dados, que justificam o investimento na formação de uma legião de pesquisadores nas próximas gerações. O exemplo que vem da Europa, onde a ocupação humana e a transformação dos habitats naturais representam uma história de milhares de anos e onde espécies como o urso e o lobo parecem apresentar sensível aumento populacional recente indica que nunca é tarde.

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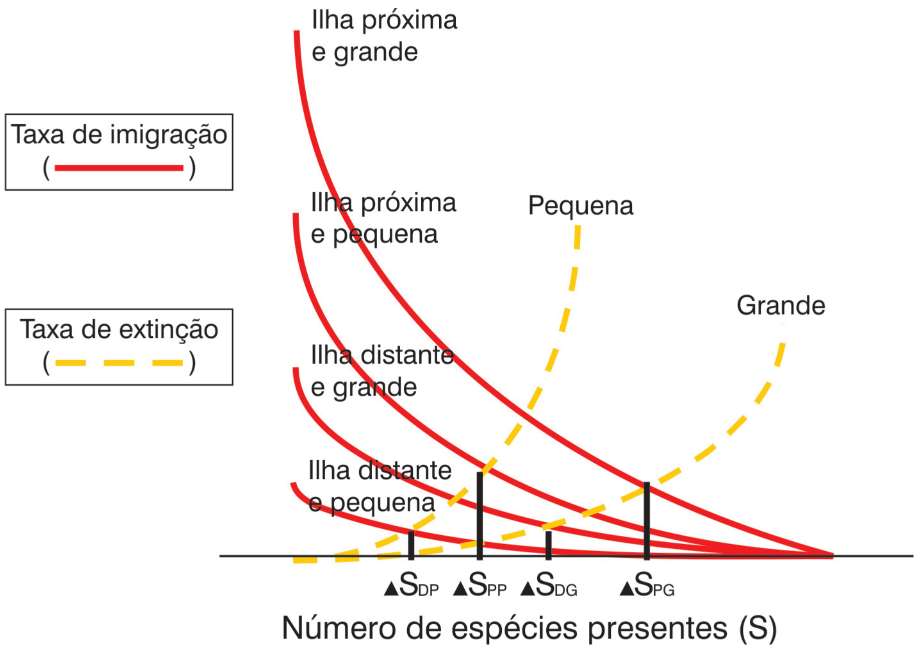


Figura 1 – Modelo de biogeografia de ilhas de MacArthur & Wilson (1967). As taxas de colonização e o número de espécies numa dada ilha dependem da distância da ilha ao continente, do tamanho da ilha e do número de espécies já existentes na ilha.

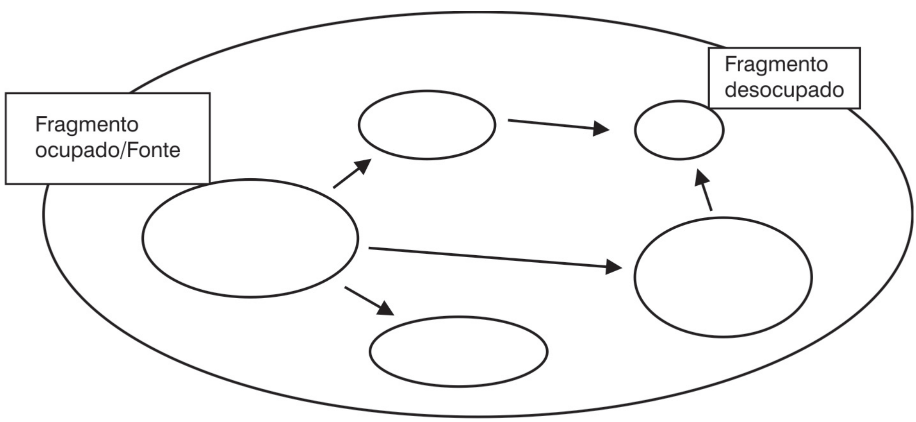
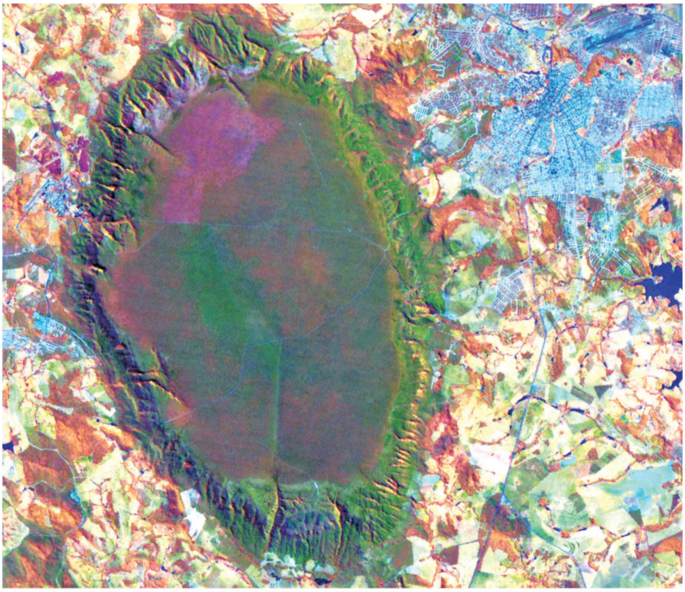


Figura 2 – O modelo de metapopulações prevê o fluxo de indivíduos/genes entre fragmentos, incluindo a colonização de fragmentos não ocupados e eventuais extinções locais em fragmentos cujas populações declinaram ou foram submetidas a catástrofes.



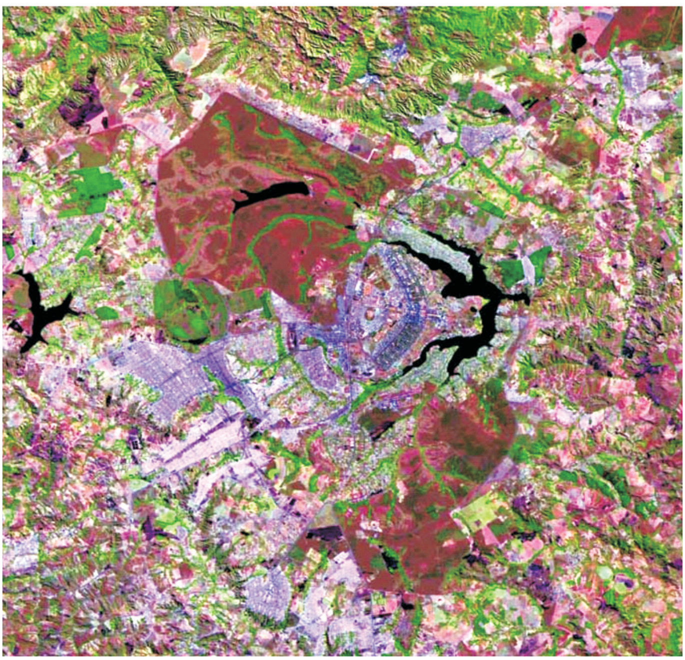


Figura 3 – O Parque Estadual de Caldas Novas, GO (acima), e o Parque Nacional de Brasília e APA Gama-Cabeça de Veado, DF (abaixo), são fragmentos de vegetação natural cercados pela malha urbana e rural, constituindo exemplos do que já ocorre em boa parte das unidades de conservação atuais e a tendência a ser observada na maior parte das UCs num futuro não muito longínquo, se permanecerem as taxas atuais de conversão dos ambientes nativos.

Capítulo 8
Carnivore distribution and abundance patterns along the Cerrado-Pantanal Corridor, Southwestern Brazil

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Introduction

In the last three decades, the Cerrado biome of southwestern Brazil experienced a drastic reduction. The conversion of natural habitats into crop fields and exotic pastures has reduced and fragmented habitats and populations. Today the survival of most of the medium to large-bodied vertebrates is regionally threatened in long-term perspectives due to the relatively small sizes of conservation units to support genetically viable populations. In this scenario, the implementation and protection of natural connections (corridors) between populations becomes extremely necessary.

Understanding species distribution and abundance is the first step to design regional conservation efforts. Also, there is clearly a need for the identification of characteristic or indicator species in the fields of nature monitoring, conservation, and management. Contrary to the common use of species richness (that can be influenced by several factors) to assess conservation value of sites, representative diversity, obtained by an analysis of indicator species can be useful in evaluating the comparative richness of sites, or the effect of isolation or fragmentation (DUFRÊNE; LENGENDRE, 1997).

Most of the data analyzed in this study was originated from camera-trapping. Camera-trapping is an efficient and reliable method for rapid assessment of species richness and abundance in short periods and on a broad scale, and thus crucial to determine regional conservation priorities. The method is efficient for inventories, especially of cryptic animals, as well as for population studies of species whose individuals can be recognized by body marks (KUCERA; BARRETT, 1993; MACE et al*.*, 1994; KARANTH, 1995; CARBONE, 2001).

Of the 21 carnivore species expected for the study site, 16 were recorded through camera-trapping. Considering the overall data, including other surveying methods, one was exclusive to the Emas National Park (Cerrado core site) and two to its surrounding eco-region. The carnivore community across the corridor presented distinct distribution patterns and abundance.

The assessment of the species distribution and abundance patterns along the corridor not only gives us an insight on the species conservation status, but also helps prioritize conservation efforts. Thus, the aims of this study were to examine the carnivore assemblage composition and abundance patterns along the Cerrado-Pantanal corridor, and to assess indicator species characterizing the different regions positioned in this corridor.

Material and methods

Study area

The study area comprises the southwestern portion of the Cerrado biome, more precisely the region of Emas National Park (53º00'- 17º92'), along the Taquari River, towards the west until the Rio Negro State Park region (56º.19'-19º.53'). For better characterization of the fauna and interpretation of the data we divided the corridor into four eco-regions: 1) Emas National Park (ENP); 2) ENP-Surroundings; 3) Taquari River and; 4) Pantanal of Rio Negro. According to physical variables these regions can be described as distinct ecological regions, where altitudes varies from 900 m to 200 m above sea level, landscapes varies from flat to rolling terrain, and habitats from grasslands to forest.

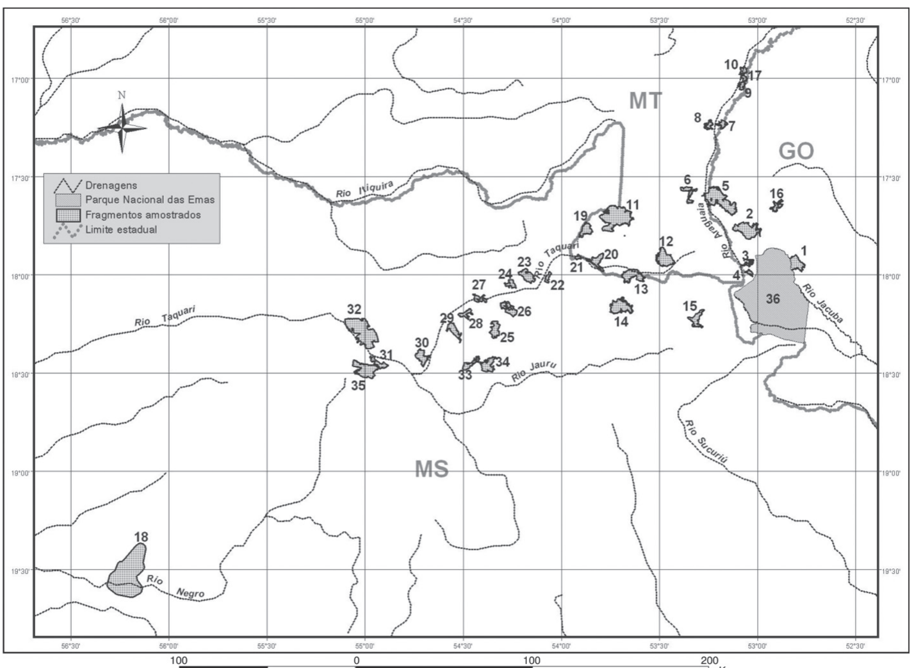

Figure 1 – Study area – The Cerrado-Pantanal Corridor region and its distinct eco-regions.

Emas National Park (ENP)

Emas National Park is situated in central Brazil in the extreme southwest of Goiás State (18º 19' S, 52º 45' W) and is one of Brazil's most representative Cerrado reserves. Its 132,000 hectares protect large tracts of grassland plains (97%), small patches of shrub fields *Cerrado* (*sensu stricto*) (1%), marshes, and riparian forest (2%). Some 1500 mm of rain fall during the October to April wet season (IBDF/FBCN, 1981). There is virtually no rain in the rest of the year, when daylight temperatures reach 39º C and may drop as low as -1,5º C at night. At least 13 endangered mammal species are found in the Park, which is considered to be one of the best sites for observing large Cerrado fauna (ERIZE, 1987; REDFORD, 1985; RODRIGUES et al*.,* 2002). ENP sits on one of the most highly productive agricultural lands of central Brazil, where soybean and cornfields are the main regional activity that fragments the regional landscape.

ENP-Surroundings

The surroundings of Emas National Park are composed of extensive crop fields, specially soybean and corn. The terrain is, in its majority, flat with low undulation closer to the watersheds of the Taquari River. Fragments of natural habitats can only be found on the valley area where agriculture is not possible.

Taquari River

The Taquari River region is characterized by the river's valley, rolling terrain and pockets of forests. The land use is mainly for beef cattle ranching and many parts of the region are inaccessible with a vehicle. Altitude in this region abruptly declines from 900 m to 600 m.

Pantanal of Rio Negro

The Pantanal wetland is considered one of the world's largest wildlife sanctuaries. It concentrates one of the highest aquatic bird diversity and mammal biomass in South American ecosystems (SCHALLER, 1983). The fauna diversity and abundance are strictly related to the flooding regime that cycles between a dry and a wet season. The flooding regime of the Pantanal has historically constrained its entire occupation with agriculture, deforestation or even ranching of beef cattle upon native grass. Mean altitude is 200 m above sea level. The terrain is flat and the natural habitats are distributed in mosaics.

Mammal survey

To generate mammal lists for each eco-region we used four distinct methods: camera-trapping, road-kill surveys, direct observation and interviews with locals. As the first method was used systematically along the area and raised the data for all statistical analyses it is described separately below. Considering that the more accurate results were obtained from the camera-trapping method, this was the first option of data source to compile the eco-regions' mammal lists. When a species was not detected through this method we then considered records obtained from road-kills, direct observation and interviews with locals. Road killed mammals and direct observations of the species were recorded on a non-systematic basis during the camera-trapping program. Interviews with locals were performed in order to complement species list for the areas.

Camera-trapping

Camera-trapping was used in this study to produce comparable data on carnivore richness and abundance across the area. Between February 2002 and March 2003, we moved 90 infrared-triggered camera-traps (Cam Trakker™ South, Watkinsville, GA, USA) along 262 distinct sites across the Cerrado-Pantanal Corridor. Cameras were set for taking pictures 24 hours a day and positioned at 45 cm above the ground. The units were set by dirt roads and game trails. During a sampling period, which varied from 30 to 160 days, cameras were set at a minimum of 1.5 km apart from each other.

The cameras were checked every 15-20 days for film and battery replacement. Photographic events of a single species were counted as independent when taken at a minimum of 60 minutes apart.

Species richness and abundance

Although to compile species lists for each eco-region we used four methods, to statistically assess species richness and abundance patterns in this study we only considered data obtained by camera-trapping. Thus, data from the literature and other methods were not included in this analysis. Species richness across the four eco-regions was assessed through the identification of the photographs produced with the camera-trapping samples. The Kruskal-Wallis test was performed to test for significant differences between species richness across the regions.

When necessary, in order to complement the lists of species occurrence and distributional maps in each eco-region, we used data from capturing events occurred during the sampling period, records of road-killed specimens and interviews with locals.

Species abundance is treated in this study as the photographic rate of a species, which means the number of pictures taken by a camera unit divided by the period (in hours) sampled.

Statistical analysis

Relationship between number of pictures and camera sampling hours

In order to validate the abundance indices (ex. photographic rates: number of pictures per time unit) a linear and positive relationship between the number of pictures and the sampling effort (hours) should be detected. One could argue that a simple solution for this problem would be to standardize the sampling effort. However, this standardization is not always desirable (e.g. larger sampling effort, measured in time sampled or number of camera units, in larger or more heterogeneous areas) or even possible. Whatever is the reason, the sampling effort used for estimating the abundance indices is usually a variable to be controlled statistically.

Thus, the effect of the eco-regions (ENP, ENP-Surroundings, River Taquari and Pantanal of Rio Negro) and the camera's sampling period over the total number of pictures were evaluated through and analysis of covariance (ANCOVA). In this case, the eco-region was considered the categorical variable of interest (factor), while the sampling effort (in hours) was included in the model as a covariable. The quantitative variables were previously log-transformed with the goals of normalizing the data and stabilizing the variance. For the validation of the photographic rates as an abundance index to be used to compare the eco-regions, it is necessary that the slope estimates, for the four eco-regions, do not differ significantly from one another. If this is true, the four regions can be compared using the adjusted means.

To assess species richness across the four eco-regions a Kruskal-Wallis test was performed.

Similarity patterns - DCA

The similarity patterns between the samples according to the carnivore photographic rates were synthesized by a detrended correspondence analysis (DCA) (HILL; GAUCH, 1980; GAUCH, 1994). The analysis was performed in the software PC-ORD (MCCUNE; MEFFORD, 1997). Rare species were down-weighted in proportion to their frequency. Twenty-six segments were used to remove the arch effect.

Indicator species

To characterize the carnivore community across the eco-regions we performed an indicator species analysis (DUFRÊNE; LEGENDRE, 1997). This method combines the species relative abundance with its relative frequency of occurrence in the various groups of sites, creating an index (called INVAL). This index is maximimum when all individuals of a species are found in a single group of sites and when the species occurs in all sites of that group.

An important characteristic of the indicator species analysis is that the INVAL values can be statistically tested by a random reallocation procedure of sites among site groups. In this study 1.000 permutations were processed using the software PC-ORD (MCCUNE; MEFFORD, 1997). The null hypotheses tested is that the highest indicator values (INVAL) found, comparing the four eco-regions for each species, could be obtained by chance alone, which means that the species is not an indicator of that eco-region.

Results

According to Fonseca et al (1996) 53 mammal species with body mass above 500 g occur in the Pantanal and the Cerrado biomes. Of this total, 43 (73%) were recorded in this study, four of which were exclusive of the Cerrado biome. Table 1 presents the mammal species list for each eco-region, recorded through all methods involved in the survey: camera-trap, direct observation, capture, road-kill and interview with locals.

Camera-trapping accumulated a total of 282,864 camera-trap hours between February 2002 and March 2003, along 262 distinct sites across the Cerrado-Pantanal Corridor, resulting in 2,877 photographs of medium to large mammals (>500g), of which 1.543 corresponded to carnivores (Figure 2). Of the 21 expected carnivore species (according to Fonseca *et al*. 1996), 18 (86%) were recorded, 16 through photographs and two (bush-dog *Speothos venaticus*) and oncilla (*Leopardus tigrinus*) after capturing events, representing 41% of the total expected terrestrial mammal community (with biomass > 500g).

Emas National Park

ENP comprised the highest sampling effort among the four eco-regions. Through all methods 38 mammals species were recorded. A total of 157,968 camera-trap hours was accumulated, resulting in 1,128 photographs of mammals. Of the total mammal species recorded, 15 belonged to the order Carnivora (13 were photo-trapped).

ENP-Surroundings

A total of 33,312 camera-trap hours was accumulated in this eco-region, resulting in 315 photographs. Through all methods 37 mammals species were recorded. Of the mammal species recorded, 16 belonged to the order Carnivora (13 were photo-trapped).

Taquari River

A total of 62,424 camera-trap hours were obtained along the River Taquari axis, resulting in 263 photographs. Through all methods 32 mammals species were recorded, 11 of which belonged to the order Carnivora (8 were photo-trapped).

Pantanal

In the Pantanal region we accumulated 29,160 camera-trap hours, resulting in 1,171 photographs. Through all methods 37 mammals species were recorded, of which 12 belonged to the order Carnivora (11 were photo-trapped).

Table 1 – List of medium-large (>500g) mammal species recorded in the Cerrado-Pantanal Corridor. Carnivores are highlighted in bold.

Popular name	Scientific name	ENP	ENP-Surr.	Taquari River	Pantanal
Marsh deer	Blastocerus dichotomus	1	1	1	1
Red brocket deer	Mazama americana	1	1	1	1
Grey brocket deer	Mazama gouazoupira	1	1	1	1
Pampas deer	Ozotoceros bezoarticus	1	1	0	1
Feral pig	Sus crofa	0	0	0	1
Collared peccary	Tayassu tajacu	1	1	1	1
White lipped peccary	Tayassu pecari	1	1	5	1
Crab-eating fox	Cerdocyon thous	1	1	1	1
Maned wolf	Chrysocyon brachyurus	1	1	1	1
Hoary fox	Lycalopex vetulus	1	1	0	0
Bush-dog	Speothos venaticus	3	5	3	5
Jaguarundi	Herpailurus yagouaroundi	1	1	1	1
Ocelot	Leopardus pardalis	1	1	1	1
Oncilla	Leopardus tigrinus	0	3	0	0
Margay	Leopardus wiedii	0	1	0	0
Pampas cat	Oncifelis colocolo	1	1	0	0
Jaguar	Panthera onca	1	1	0	1
Puma	Puma concolor	1	1	1	1
Hog-nosed skunk	Conepatus semistriatus	1	1	0	0
Tayra	Eira barbara	1	1	1	1
Grison	Galictis vittata	1	0	0	0
River otter	Lontra longicaudis	3	5	5	1
Giant otter	Pteronura brasiliensis	0	0	5	1
Coati	Nasua nasua	1	1	1	1
Crab-eating raccoon	Procyon cancrivorus	1	1	1	1
Rabitt	Sylvilagus brasiliensis	0	0	1	1
Opossum	Chironectes minimus	3	0	0	1
Opossum	Didelphis albiventris	1	1	1	1
Thick-tailed opossum	Lutreolina crassicauda	3	0	0	1
Tapir	Tapirus terrestris	1	1	1	1
Howler monkey	Alouatta caraya	3	5	5	1
Capuchin monkey	Cebus apella apella	1	1	3	1
Paca	Agouti paca	1	1	1	1
Agouti	Dasyprocta azarae	1	1	1	1
Coendou	Coendou prehensilis	3	3	3	1
Capybara	Hydrochaeris hydrochaeris	1	3	1	1
Tatu-de-rabo-mole	Cabassous unicintus	3	3	1	1
Nine-banded armadillo	Dasypus novemcinctus	1	1	1	1
Seven-banded armadillo	Dasypus septemcinctus	3	5	5	1
Hairy armadillo	Euphractus sexcinctus	1	1	1	1
Giant armadillo	Priodontes maximus	1	1	1	1
Giant anteater	Myrmecophaga tridactyla	1	1	1	1
Lesser anteater	Tamandua tetradactyla	1	1	1	1
Total nº. of species		**38**	**37**	**32**	**37**

1 - camera-trap; 2 - capture; 3 - direct observation; 4 - road-kill; 5 - interview

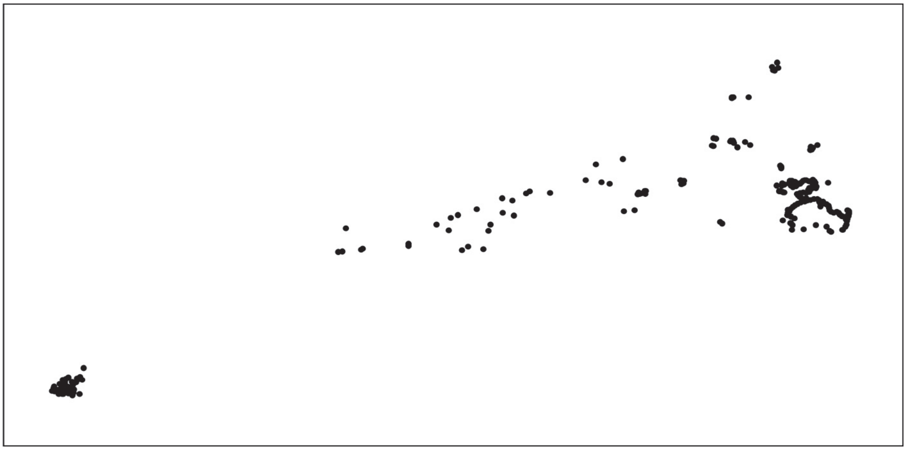

Figure 2 – Distribution of the 262 camera-trap sites along the Cerrado-Pantanal Corridor between February 2002 and March 2003

Species richness

Species richness varied significantly between the eco-regions of the Cerrado-Pantanal Corridor according to the Kruskal-Wallis test (H = 48.3; P = 0.0000) (Figure 3). For this analysis only camera-trapping data was used. However, to complement the eco-region's carnivore lists we considered other data sources: captured species, observed, road-killed and interview with locals (see Table 1).

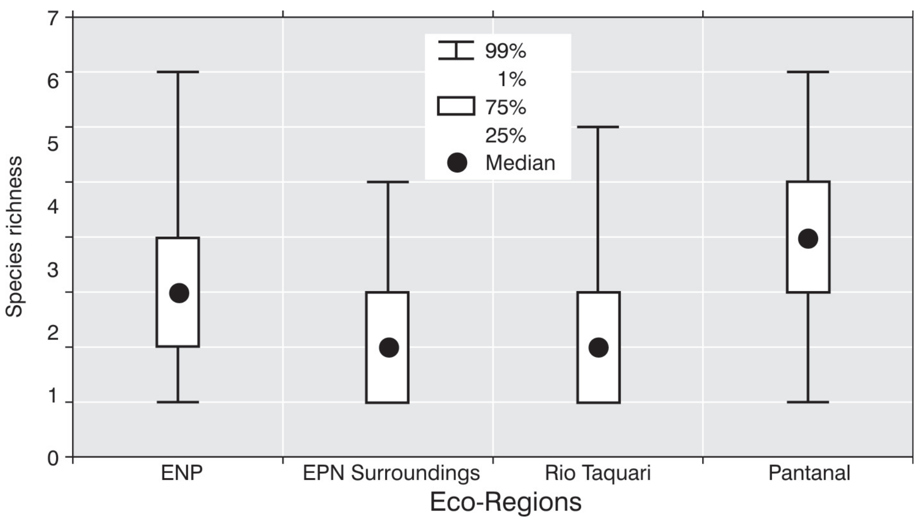

Figure 3 – Species richness in the four eco-regions of the Cerrado-Pantanal Corridor varied significantly according to the Kruskal-Wallis test (H=48.3; P = 0.0000)

The ANCOVA results indicated that there is a significant relationship between the number of pictures and the cameras' sampling effort (F = 127.1; P = 0.000). The parallelism hypothesis (the homogeneity of the slopes) was accepted (F = 0,211; P = 0,889). Considering that the two major prerequisites of the ANCOVA were observed (relationship between effort/number of pictures and homogeneity of the angular coefficients), the adjusted means (average number of pictures for a given sampling effort) can be considered as abundance indices, and thus were used to compare the regions. The results showed that there are significant differences between the eco-regions sampled (F = 26,9; P = 0,000). The highest mean abundance (adjusted mean) was found in the Pantanal, while the lowest abundance indices were estimated, from highest to lowest, for the eco-regions PNE, River Taquari and PNE-Surroundings, respectively (Figure 4).

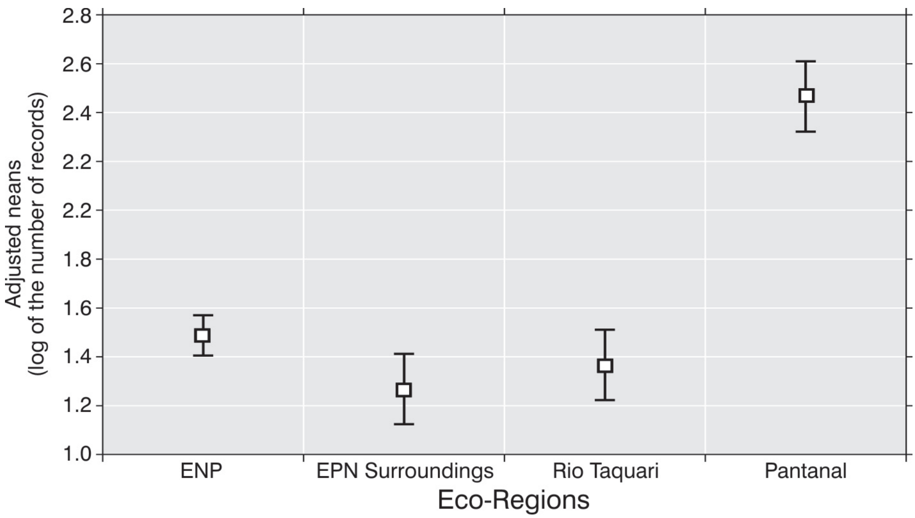

Figure 4 – Abundance means adjusted by the camera sampling effort (in hours) of the number of pictures in the distinct eco-regions analyzed. Whiskers = ± standard error. *n* ENP = 69; *n* ENP-Surroundings = 20; *n* River Taquari = 21; *n* Pantanal of Rio Negro = 45

Detrended correspondence analysis - DCA

The first DCA axis (eigenvalue = 0.7) contrasted Pantanal sites (with lower scores) to ENP ones (higher scores). The eco-regions "ENP-Surroundings" and "River Taquari" presented intermediate scores. The second axis (eigenvalue = 0.43) indicates that the sites located in ENP were more heterogeneous and also, for most of the observations, differentiated ENP from the other eco-regions (Figure 5). The similarity patterns among the regions can be interpreted considering the species scores that indicate, approximately, where the photographic rates of each species were higher (Figure 6). Therefore, the following species presented the highest photographic rates at the Pantanal sampling sites: crab-eating-fox (*Cerdocyon thous)*, raccoon (*Procyon cancrivorus)*, coati (*Nasua nasua*) and ocelot (*Leopardus pardalis*). Some less-abundant species were only detected in this region, such as giant-otter and river-otter. In another way, ENP was distinct from the other eco-regions in relation to the highest photographic rates for the maned-wolf (*Chrysocyon brachyurus*), hoary-fox (*Lycalopex vetulus*) and pampas-cat (*Oncifelis colocolo*). In addition to the hoary-fox, the grison (*Galictis spp.*) was only recorded in ENP (albeit with a very low photographic rate).

Only the margay cat was exclusive to the ENP-Surroundings. In the River Taquari eco-region, the jaguarundi was the only species to present a higher photographic rate in relation to the other sites.

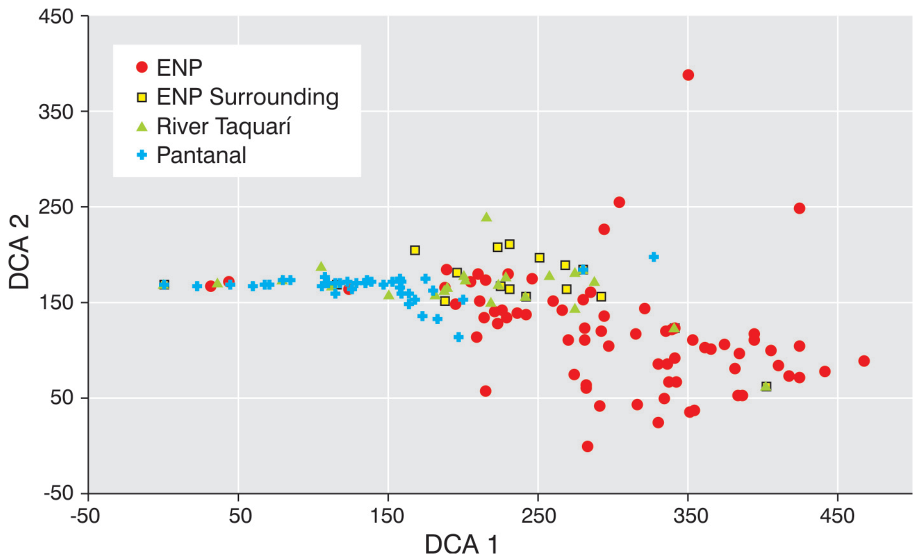

Figure 5 – Scores from the sites across the first two axes of the DCA. The regions are differentiated by the symbols in the figure.

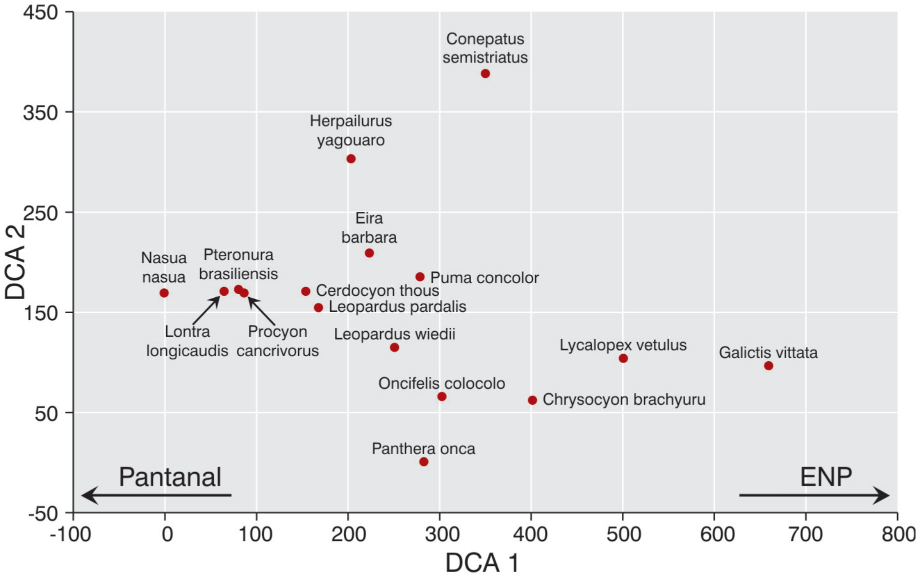

Figure 6 – Species scores across the first two axes originated from the DCA.

Indicator species

Of the 16 species used in the indicator analysis across the four eco-regions, seven presented significant indicator values, according to the Monte Carlo test (Table 2). Considering the probabilities of type I error lower or equal to 5%, three species, the maned-wolf, pampas cat and the hoary-fox can be considered as indicators for the Emas National Park eco-region, and four, the crab-eating fox, crab-eating raccoon, coati, and ocelot, for the Pantanal eco-region. With an indicator value of 39% the maned-wolf was the species that best characterized the Emas Park eco-region. Meanwhile the crab-eating-fox was the species that best represented the Pantanal region, with an INVAL of 60% (Figure 7).

When considering the three major habitat types (grassland, cerrado and forest), six species presented significant INVAL (Table 3). For the grassland habitat the maned-wolf, hoary-fox and the pampas-cat presented significant INVAL ($P<0.001$), puma was the indicator for the cerrado habitat ($P<0.000$), and coati and crab-eating raccoon best represented the forest habitat ($P<0.001$).

Table 2 – Species Indicator Values (INVAL) for the four eco-regions surveyed (Emas National Park (ENP) = 1; ENP-Surroundings = 2; Taquari River = 3; and Pantanal = 4). *P* indicates the probability of Type I error obtained by the Monte Carlo test. The values in bold indicates if the species is an indicator for the region sampled.

	Frequency of Occurrence				Abundance				Indicator Value (INVAL)				
	1	2	3	4	1	2	3	4	1	2	3	4	P
Crab-eating fox	6	7	8	79	37	28	30	76	2	2	2	**60**	0.00000
Maned-wolf	56	26	17	1	62	24	27	2	**35**	6	5	0	0.00000
Raccoon	2	11	5	82	6	16	11	61	0	2	1	**50**	0.00000
Coati	3	10	14	74	3	8	16	43	0	1	2	**32**	0.00000
Hoary-fox	100	0	0	0	19	0	0	0	**19**	0	0	0	0.00100
Ocelot	3	25	27	45	13	32	41	61	0	8	11	**27**	0.00100
Pampas-cat	75	25	0	0	13	4	0	0	9	1	0	0	0.04000
Jaguar	45	0	0	55	33	0	0	17	15	0	0	10	0.06300
Margay	0	100	0	0	0	4	0	0	0	4	0	0	0.13400
Skunk	66	20	14	0	12	4	3	0	8	1	0	0	0.14000
Tayra	3	34	26	36	4	24	22	15	0	8	6	6	0.21300
Giant-otter	0	0	0	100	0	0	0	2	0	0	0	2	0.47700
Jaguarundi	2	32	37	28	1	4	8	4	0	1	3	1	0.47700
Otter	0	0	0	100	0	0	0	2	0	0	0	2	0.51300
Puma	17	32	28	23	48	48	51	41	8	15	15	9	0.62700
Grison	100	0	0	0	1	0	0	0	1	0	0	0	0.99900

Table 3 – Relative abundance, relative frequency and species indicator values (% of perfect indication, based on combining the values for relative abundance and relative frequency), for the three habitat types sampled along the study area (1 - grassland; 2 - Cerrado; 3 - Forest). *P* indicates the probability of type I error obtained from the Monte Carlo test of significance.

	Frequency of Occurrence			Abundance			Indicator Value (INVAL)			
	1	2	3	1	2	3	1	2	3	P
Maned-wolf	63	32	5	65	48	5	**41**	15	0	0.0000
Puma	18	54	28	44	73	37	8	**40**	10	0.0000
Coati	2	2	96	2	2	34	0	0	**33**	0.0000
Hoary-fox	100	0	0	23	0	0	**23**	0	0	0.0000
Raccoon	28	8	65	7	14	37	2	1	**24**	0.0010
Ocelot	11	42	47	8	34	51	1	14	**24**	0.0040
Jaguar	61	16	23	31	14	11	19	2	3	0.0080
Pampas-cat	81	19	0	14	5	0	11	1	0	0.0090
Crab-eating fox	21	16	63	40	41	48	8	7	30	0.0320
Skunk	63	37	0	10	11	0	7	4	0	0.1300
Tayra	8	37	55	3	20	16	0	8	9	0.1880
Jaguarundi	5	32	62	1	5	5	0	1	3	0.3630
Otter	0	0	100	0	0	1	0	0	1	0.5800
Margay	0	0	100	0	0	1	0	0	1	0.5920
Giant-otter	0	0	100	0	0	1	0	0	1	0.5950
Grison	100	0	0	1	0	0	1	0	0	0.9990

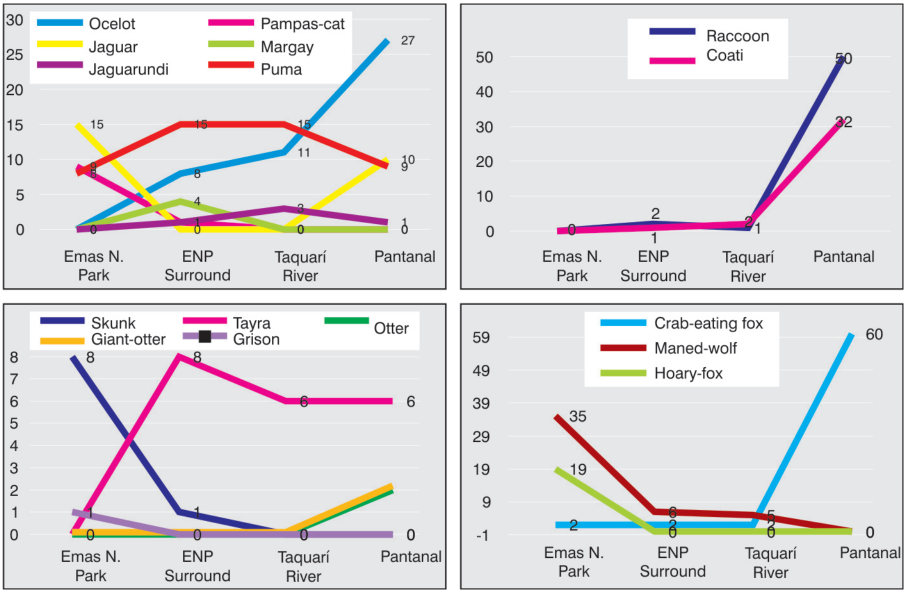

Figure 7 – Indicator Values (%) for the Carnivora Species photo-trapped along the Cerrado-Pantanal Corridor.

Discussion

Species richness

Based on the camera-trap data the species richness along the four eco-regions varied significantly. The ecological and physical variables characterizing the predominant grassland habitat of Emas National Park and its close surroundings, selects exclusively open habitat species such as the hoary-fox, pampas-cat, grison and hog-nosed skunk. The River Taquari eco-region is characterized by a mosaic of natural and converted habitat, mostly exotic pastures for beef cattle ranching. The conversion of grassland habitats for cattle ranching can probably be an explanation for the absence of records of species such as the hoary-fox, pampas-cat and hog-nosed-skunk, which probably have historically occurred in the area. Of the two species that based on the camera-trapping appeared exclusive to the Pantanal eco-region, the giant-otter and the river-otter, only the former does not occur in ENP. Their occurrence was probably not detected through this method because these specie's habitat outside the Pantanal is limited to the river and river margins, therefore it would be little likely that they would get photo-trapped away from riverbanks. Contrary to these situations, in the Rio Negro Pantanal area the otters regularly use the dry habitats to cross between the numerous natural lakes scattered in region. Thus, their probability of being photographed is much higher.

The significant differences of mean abundance between the eco-regions sampled may be a reflection of habitat heterogeneity and distribution along the distinct areas. Other ecological and physical variables not assessed in this study can also be influencing the abundance variations. But, if we just consider the extent of continuous natural habitat, the distribution of habitat (in mosaic), and the seasonal water dynamics (flooding and drought), the Pantanal would be a natural candidate to hold relatively higher fauna abundance.

DCA and indicator species

Despite the similarity regarding the species composition (low turnover), the DCA results indicated a gradient across the ENP-Pantanal axis. At one end there seems to be a clear congregation of a carnivore community more specialized in wet environments and forests, such as the crab-eating-fox, crab-eating raccoon, ocelot and coati, while at the ENP end the community is highlighted by species typical of drier open habitats such as the hoary-fox, maned-wolf, pampas cat and grison. Species with extreme scores such as the otter species in the Pantanal region, hoary-fox and grison in the Emas Park region and the skunk in the middle gradient reflects the combination of their high or exclusive occurrence and abundance (photographic rate). In general, we can interpret that species found in the middle of the gradient tended to be more homogeneously distributed along the distinct eco-regions. Therefore, although the jaguar, pampas-cat and skunk have this middle position in the gradient they need further interpretation. For instance, although the jaguar was not found in between the extreme corridor region, its position in the middle gradient reflects the species occurrence in the extreme sites. The skunk was most abundant at the Emas Park site but had some records for the surrounding region, which may justify its high score in the middle-left position of the graph. A similar interpretation can be used to explain the central position of the pampas-cat that, although mainly present in Emas Park, also occurred in the surrounding eco-region.

Except for the otters, the species shaping the Pantanal gradient in the graph are not exclusive for this eco-region, but reflect their relatively higher abundance across the entire corridor area.

In an overview interpretation of the gradients resulted from the DCA we could infer that there might be some correlation with water availability, heterogeneity of habitats and other physical variables on a regional scale. The species congregated in the Pantanal gradient are more commonly found in wet environments while at the Emas Park end are more commonly found in drier habitats.

The indicator species analysis reflects similar results obtained from de DCA, in which the species with INVAL higher than 11% were the same that presented the highest scores in both axes.

In summary, the Pantanal presented the highest abundance indices, followed by Emas Park, River Taquari and the PNE-surroundings. Several landscape features can be used to describe and interpret the eco-regions in a similar order as determined by the mean abundance indexes. Along the corridor the Pantanal is the largest continuous area with the higher heterogeneity of habitat, distributed in mosaic. The dynamics of the water regime ruled by a conspicuous flooding season followed by severe droughts should play its role in the high primary productivity and subsequent effects on the food web. This environmental complexity is probably the major factor responsible for the high abundance indices found in this eco-region. Emas Park presented the second highest abundance index and also represents the second largest continuous natural area along the corridor. The River Taquari eco-region presented the third highest abundance index and in the same order, represents the largest tracts of natural habitats, although mostly fragmented. The fourth and last eco-region, reflecting the lowest abundance index also reflects the most converted area. Despite its proximity to a large "potential" source population such as that of Emas Park, the region is dominated by extensive agriculture, limiting the natural refuges to smaller isolated fragments.

The difference in richness between the distinct eco-regions, especially ENP, can probably be explained by the area size, heterogeneity and distribution of the available habitats. ENP for instance is predominantly covered by a flat terrain and grassland habitat (98%) in such a way that the uniformity of the landscape leads to different fauna compositions whenever sampled at habitat's ecotone. For example, cameras sampling at the few strips of gallery forest or at the border of valleys or marshes, presented distinct compositions. Despite the apparent landscape homogeneity, sampling in ecotones may explain higher composition variation in ENP.

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Capítulo 9
Levantamento e monitoramento de populações de carnívoros

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Introdução

O conhecimento do tamanho ou densidade de uma população muitas vezes é um requerimento vital para seu manejo efetivo (CAUGHLEY; SINCLAIR, 1994); é um dos meios mais diretos de medir o sucesso de planos de manejo ou conservação. Além disso, esse conhecimento permite ainda fazer inferências sobre as tendências da população estudada. A efetividade de áreas protegidas em manter populações viáveis de uma dada espécie pode também ser medida, numa primeira abordagem, se boas estimativas do tamanho e/ou densidade das populações de interesse e suas tendências forem obtidas. Finalmente, estudos ecológicos podem requerer estimativas de tamanho ou densidade para explicar padrões em ecossistemas ou comunidades.

Populações não são absolutamente estáveis ao longo do tempo. Flutuações na abundância podem existir, e elas existem, em vários níveis, desde imperceptíveis até flutuações dramáticas tanto positivas quanto negativas. Flutuações podem não ser tão importantes sob o ponto de vista de manejo ou conservação desde que algum critério de nível aceitável de variação seja adotado e elas não ameacem a viabilidade da população ou os objetivos do plano de manejo.

Estimativas do tamanho de população não são sempre necessárias. Em alguns casos, um índice de abundância é suficiente para inferir sobre mudanças na população-alvo ao longo do tempo. Um índice também pode permitir comparações entre diferentes populações, num mesmo momento.

Carnívoros tendem a ser crípticos, noturnos e ocorrerem em densidades relativamente baixas. Estes aspectos tornam mais difícil o trabalho de biólogos de campo, quando o objetivo é fornecer estimativas de abundância e/ou monitorar tendências de populações. Grandes carnívoros são um caso extremo, já que suas densidades tendem a ser baixas, e os levantamentos requerem a inclusão de grandes áreas amostradas e um esforço amostral enorme para se obter tamanho adequado de amostras e precisão das estimativas. Além disso, a captura de grandes carnívoros é mais difícil e cara do que espécies de tamanho pequeno. Finalmente, espécies raras são quase sempre difíceis de detectar, fazendo o trabalho de monitoramento das tendências de suas populações se tornar um objetivo bastante complicado.

No que se refere aos levantamentos de abundância de populações, esses aspectos irão influenciar várias características do estudo, como detectabilidade, precisão, acurácia, relação índice/abundância, poder estatístico, e suficiência de amostras, as quais devem ser consideradas quando se planeja o levantamento ou monitoramento de população.

Detectabilidade

Um dos maiores erros que continuam sendo cometidos por biólogos de campo é acreditar que todos os animais em uma dada área amostrada podem ser registrados e contados. Contagens totais raramente são possíveis, e nesse caso, são chamadas de censo. Quando contagens totais não são possíveis, diz-se que as estimativas são baseadas em contagens incompletas. Em abordagens de estatística básica, assume-se que a variável de interesse é registrada sem erro em cada unidade amostral. Entretanto, na maioria das situações de amostragens de populações animais, isso não é verdade. A probabilidade de um alvo ser detectado em uma unidade amostral, não importando se avistado, ouvido, capturado ou se sua presença é registrada por meio de um outro meio qualquer, é chamada de detectabilidade. Quase sempre, esta probabilidade (p) não pode ser conhecida, mas pode ser estimada por diferentes métodos, tais como contagem dupla, esquemas de captura-recaptura, amostragem de distâncias, etc. Na verdade, a maioria das técnicas de amostragem para levantamentos de populações animais são estratégias para se estimar e corrigir erros associados à probabilidade de detecção.

Índices de abundância

A obtenção de estimativas acuradas de tamanho absoluto de populações ou suas densidades é difícil (GIBBS, 2000). Uma solução freqüentemente usada por biólogos de campo é utilizar índices de abundância. Um índice é um valor que se espera que mude proporcionalmente com as mudanças na abundância absoluta (THOMPSON et al., 1998). Caughley (1977) chamou isso de um co-relativo mensurável da densidade, o qual é presumivelmente relacionado com a abundância verdadeira (GIBBS, 2000). A natureza da variação na abundância pode ser temporal ou espacial, e o índice nos auxilia a inferir sobre a magnitude desta variação. Quanto mais forte for a relação linear entre um índice e a abundância real da população, melhor é o índice. Apesar de estudos analisando a relação índice/abundância serem raros, eles seriam essenciais para inferir sobre a confiabilidade do índice de abundância. Na maioria das vezes, a abundância real não pode ser estimada com base em índices, a menos que um modelo de regressão entre o índice e as respectivas estimativas de abundância tenha sido obtido em áreas com diferentes tamanhos de população. Geralmente, esse não é o caso em estudos de campo envolvendo levantamentos de populações. Finalmente, a relação entre um índice e o tamanho de populações é influenciada por vários fatores, tais como desenho amostral adequado, probabilidade de detecção, precisão das estimativas e padronização de métodos. Existem duas premissas principais que devem ser respeitadas quando se decide utilizar índices: o índice deve ser monotonicamente relacionado com a variável que está sendo indexada (uma relação linear com intersecção na origem) e a variância amostral do índice deve ser pequena ou a coleta de dados deve ser suficientemente fácil de forma que a variância possa ser reduzida pelo aumento do tamanho da amostra (THOMPSON et al*.,* 1998).

Índices podem ser obtidos com base em vários sinais indicando a presença de mamíferos carnívoros. Rastros são comumente utilizados para um amplo leque de espécies, tais como o leopardo-das-neves, *Panthera uncia* (Schaller, 1977), o dingo, *Canis familiaris dingo* (ALLEN et al*.,* 1996), a onça-parda, *Puma concolor* (Van DYKE et al*.,* 1986), a lontra-de-rio norte-americana, *Lutra canadensis* (REID et al*.,* 1987; SMALLWOOD; FITZHUGH, 1995), a raposa-vermelha, *Vulpes vulpes* (SERVIN et al*.,* 1987; STANLEY; BART, 1991), o leão, *Panthera leo* (STANDER, 1998), o coiote, *Canis latrans* (O'DONOGHUE et al*.,* 1997), o lobo-guará, *Chrysocyon brachyurus* (LACERDA, 2002; RUMIZ; SAINZ, 2002), e muitas outras. Rastros podem ser utilizados de várias maneiras e para diferentes técnicas, tais como contagens ao longo de transectos (Van DIKE, 1986; SMALLWOOD; FITZHUGH, 1995; EDWARDS et al., 2000; O'DONOGHUE et al*.,* 1997; STANDER et al*.,* 1998; SERVIN et al*.,* 1987) ou estimando a freqüência de ocorrência em um conjunto de estações de rastros (p.e., ALLEN et al*.,* 1996; MAHON et al*.,* 1998; SARGEANT et al*.,* 1998; WOOD, 1959; LINDZEY; THOMPSON, 1977; SMITH et al*.,* 1994; TRAVAINI et al., 1996; DIEFENBACH et al*.,* 1994). Lacerda (2002) registrou a freqüência de rastros de lobo-guará e mão-pelada em caixas de areia distribuídas sistematicamente ao longo de estradas internas do Parque Nacional de Brasília, no Brasil. Atrativos podem ser utilizados, especialmente em estações de rastros (p.e., WOOD, 1959; LINHART; KNOWLTON, 1975; LINDZEY; THOMPSON, 1977; SMITH et al*.,* 1994; TRAVAINI et al., 1996). Entretanto, Wilson e Delahay (2001) alertam para o fato de que pode ser difícil relacionar tendências da população absoluta com valores de índices baseados em rastros, e que a relevância biológica das mudanças do índice deve ser vista com cuidado sob a luz de conhecimento da ecologia da espécie estudada.

Índices podem ser obtidos também através da contagem de outros tipos de sinais da presença de carnívoros, tais como fezes, desde que as fezes possam ser acuradamente atribuídas à espécie de interesse. Entretanto, deve-se levar em conta o fato de que a taxa de defecação pode variar com a estação do ano, idade e estado fisiológico. Além disso, a distribuição das fezes em uma dada área pode mudar sazonalmente, especialmente para carnívoros que apresentam variações no comportamento de marcação através de odores (DELAHAY, 2001). Para grandes carnívoros, um outro problema é a pequena chance de encontrar fezes em grandes áreas, já que a densidade dessas espécies tende a ser baixa, requerendo um enorme esforço amostral, o qual pode não ser realista.

De acordo com Wilson e Delahay (2001) existem duas abordagens principais para estimar índices de abundância por meio de contagens de fezes: quantificando-se o estoque de fezes em um determinado instante ou quantificando-se a sua taxa de acumulação. A quantificação do número de pilhas fecais pode ser feita ao longo de transectos em faixa (p.ex., LOCKIE, 1964) ou em quadrados ou segmentos de transectos em faixa (p.ex., STRATCHAN; JEFERIES, 1996). Para se quantificar a taxa de acumulação é necessário remover as pilhas fecais das unidades amostrais (quadrados, subunidades em transectos em faixa, etc.) e recontá-las após um dado período de tempo. Dois fatores podem afetar este tipo de levantamento: a taxa de decomposição das fezes e a possível influência de sua remoção sobre o comportamento da espécie de interesse (WILSON; DELAHAY, 2001).

A contagem de estruturas visíveis relacionadas com a presença de carnívoros pode ser também usada para estimar índices. Alguns carnívoros constroem tocas conspícuas para proteção e/ou reprodução, as quais podem facilmente ser utilizadas como um indicador da abundância em uma dada área (WILSON; DELAHAY, 2001). Várias espécies têm sido amostradas com base neste tipo de sinal, tais como texugos (Cresswel et al., 1990), lontras (KRUUK et al., 1989; Tomas et al., no prelo), e a raposa-vermelha (STORM et al., 1976; HARRIS; TREWHELLA, 1988; MARKS; BLOOMFIELD, 1999). Entretanto, o uso de tocas como indicador da abundância de carnívoros deve ser examinado com cuidado, com base em informações sobre o comportamento social, já que muitas espécies vivem em grupos e usam tocas comunais. Além disso, algumas espécies parecem usar mais de uma toca em dado período, o que pode levar a conclusões distorcidas sobre a abundância.

Observações diretas também podem fornecer dados para se estimar um índice de abundância. Neste caso, os números são usualmente relacionados com um dado esforço amostral, como o tempo despendido no levantamento, distância amostrada em transectos e assim por diante (WEBER et al., 1991; MAHON et al., 1998; DUCKWORTH, 1992). Para obter um bom índice, o esforço deve ser padronizado ao longo do levantamento e em toda a área amostrada, e também entre diferentes áreas amostradas, para ser comparável.

Geralmente, a taxa de encontro é o índice mais utilizado, para a qual correções de erros de visibilidade (detectabilidade) não são utilizadas. Assim, não se tem nenhuma idéia sobre a abundância real, o que não impede que a taxa de encontro seja utilizada para detectar tendências ou para comparar duas ou mais áreas de estudo. Quando contagens são obtidas de unidades amostrais definidas, como em transectos em faixa, e correções de erros de visibilidade não são feitas, a densidade aparente pode também ser utilizada como um índice de abundância. Contagens feitas à noite com auxílio de um facho de luz têm sido usadas para gerar índices de abundância baseados em observação direta de algumas espécies, como a raposa-orelhuda *Vulpes macrotis*, mas quase sempre com pouca correlação com estimativas de tamanho das populações (WARRICK; HARRIS, 2001). Por outro lado, Gehrt (2002) indicou que este método é útil para monitorar tendências em populações de guaxinim (*Procyon lotor*), mas recomenda cautela quanto ao uso deste método para comparar índices entre populações em habitats de características diferentes ou em avaliações de populações cujas densidades são baixas.

Similarmente, detecção remota através de fotografias ou vídeos pode produzir índices de abundância confiáveis (TOMAS; MIRANDA, 2003). Este tipo de amostragem é interessante se não há um meio de identificar indivíduos na população de forma a permitir o uso de marcação-recaptura para estimar seu tamanho, como é o caso de muitas das espécies de carnívoros neotropicais (MAFFEI et al., 2002). Um exame cuidadoso dos dados deve ser feito, considerando especialmente os conhecimentos sobre comportamento social da espécie estudada, já que agrupamentos sociais quase sempre resultam em falta de independência entre detecções dos indivíduos. Neste caso, câmeras de vídeo podem funcionar melhor do que câmeras fotográficas (WILSON; DELAHAY, 2001), já que permitem a identificação de grupos detectados ao longo do tempo de exposição, em vez de indivíduos. O sucesso do uso de câmeras é extremamente dependente do local onde o equipamento é colocado. Mais ainda, até mesmo a direção para a qual uma câmera é focada pode alterar significativamente a probabilidade de detecção de uma dada espécie e, portanto, seu uso exige uma boa experiência de campo. Uma das grandes tentações resultantes do uso de câmeras fotográficas automáticas é a de se aproveitar as fotos de todas as espécies detectadas numa dada área. Seria racional do ponto de vista econômico fazer isso, mas nunca se deve esquecer que um conjunto de câmeras armadas numa área tem algumas características fixas: a densidade das câmeras, os habitats e microhabitats nas quais elas se encontram e a direção para a qual cada máquina está focada. Estes aspectos são fundamentais para definir as chances de detecção de cada uma das espécies. Sabe-se que o mesmo protocolo não serve para amostrar espécies com áreas de vida, densidade e preferências por habitats diferentes, o que pode levar à obtenção de números sem nenhum significado demográfico, biológico ou estatístico, para a maioria delas. Assim, cautela no uso de câmeras (ou qualquer outro tipo de amostragem por ponto) é altamente recomendada. Uma outra alternativa é atrair animais para pontos de amostragem através da técnica de gravação e uso de vocalizações, como aplicado em leões por Ogutu e Dublin (1998). Esta técnica pode ser facilmente associada com câmeras para amostrar populações de carnívoros solitários, mas atenção especial deve ser dada às possíveis respostas comportamentais associadas às diferenças sexuais, sazonalidade e outros fatores. Para onças-pintadas, por exemplo, esta técnica pode ser problemática, já que efeitos de dominância associada às vocalizações podem levar a respostas opostas, ou seja, inibir a aproximação de certos indivíduos ao ponto de amostragem (PETER CRAWSHAW, comunicação pessoal). Estações de odores equipados com câmeras podem também ser alternativas interessantes, e um índice baseado em presença-ausência pode ser facilmente obtido.

Índices apresentam algumas características que devem ser levadas em conta. A relação entre o índice e a abundância real pode não ser linear, tomando formas nas quais as mudanças do índice não refletem as mudanças na população (GIBBS, 2000). Uma relação não linear pode ocorrer quando um índice se torna "saturado" em populações em altas densidades. Outro exemplo ocorre quando presença/ausência é usada para estimar um índice. A um dado ponto, quando todas as unidades amostrais são usadas (100% delas contêm sinais da presença da espécie de interesse) o índice não varia mais, mesmo que a abundância real aumente. Um outro problema que deve preocupar quem conduz levantamentos de populações de carnívoros através de índices está relacionado com flutuações abaixo do limite inferior de sensitividade do índice. Em densidades muito baixas, se o número de unidades amostrais é pequeno, observadores podem simplesmente falhar em registrar indivíduos ou sinais mesmo que eles estejam presentes na área estudada. Conseqüentemente, a detecção de mudanças abaixo deste limite é "bloqueada" (GIBBS, 2000). Esta situação tende a ocorrer para espécies raras, ameaçadas ou incomuns (ZIELINSKI; STAUFFER, 1996). Devido às premissas inerentes ao uso de índices e à falta generalizada de informações que demonstrem que é realista obedecê-las, Lancia et al. (1994) e Thompson et al. (1998) recomendam cautela em seu uso, exceto quando não existirem alternativas razoáveis. Finalmente, a validação do índice em contraste com estimativas da abundância absoluta é necessária, e isso tem sido feito com sucesso para algumas espécies, como leões, leopardos *Panthera pardus*, e cães-selvagens *Lycaon pictus* (STANDER, 1998), raposa-vermelha (SERVIN et al., 1987; O'DONOGHUE et al., 1997), guaxinim, "bobcats" *Felis rufus*, e raposa-cinzenta *Urocyon littoralis* (Conner et al., 1983), e raposa-orelhuda (WARRICK; HARRIS, 2001). Inconsistências têm sido evidenciadas em vários casos (DIEFENBACH et al., 1994; SARGEANT et al., 1998; SMITH et al., 1994; Gehrt, 2002), e muitas publicações também registram uma falta de correlação entre índices e estimativas de tamanho de populações de outras espécies não carnívoras (HAY, 1958; UHLIG, 1956; DOWNING et al., 1965; FULLER, 1991).

Tamanho e densidade de populações

Quando informações sobre a abundância absoluta são necessárias, as técnicas disponíveis podem ser separadas em duas classes: A) contagens completas, nas quais todos os indivíduos presentes na área de estudo ou em unidades amostrais são observados e B) contagens incompletas, nas quais apenas uma parte dos indivíduos existentes é detectada (CAUGHLEY; SINCLAIR, 1994; LANCIA et al., 1994; WILSON; DELAHAY, 2000). Contagens incompletas podem também ser divididas em métodos diretos e indiretos (CAUGHLEY; SINCLAIR, 1994; LANCIA et al., 1994). Métodos diretos são aqueles nos quais os animais observados são direta e acuradamente registrados pelo observador (como em contagens aéreas, transectos em faixas, transectos lineares, capturas, etc.), enquanto em métodos indiretos a estimativa pode ser feita através de estratégias ou artefatos que não requerem contagens acuradas dos animais, como em captura-recaptura, "change-in-ratio" ou índice-manipulação-índice (CAUGHLEY, 1977; CAUGHLEY; SINCLAIR, 1994).

Quando se planeja estimativas de tamanho de populações, uma preocupação deve ser um protocolo de amostragem adequado, e uma extensiva literatura sobre estes aspectos está disponível (e.g. CAUGHLEY; SINCLAIR, 1994; LANCIA et al., 1994; SUTHERLAND, 1996; THOMPSON et al., 1998; GIBBS, 2000). Cuidado deve ser direcionado para aspectos importantes, como precisão e acurácia, independência entre unidades amostrais, estratégia de amostragem, implicações de amostragem aleatórias e não aleatórias, estratificação da área de estudo e, finalmente, a biologia e o comportamento da espécie de interesse.

Para carnívoros, as técnicas são basicamente aquelas consideradas padrões para levantamentos de fauna, com algumas adaptações às características das espécies. Contagens completas são praticamente impossíveis, como discutido anteriormente, e contagens incompletas têm sido a regra. Contagens incompletas, também chamadas métodos de enumeração, somente produzem resultados sem viés quando as premissas de cada método são razoavelmente satisfeitas (THOMPSON et al., 1998).

Métodos diretos apresentam um amplo leque de dificuldades, dependendo de vários fatores, tais como tamanho corporal, densidade, tipo de habitat, comportamento, período de atividade, e custo. Um dos mais consistentes métodos é o de amostragem de distâncias (Buckland et al., 1993). As 3 premissas mais importantes são: A) todos os animais sobre a linha do transecto são detectados sem erro, B) indivíduos detectados são registrados em sua posição original e C) as distâncias são registradas sem erro. Entretanto, em algumas situações é difícil obedecer estas premissas (Duckworth, 1998). Transectos ou contagens por pontos em amostragem de distâncias são úteis para espécies que podem gerar uma taxa de encontro que resultam em um custo-benefício aceitável, significando que ela não é baixa ao ponto de requerer um esforço amostral impraticável para que se obtenha um número mínimo de registros, permitindo estimativas acuradas e precisas (cerca de 80 registros, dependendo da variância nas amostras). Para carnívoros, especialmente espécies de grande porte, esta meta pode ser muito difícil de se atingir, já que as observações tendem a ser bastante raras. Entretanto, este método tem sido aplicado para espécies como a raposa-vermelha (HEYDON et al., 2000) e a raposinha-do-campo (*Pseudalopex vetulus*) através de contagens noturnas (o método tem sido usado atualmente para estimar densidades desta espécie em Nova Xavantina, Mato Grosso, com resultados promissores). A raposinha-do-campo utiliza habitats abertos, bons para esse tipo de levantamento. Para espécies que vivem em habitats florestais, o método de amostragem de distâncias pode não ser adequado (DUCKWORTH, 1998), mas CULLEN et al. (2001) obtiveram estimativas acuradas de densidades de quatis *Nasua nasua*, bem como outras espécies que não carnívoros, em áreas de Mata Atlântica. No Pantanal, um levantamento de várias espécies vem sendo conduzido, incluindo mesocarnívoros, com resultados similares para quatis (Desbiez, comunicação pessoal). Transectos em faixa têm sido usados em algumas situações, tais como o levantamento multiespecífico conduzido por Glanz (1990) na Ilha de Barro Colorado, no qual carnívoros foram incluídos. Este método apresenta problemas, já que tende a subestimar o tamanho e a densidade de populações devido a inconsistências na correção dos efeitos de detectabilidade menor que 1 na faixa do transecto.

Levantamentos aéreos são adequados para espécies conspícuas, de grande porte e que vivem em habitats abertos (CAUGHLEY; SINCLAIR, 1994; MOURÃO; MAGNUSSON, 1997; WILSON; DELAHAY, 2001), e podem não ser aplicáveis para a maioria das espécies de carnívoros neotropicais. Levantamentos aéreos são um caso especial de transectos em faixa, nos quais as bordas da faixa de amostragem são fixas. Dado que a premissa básica em levantamentos em transectos em faixa ao nível do solo é que todos os animais dentro das faixas de amostragem são registrados (e nós sabemos que isso não é verdade devido à detectabilidade diferencial), uma estratégia é usar a técnica de contagem dupla para corrigir erros de visibilidade (MAGNUSSON et al., 1978; CAUGHLEY; GRICE, 1982; BAYLISS, 1986; BAYLISS; YEOMANS, 1989). Espécies de carnívoros vivendo em grandes grupos sociais podem ser boas candidatas para levantamentos aéreos (SHUTERLAND, 1996), já que as contagens são baseadas em registros de grupos ou agrupamentos em vez de indivíduos e grupos têm melhor chance de serem detectados a partir de um avião. Desta forma, as estimativas de tamanho e densidade populacionais são baseadas no cálculo do número de grupos existente em uma dada área. Em condições climáticas especiais, algumas estratégias podem melhorar a qualidade das estimativas, como a utilizada por GASAWAY et al. (1992) para lobos (*Canis lupus*) no Canadá e Alaska. O levantamento aéreo foi conduzido logo após nevascas através de transectos nos quais se buscou pegadas de matilhas de lobos, as quais foram seguidas até se encontrar a matilha e se contar o número de indivíduos. Para a grande maioria das espécies de carnívoros neotropicais, contagens aéreas podem ser inúteis e, quando for possível realizá-las, o esforço amostral pode ser muito grande e caro para que seja viável.

Uma outra técnica que pode ser classificada como direta é a de captura-recaptura, baseada no estimador de Lincoln-Petersen (PETERSEN, 1896), no qual uma amostra da população é capturada, marcada e liberada de volta na população em uma primeira ocasião. Numa segunda ocasião, outra amostra da população de interesse é capturada, e os indivíduos são classificados como recapturados (previamente marcados na primeira ocasião) ou não marcados. Baseando-se neste conjunto de informação, estimativas do tamanho da população podem ser obtidas se as 3 principais premissas deste método são obedecidas: A) a população é fechada (não houve mortes, nascimentos, imigração ou emigração entre a primeira e a segunda ocasião de captura), B) todos os animais têm a mesma probabilidade de ser capturados em cada ocasião, e C) todos os indivíduos previamente marcados podem ser distinguidos com precisão daqueles não marcados (marcas não são perdidas). Variações deste modelo básico têm sido cuidadosamente desenvolvidas, numa tentativa de resolver problemas relacionados com a violação destas premissas, e vários modelos alternativos têm sido propostos e validados, com uma extensa literatura disponível (JOLLY, 1965; SEBER, 1965; SEBER, 1982; SEBER, 1986; SEBER, 1992; OTIS et al*.*, 1978; WHITE et al*.*, 1982; POLLOCK et al., 1990; BURNHAM et al., 1994; FERNANDEZ, 1995; THOMPSON et al., 2000). A história de capturas de cada indivíduo em populações fechadas é usada para calcular a probabilidade de captura e assim incorporar nas estimativas o conceito de detectabilidade. Para populações abertas, várias abordagens alternativas têm sido propostas (LEBRETON et al*.*, 1992; LEBRETON et al*.*, 1993). Para carnívoros, estes métodos têm sido aplicados de várias maneiras, mas existem restrições porque carnívoros às vezes são difíceis de capturar em um período relativamente curto e em número adequado. Num levantamento populacional do mangusto *Herpestes javanicus*, o método de captura-recaptura não funcionou adequadamente quando comparado com o método de amostragem de distâncias através de uma teia de armadilhas (CORN; CONROY, 1998). Diferentes estratégias de amostragem têm sido usadas, como as de levantamento indireto através de fotografia remota, escatologia molecular, e mesmo a identificação de indivíduos através de mensuração de suas pegadas. Fotografia remota é uma opção bastante interessante se os animais podem ser capturados em números relativamente altos, marcados e liberados na população para serem "recapturados" através de fotografias (GRIFFITHS; van SCHEIK, 1993; MACE et al*.*, 1994; CUTTLER; SWAN, 1999; TOMAS; MIRANDA, 2003). O problema é que marcas devem ser conspícuas e facilmente identificadas, o que nem sempre é possível através de fotografias. Se o levantamento requer mais do que duas ocasiões de captura, então uma acurada identificação de cada indivíduo é necessária para permitir a construção de uma tabela com a história de capturas de cada um deles. Assim, a qualidade e visibilidade do artefato de marcação é um fator chave. Uma outra estratégia se aplica a espécies que possuem marcas naturais, como os gatos pintados. Todas as capturas podem ser obtidas através de fotografia remota automática, permitindo o uso da abordagem de múltiplas capturas e recapturas. Este método tem sido usado em tigres (KARANTH, 1995; KARANTH; NICHOLS, 1998), jaguatirica *Leopardus pardalis* (TROLLE; KÉRY, 2003; MAFFEI et al., 2002; JACOB, 2002) e onça-pintada *Panthera onca* (MAFFEI et al., 2002; SILVEIRA, 2003). Atualmente, esta técnica está sendo usada em vários projetos sobre onça-pintada no Brasil e Bolívia. Capturas em vídeo têm sido usadas para estimar o tamanho de populações de ariranhas (*Pteronura brasiliensis)* no Pantanal, Brasil, através de métodos de remoção (W. M. Tomas, não publicado).

Marcação-recaptura tem sido conduzida também por meio do uso de radioisótopos, com sucesso (PELTON, 1979; SHIRLEY et al., 1988; CRABTREE et al., 1989). Recapturas visuais também são uma alternativa viável (ARNASON et al., 1991), como aplicada por HEIN; ANDELT (1995) em coiotes. Tetraciclina é outro marcador químico utilizado para estimar tamanho de populações de carnívoros baseado em abordagem de captura-recaptura, como em urso-polar *Ursus maritimus* (TAYLOR; LEE, 1994). Associação de radiotelemetria e esquemas de captura-recaptura também tem sido usada em várias espécies (GREENWOOD et al., 1985; HALLET et al., 1991; MILLER et al., 1997). Tentativas de registrar todos os indivíduos em uma dada área com base em fotografia remota têm sido publicadas (MAFFEI et al., 2002), mas o melhor seria assumir as estimativas como índices.

Escatologia molecular é uma alternativa muito promissora entre os métodos indiretos, apesar de seu custo e problemas técnicos que ainda precisam ser melhorados. O isolamento de DNA de células do excretor presentes em suas fezes permite não apenas identificar sua espécie e sua população de origem, mas também sua diferenciação de outros indivíduos da mesma espécie e população (HOSS et al., 1992; KOHN; KNAUER, 1997; KOHN et al., 1999). O DNA nuclear fornece informação baseando-se em locos microsatélite, os quais permitem identificar os indivíduos presentes em uma dada área. O DNA mitocondrial permite a correta definição da espécie de um dado indivíduo excretor, eliminando erros devido à identificação incorreta (WASSER et al., 1997; Kohn et al., 1999). A abordagem básica é tratar cada visita para coleta de amostras de fezes em uma dada área como ocasiões de captura (WILSON; DELAHAY, 2001). Esta abordagem tem sido usada em alguns carnívoros, como urso-pardo *Ursus arctos* (TARBELET et al., 1997), coiote (KOHN et al., 1999) e onça-parda (Ernest et al., 2000). Os principais problemas relacionados com esta técnica são a necessidade de obtenção de amostras relativamente grandes de fezes frescas (KOHN et al., 1999; WILSON; DELAHAY, 2001), a estocagem apropriada para minimizar a degradação do DNA (TARBELET et al., 1999), os erros inerentes ao processo de PCR, que podem produzir falsos alelos (TARBELET et al., 1999; WILSON; DELAHAY, 2001), e o custo. A coleta de amostras de pêlos também permite este tipo de levantamento indireto para estimar tamanho de populações baseando-se em técnicas de genética molecular, e tem sido usada com sucesso por Foran et al. (1997), Tarbelet et al. (1999) e Mowat e Strobeck (2000).

Algumas tentativas de identificação de indivíduos com base na mensuração de suas pegadas têm sido publicadas (SMALLWOOD; FITZHUGH, 1993; GRIGIONE et al., 1999, LEWISON et al., 2001; RUMIZ; SAINZ, 2002). O uso desta técnica indireta em esquemas de captura-recaptura é perigoso porque a premissa de correta identificação ou enumeração de indivíduos nas amostras é crítica. Qualquer identificação errônea baseada na medição das pegadas irá produzir dois tipos de problema: superestimar o número de indivíduos presentes por gerar "novas" capturas (e assim diminuindo a probabilidade de "recaptura"), ou subestimar o número de indivíduos por não detectar diferenças nas medidas das pegadas de indivíduos diferentes (e assim deixar de fazer novas "capturas" e aumentar a probabilidade de "recapturas"). Apesar de controvérsias existirem sobre este assunto, é inegável que este método pode ser muito sensível à precisão e acurácia do processo de medida das pegadas. A medição de pegadas é fortemente influenciada pelo observador, pelo substrato, pelas condições climáticas e outros fatores que são fonte de variância, fazendo com que a confiabilidade do método para uma abordagem de captura-recaptura seja, no mínimo, discutível. Em situações especiais, o uso de intersecção linear pode fornecer estimativas melhores, como a técnica desenvolvida por Becker (1991) para estimar as populações de glutões (*Gulo gulo*) e linces no Alaska.

Métodos de remoção podem ser utilizados para estimar o número de indivíduos em populações fechadas de carnívoros, especialmente se a remoção não for física. Fotografia remota, genética molecular e medidas de pegadas podem ser usadas para identificar indivíduos em uma população ("capturas"), sendo que apenas indivíduos novos (não "marcados") são considerados em cada ocasião de "captura". O declínio no número de animais não "marcados" é usado para estimar o tamanho da população (SUTHERLAND, 1996). Este método permite uma variação do esforço amostral, mas terá um viés se a detectabilidade variar de indivíduo para indivíduo. Além disso, uma reta deve se ajustar a uma regressão entre o número de indivíduos não "marcados" e o número acumulativo de indivíduos "capturados" (SUTHERLAND, 1996).

Radiotelemetria tem sido usada para estimar tamanho de populações em grandes carnívoros, com base no tamanho e sobreposição de suas áreas de vida. Esta estratégia apresenta problemas, já que se assume que todos os indivíduos usando uma dada área foram capturados e monitorados por um dado período de tempo para se obter estimativas de área de vida. O método tende a produzir subestimativas se alguns animais presentes na área não foram capturados e incluídos na amostra (não detectados). Estas restrições são mais importantes em espécies com grandes sobreposições de áreas, mas podem ser minimizadas em espécies mais territoriais. Esta abordagem foi utilizada em lobo-guará, no Brasil (RODRIGUES, 2002). Um outro problema desta técnica é seu custo relativamente alto, o tamanho da amostra quase sempre pequeno, e a corriqueira falta de réplicas.

Usualmente, a técnica é aplicada em estimativas de tamanho ou densidade de populações em associação com estudos cujos objetivos exigem o uso de radiotelemetria. O método pode ser interessante se não existem técnicas alternativas viáveis, dadas as características do relevo, a abundância e o comportamento da espécie de interesse. Radiotelemetria também pode ser utilizada em esquemas de captura-recaptura, bem como para validar outras técnicas através de comparações das estimativas e identificação correta de indivíduos em levantamentos baseados em pegadas (DEMASTER et al., 1980; GREENWOOD et al., 1985; SERVIN et al., 1987; MILLER et al., 1997).

Monitoramento de tendências populacionais

Estimar a abundância em populações de carnívoros às vezes é difícil, mas monitorar suas tendências impõe um nível maior de complicações. Monitorar tendências de populações significa avaliar as flutuações no número de indivíduos ao longo do tempo e avaliar se existe uma tendência positiva ou negativa. Estes objetivos podem ser atingidos usando índices de abundância ou estimativas de tamanho absoluto da população ou de sua densidade.

Monitoramentos permitem avaliar as respostas da população de interesse às práticas de manejo e conservação, bem como aos impactos de fatores externos (doenças, caça, perda de habitat, mudanças climáticas). Além disso, um programa de monitoramento pode dar suporte a processos de tomada de decisão com base em informação consistente sobre a população e suas tendências. Apesar de ser conceitualmente simples, a tarefa de monitorar populações ao longo do tempo pode ser uma empreitada decepcionante e difícil (GIBBS, 2000; EAGLE et al., 2001). A habilidade de um dado protocolo de amostragem em detectar com sucesso uma tendência existente na população de interesse, manifestada como significância estatística, é conhecida como poder (GERRODETTE, 1987; THOMPSON et al.,1998; GIBBS, 2000; EAGLE et al., 2001). Análise de poder é, portanto, uma estratégia para fornecer direções para a definição de um protocolo de amostragem (esforço amostral versus período mínimo de monitoramento) que atinja adequadamente o nível de sensibilidade requerido para detectar tendências. Estatisticamente, poder é definido como $1 - b$, sendo b a probabilidade de se aceitar erroneamente a hipótese nula quando ela na realidade é falsa (Erro Tipo II). Além disso, o poder é complementado pela probabilidade desejável de se rejeitar corretamente a hipótese nula, que é influenciada por muitos fatores, tais como variabilidade nas contagens, tamanho da amostra, esforço anual, duração do levantamento, nível escolhido da magnitude da tendência a ser detectada, e nível de significância estatística a (THOMPSON et al., 1998; EAGLE et al., 2001). Esta é a probabilidade de erroneamente se rejeitar a hipótese nula, ou Erro Tipo I. A variação nas contagens é o “ruído” que um programa de monitoramento deve diminuir para detectar o “sinal” que é a tendência da população (GIBBS, 1996).

A sensibilidade de um esquema de monitoramento depende muito de estimativas de abundância ou índices de abundância precisos. Quando se trata de índices, a relação entre o tamanho real da população e o índice deve ser forte, linear e monotônico para ser útil em um programa de monitoramento. Estimativas de tamanho populacional não devem apresentar viés, ou ser quase sem viés e, de forma válida, poderem ser estendidas para toda a área de interesse (THOMPSON et al*.,* 1998). Usualmente, uma análise de poder deve ser conduzida com base em um levantamento piloto, de forma que a definição de um protocolo de amostragem ajude a evitar Erros Tipo I e II.

Para carnívoros, monitoramentos têm sido têm sido feitos através de métodos indiretos (WILSON; DELAHAY, 2001; WARRICK; HARRIS, 2001; HAYARD et al*.,* 2002; SCHAUSTER et al*.,* 2002), com a premissa de que o índice tem uma correlação positiva com a população real. HAYARD et al*.,* (2002), por exemplo, usou um índice baseado em levantamentos de pegadas para monitorar tigres em Amur (*Panthera tigris altaica*), na Rússia. Os autores conduziram uma análise de poder para definir um programa de monitoramento, e sugeriram que a detecção de declínios na população de tigres poderá ser melhor obtida através de contagens de pegadas do que com levantamentos baseados em presença-ausência.

Levantamento e monitoramento de populações de carnívoros neotropicais

Existem muito poucos estudos sobre carnívoros neotropicais envolvendo estimativas e monitoramento de populações. Os critérios da IUCN para identificar espécies ameaçadas requerem dados sobre abundância de populações e suas tendências mas, com exceção de poucos estudos sobre abundância em escala local (CULLEN et al*.,* 2001; LACERDA, 2002; RUMIZ; SAINZ, 2002; RODRIGUES, 2002; MAFFEI et al*.,* 2002; JACOB, 2002; TROLLE; KÉRY, 2003), dados de longo prazo não têm sido publicados para carnívoros neotropicais.

Os vários métodos discutidos neste capítulo apresentam validade variável para espécies neotropicais, considerando-se as variações em sua biologia, comportamento e abundância (ver Tabela 1). Índices de abundância baseados em levantamento de pegadas podem ser úteis para a maioria das espécies, mas recomenda-se cautela quanto ao significado biológico e estatístico dos números obtidos no campo. Levantamentos baseados em contagens de fezes e observação direta também se aplicam diferencialmente entre as diferentes espécies (Tabela 1). Recomenda-se bastante que se façam estudos relacionando índices com estimativas de abundância para validar seu uso. Técnicas mais caras são usualmente mais consistentes para algumas espécies e situações, tais como câmeras automáticas, análise de DNA das fezes, e radiotelemetria. O julgamento do que deve ser usado deve ser feito guiando-se pela definição de que tipo de informação é requerido e os objetivos do estudo ou plano de conservação, de forma que todos os aspectos possam ser considerados para que se atinja uma relação custo-benefício equilibrada. Em alguns casos, estas técnicas mais dispendiosas podem ser as únicas alternativas, como câmeras fotográficas ou genética molecular, para se estimar a abundância de espécies difíceis de serem detectadas por métodos convencionais ou monitorados por radiotelemetria, como o urso-andino (*Tremarctos ornatus*), por exemplo. Por outro lado, espécies que possuem marcas naturais, como os felinos pintados e a ariranha, são boas candidatas para levantamentos através de fotografia remota ou vídeo, mas o uso de DNA pode ser complicado em ariranhas porque os indivíduos costumam defecar em latrinas comunais e as fezes são misturadas com urina e terra logo após a defecação. Para aquelas espécies que ocorrem em densidades muito baixas, amostras de fezes podem ser obtidas através de cães treinados, aumentando assim a chance de encontro de material adequado para extração de DNA. Finalmente, uma análise cuidadosa deve ser conduzida para cada espécie de interesse antes de se decidir por uma técnica ou protocolo de amostragem. A Tabela 1 é um exercício que tenta fornecer uma síntese da aplicabilidade dos diferentes métodos em levantamento de espécies representativas da fauna de carnívoros neotropicais.

É fundamental que biólogos de campo entendam a importância de se obter dados confiáveis sobre a abundância de populações, bem como as premissas, os problemas e vantagens de cada método e protocolo. Treinamento e orientação, incluindo análise cuidadosa dos objetivos do estudo ou do programa de manejo, o tipo e a qualidade dos dados requeridos, e o máximo possível de padronização de metodologias e esforços são aspectos fundamentais para a melhoria da quantidade e qualidade da informação sobre de carnívoros neotropicais. Monitoramento, por outro lado, deve ser incluído na agenda, já que não existem informações disponíveis sobre as tendências das populações destas espécies. Nós encorajamos biólogos de campo e agências financiadoras a investir em projetos consistentes, baseando-se em programas de monitoramento prioritários e bem definidos para se detectar tendências de longo prazo nas populações de carnívoros. O sucesso dos esforços de conservação vai depender bastante da capacidade que tivermos em fornecer dados claros e consistentes, sobre a situação das populações, principalmente de espécies ameaçadas de extinção.

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Tabela 1 – Síntese de tentativa da aplicabilidade de diferentes metodologias para levantamentos populacionais de espécies representativas de carnívoros sul-americanos.

Espécie	Indice							
	Rastros	Fotos	Fezes	Tocas	Contagem aérea	Observação direta	Capturas	Rastros
Onça-pintada	***	****	***			*	*	**
Onça-parda	****	****	***			*	*	**
Jaguatirica	****	****	**			**	**	*
Gatos pintados	***	****	*			*	**	*
Jaguarundi	***	***	*			**	**	*
Gato-palheiro	****	***	*			*	*	*
Lobo-guará	****	****	****		*	****	**	*
Cachorro-vinagre	**	**	*			*	*	*
Lobinho	****	****	****		*	****	****	*
Raposinha	****	****	****		*	****	****	*
Irara	****	***	*			**	**	*
Ariranha	*	**		***	**	****	*	*
Lontra	*	*	****	***	*	***	*	*
Furão	****	*	**	**		**	**	*
Jaritataca	****	***	*	**		****	***	*
Quati	****	****	**		**	****	***	*
Mão-pelada	****	****	*			***	**	*
Kinkajou	*	*	*			**	*	*
Olyngo	*	*	*			**	*	*
Urso andino	**	****	***		*	**	*	*

Célula em branco – não aplicável
* Praticamente impossível
** Baixa
*** Médio
**** Alta

Tamanho/densidade							
Captura-recaptura/método de remoção				Telemetria	Transectos Lineares	Contagens aéreas	
Observação direta	Fotos	Videos	Genética molecular	Área de vida	Amostragem de distâncias	Contagem dupla	Amostragem de distâncias
*	****	**	****	***			
*	*	*	****	***			
**	****	**	****	***	*		
*	****	**	**	***	*		
*	*	*	*	***	*		
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**	***	***	**	**	**		
***	*	*	*	**	****	*	*
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*	*	*	*	**	*		
*	*	*	*	**	*		
*	****	***	***	***	*	*	*

Parte IV
Predação de animais domésticos por carnívoros silvestres

Capítulo 10
The impact of domestic animal predation by large carnivores. How does this affect the conservation of keystone species?

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Introduction

One of the main problems facing conservation of large carnivores is the level of livestock depredation in areas where they live in close contact. Why should we preserve species that sometimes cause considerable economic losses to certain human populations? Why should we bring back species that were exterminated in several regions of the world in retaliation to livestock depredation? Why should we protect species that require considerable resources and time to be studied? Are large carnivores so important that they warrant special conservation efforts? In a world that natural landscapes have been altered or destroyed to satisfy human need for development, species requiring large areas to survive have undergone local extinctions or drastic reductions. Furthermore, large carnivores are in many cases, forced to live in close contact with domestic stock and therefore, use them as prey. As this source of conflict mounts, our chances of understanding the importance of large carnivores and their relationships with other species and with their ecosystems decrease considerably. Indeed, for the Neotropics, information on the population dynamics of large carnivores and their relationships with domestic stock is largely lacking.

In this chapter, I will investigate a possible link between depredation of domestic animals by large carnivores and the conservation of keystone species in some Neotropical environments. Can depredation of livestock by large carnivores be responsible for the elimination of some keystone species? Is there a positive aspect in conserving keystone species even when these are responsible for livestock losses? I will review several case studies where the role played by some predatory mammals in their terrestrial ecosystems is discussed. Furthermore, I will consider the complex interaction between large predators and their prey base. Finally, I will use existing information to discuss how the absence of some keystone species can alter the whole balance of terrestrial ecosystems.

Depredation of domestic stock

Among the forces that menace the conservation of large carnivores, retaliation against animals that prey upon domestic stock has been one of the most effective ways of reducing populations of large carnivores. In North America and Eurasia, species like the wolf (*Canis lupus*) have a long history of persecution and elimination by local people, mostly because of predation on livestock (BOITANI, 1995; MECH, 1996; CIUCCI; BOITANI, 1998). In European countries, the main reasons for this persecution seemed to be the alteration of natural habitats and elimination of wolf's prey populations (CIUCCI; BOITANI, 1998). In North America, wolves have been eradicated over the past 350 years (CLUFF; MURRAY, 1995), being eliminated from 97% of their original range (WEBER; RABINOWITZ, 1996). In addition to the alteration of the original wolf's habitat, persecution and elimination of some of the wolves' main prey, such us the bison (*Bison bison*) and other prey species, contributed for increasing the numbers of domestic livestock in North America. In turn, this alternative source of prey became one of the preferred prey species for wolves (PATE et al., 1996).

Other large carnivores have suffered the same fate of reduction due to human pressure. Populations of brown bears (*Ursus arctos*) have declined considerably mostly due to habitat destruction and human overexploitation (SERVHEEN, 1990). In Europe, areas where extensive grazing is practiced, populations of brown bears have suffered considerable human retaliation due to predation on livestock (COZZA et al., 1996). In North America and Canada, predation upon domestic stock has become one of the sources of illegal killing of brown bears and contributes for the negative perception toward this carnivore species (KELLERT et al., 1996). This situation is aggravated in the case of another predator, the mountain lion or puma (*Puma concolor*). In addition to be considered as livestock predators in several regions of North America (SHAW, 1975; BROWN, 1986, ANDERSON et al., 1992; KELLERT et al., 1996), pumas are reported to cause either injuries or killings of several domestic animals in just one attack (SHAW, 1975; ANDERSON et al., 1992). This behavior also contributes for the negative attitude towards this predator.

Jaguars (*Panthera onca*) and pumas are the two largest predators in the lower Neotropics. The jaguar originally ranged from southwestern United States to south of the Rio Negro, in Argentina (45º S) (HALL, 1981). The jaguar now occupies only 33% of its original range in Mexico and Central America, and 62% in South America (SWANK; TEER, 1989). The puma's range extends from southern Canada through to southern Chile (JACKSON, 1991), and although generally not considered as threatened, some subspecies, such as the Florida panther (*Puma concolor coryi*) are considered to be endangered (JACKSON, 1991). Occurring sympatrically over most of their ranges, the jaguar and the puma have faced two main threats to their survival. Habitat loss and retaliation after livestock killings have contributed to the decline of both carnivore populations (FARRELL et al., 2000). Indeed, populations of jaguars have suffered more with deforestation (HOOGESTEIJN et al., 1993) mostly because jaguar requirements for high habitat quality comprised by extensive portions of forest cover, abundance of water and prey species. Specifically, the destruction of tropical and subtropical forests, as well as cattle grazing and shifting cultivation patterns, has vastly reduced habitat availability for Neotropical cats. Jaguars also have been subject to direct killing by man as a means of reducing predation on livestock (SCHALLER; CRAWSHAW, 1980; RABINOWITZ, 1986; MONDOLFI; HOOGESTEIJN, 1986). In addition to these factors, rudimentary livestock management and illegal hunting of jaguars by poachers when searching for jaguar staple prey species have contributed for the decline of jaguar populations throughout their range (SWANK; TEER, 1989; HOOGESTEIJN et al., 1993; CRAWSHAW, 1995). Puma populations have experienced lesser declines, due to predator control activities and sport hunting (YANEZ et al., 1986; LINDZEY et al., 1988).

Certain predisposing factors seem to influence jaguar and puma depredation on livestock. Livestock roaming throughout jaguar's habitat, low abundance of these two predators' natural prey, habitat characteristics and predation by sick or injured predators often turned into killers because of shooting by ranchers or poachers, have been reported as the main factors contributing for increasing the level of predation on livestock (MONDOLFI; HOOGESTEIJN, 1986; RABINOWITZ, 1986, HOOGESTEIJN et al., 1993; KELLERT et al., 1996; WEBER; RABINOWITZ, 1996). Hoogesteijn et al. (1993) reported that loss of habitat, poaching of jaguars and their prey and rudimentary cattle management, were the main causes promoting jaguar predation on livestock. Those factors led jaguars to incorporate livestock on their diets, and cattle constituted 35-56% of total prey killed by jaguars in three ranches in Venezuela. Geographic variation and suitability of habitats were reported to be main factors driving depredation on sheep by pumas (TORRES et al., 1996). Suitable habitat was related to increases in levels of depredation of sheep, while fragmentation of habitats due to human development inside puma's habitats was related to increases in predation of pet animals in some regions of North America. Furthermore, in the case of pumas, some other factors such as weather and light conditions unfavorable to human activities are also reported to promote depredation on livestock (MAZZOLLI et al., 2002).

Jaguars can subsist on a wide variety of prey. Schaller (1980) reported that in Pantanal, Brazil, cattle became the jaguar's main food source because cattle were the most important prey in terms of available biomass. The increasing frequency of jaguars attacking livestock in several regions of Venezuela has been a direct consequence of the decrease in wild prey combined with the loss of forested habitat (MONDOLFI; HOOGESTEIJN, 1986). This reinforces the idea that jaguars are able to adapt their land-tenure system and related behavior to suit local circumstances, thereby using alternative sources of prey such as domestic stock.

Changes in wild prey populations may be important in determining the predation impact of predators on livestock. However, livestock depredation may not be positively correlated to the availability of wild prey species. Stahl et al. (2001) reported that Lynx (*Lynx pardina*) did not kill sheep due to lack of wild prey. Kaczensky (1996) observed that predation on sheep by lynx was high only where large flocks of unattended animals were put to graze in forested sites. The same pattern was observed by Schaller (1980), where cattle were usually left unattended grazing in areas with close contact with jaguars. Other large cats seem to behave in a similar way. When livestock is raised in close contact with predators, missing animals were reported to be preyed upon by leopards (MIZUTANI, 1993). However, the availability of wild prey was suggested to be an important factor in maintaining low levels of predation on livestock (MIZUTANI, 1993). It appears that a variety of factors such as herding management, habitat characteristics, availability of wild prey, predators with injuries, and abundance of predators, act together in shaping patterns of large cats depredation on livestock. Nevertheless, it seems that the most appropriate approach in understanding patterns of livestock predation by jaguars and pumas should involve a comprehensive study of their prey characteristics, habitat, predator and prey abundance, predator's health condition and livestock husbandry practices.

As the pattern of livestock depredation persists, some important carnivore species are pushed to the brink of extinction in some areas where they were abundant in the past. The conservation of Neotropical carnivores, such as the jaguar and the puma, now faces the threats and difficulties that eliminated important species of carnivores from the earth. For instance such as three out of eight subspecies of tigers have disappeared in the last 50 years (WEBER; RABINOWITZ, 1996). We are now called to make a difficult choice that may sustain populations of important Neotropical carnivores in the future. The future for these large carnivore species lies on our capacity to maintain and study healthy populations before they get too isolated and forced to alter their natural behavior through predation on livestock.

Predators and their prey base

It appears that populations of large cats generally are regulated largely by social behavior and prey numbers. Factors other than prey abundance can also determine predator density, including availability, prey distribution in space and time, and prey quality and size (SUNQUIST; SUNQUIST, 1989). When prey availability is adequate, a land tenure system based on prior residency limiting the number of resident animals seems to be the main factor regulating populations of pumas (HORNOCKER, 1969, 1970; SEIDENSTICKER et al., 1973) and bobcats (BAILEY, 1974, HORNOCKER; BAILEY, 1986). This system seems to break down when prey density is low, which leads to the dispersion of resident cats thereby collapsing the social system (HORNOCKER; BAILEY, 1986; POOLE, 1995).

Jaguars have been reported as being territorial animals with large home ranges (SCHALLER; CRAWSHAW, 1980; RABINOWITZ, 1986). Their movements and habitat use have been related to the presence of water sources, availability of prey, and forest cover (EMMONS, 1987; CRAWSHAW, 1995). More recently, factors such as the close contact with livestock and human activities also have also been responsible in shaping jaguars' habitat use (CRAWSHAW, 1995). However, the influence of the presence of other predators such as pumas and ocelots in shaping jaguar's space and habitat use remains unclear yet potentially important.

It is unclear if overlap in resource utilization among Neotropical cats is due to competitive or noncompetitive factors. Large overlap among jaguars and pumas has been reported in areas where prey seemed to be abundant, but where spatial interaction among them remained unclear (EMMONS, 1987; CRAWSHAW, 1995). EMMONS (1987) reported that pumas overlapped the entire geographic range of jaguars, with the only observed difference being in the use of riparian areas by jaguars and avoidance of such areas by pumas. In Iguaçu National Park, Brazil, the lack of permanent small river courses might lead jaguars to compete more intensively with pumas for food and space. Although no evidence of spatial or temporal separation between puma and jaguar was observed by Crawshaw (1995), the lack of pumas with radio-collars during the course of the study in Iguaçu National Park, caused low statistical power.

For large felids, the most profitable food source seems to be the largest available prey that can safely be killed (SUNQUIST, 1989). However the abundance of prey is not directly related to the availability of prey, but rather to prey vulnerability to predation. Emmons (1987) noted that jaguars in southeastern Peru took agouti (*Dasyprocta variegata*), paca (*Agouti paca*), deer (*Mazama americana*) and capybara (*Hydrochaeris hydrochaeris*) according to their relative abundance. However, peccaries were taken disproportionately often. Pumas, in contrast, took their main prey, such as paca and agouti, close to their estimated abundance. Ocelot concentrated on prey weighing less than 1 kilogram, which included reptiles and birds. Prey abundance and vulnerability determining the jaguar's diet were observed in Belize, where they preyed upon armadillo (*Dasypus novemcinctus*). Crawshaw (1995) discussed that jaguars in Iguaçu took peccaries much more often than the estimate relative abundance would predict, while deer were the only prey taken in similar ratios to their estimated abundance. In contrast, ocelots concentrated upon armadillo, agouti and opossum, and thus did not show substantive dietary overlap with jaguars and pumas. Despite the importance in defining the ecological parameters that might influence the regulation of populations of Neotropical cats described in the studies mentioned above, the effects of the dietary and habitat overlap among these felid species require further attention.

More information on the dynamics of the interaction among these three cats would be relevant in explaining how fluctuations on prey numbers as well as on predator densities can determine the organization of Neotropical communities.

Conservation of keystone species in the neotropics

Although still controversial, the role of some carnivores as keystone species may be demonstrated through removal from their original environments. As defined by Power (1986), when a species with low abundance has a relatively large impact on its community or ecosystem, this species is considered as keystone. In most cases, predation is the way in which the role of keystone species is demonstrated (ESTES, 1996). The absence of predation through the elimination of keystone species can lead to local extinction of prey species or even predators. As reported by Berger et al. (2001) the local extinction of some important carnivore species has been shown to trigger important ecological events. Their findings supported the theory of top-down effect of large carnivores in terrestrial communities. The removal of important carnivores, such as the wolf and the grizzly bear from the southern Yellowstone Ecosystem caused the increase on population numbers of one of their main prey, the moose (*Alces alces*). In addition to that, other effects were reported, such as the alteration of riparian willow vegetation structure and density due to the effect of herbivory and the reduction of avian Neotropical migrants in the communities affected by moose herbivory (BERGER et al., 2001). The interaction between wolves and moose is also demonstrated through predation on Isle Royale, where the number of wolves and the level of predation probably affects moose populations. It was demonstrated by McLaren and Peterson (1994) that when there is decrease on wolf numbers, the rate of growth in young fir trees is depressed due to moose predation, process that was called wolf-induced trophic cascade.

The effects of the absence of important predator species have been suggested for coyotes in North America. Considered as the most important predator of domestic sheep in North America (WAGNER, 1988; ANDELT, 1996; NEALE et al., 1998) coyotes have been exterminated due to predation on domestic stock in several regions of North America. Coyote disappearance was reported to cause the increase on numbers of smaller wild carnivores such as stripped skunks, raccoons, and grey foxes, as well as exotic carnivores, such as the domestic cat, and the opossum. This increase on smaller predators due to the absence of a top predator resulted on the increase rate of predation upon birds and other small vertebrates (mesopredator release) (CROOKS; SOULÉ, 1999).

In the Neotropics, the complexity and organization of ecological communities generally has been related to the importance of resource availability. Indeed, populations of herbivore mammals seem to be regulated by the abundance of leaves and fruits in Neotropical forests. Seasonal shortage of fruits and new leaves appears to be the limiting factor for herbivore populations, as reported for Barro Colorado Island, Panama, and for several rainforests (LEIGH, 1999). If herbivore mammals are restraint by the availability of leaves and fruits, what role do predator mammals have on the regulation of Neotropical communities?

As suggested by Paine (1966) and numerous other authors, keystone predators control community organization through their impact on prey species. When present in Neotropical forests, predators can exert control on species proportions and densities. Eisenberg (1980) reported the abundance of small predators as having a negative impact on biomass values of rodents in a forest in Venezuela. He also suggested that the abundance of species of rodents at Barro Colorado Island might be correlated to the absence of certain species of carnivores. Opposing the idea of predators regulating Neotropical communities, Glanz (1982) suggested that higher abundance of herbivore mammals at Barro Colorado than in other forest sites might be related to the availability of resources, not the effect of predation.

Three species of top carnivores, jaguar, puma and ocelot (*Leopardus pardalis*) are known to occur sympatrically in most Neotropical forests. Their function in regulating other mammal populations through predation is still a controversial issue. It has been reported that these three cats in the forest of Cocha Cashu, Peru, presented a selective hunting behavior (EMMONS, 1987). Despite the fact that food might be a limiting factor for herbivore populations in some years (GLANZ, 1982). Emmons (1987) suggested that predation by these three cats could limit prey population growth in other years. Moreover, Terborgh (1992) suggested that the non-selective behavior presented by these cats in Peru, might control what he called "apparent competition" among prey species. Species with high fecundity rate, such as peccaries (*Tayassu peccary*) and capybaras (*Hydrochaeris hydrochaeris*) could force species with low fecundity rates, such as agoutis (*Dasyprocta sp*.), pacas (A*gouti paca*) and coatis (*Nasua narica*) to decline, due to competition in the absence of top predators. With the presence of jaguars, pumas and ocelots, the interaction among those prey species is mediated by predation, leading to a balance on prey availability.

In a comparative analysis, Terborgh (1988) suggested that mammal populations are higher in Barro Colorado than in Peru, due to the lack of predators, such as jaguars, pumas and ocelots. According to Terborgh and Winter (1980) their absence of predators at Barro Colorado led to a dramatic increase in the population of terrestrial vertebrates that prey on seeds. Therefore, the higher numbers of seed predators may have a considerable impact on determining the composition of Neotropical forests.

The idea of predators controlling the apparent competition among prey species, acting as keystone species in Neotropical forests by limiting mid-sized terrestrial mammals, and predators having indirect effects on determining the composition of Neotropical forests (TERBORGH, 1988, 1990, 1992) has been recently challenged. Other studies have shown that for some forest sites, densities of herbivore mammals do not differ systematically at forests with or without large felids (WRIGHT, 1994). Differences in food habits of felids among sites (WRIGHT, 1994; LEIGH, 1999) and the effect of habitat fragmentation and poaching (JORGENSON; REDFORD, 1993) might play a more important role in controlling fluctuations of mid-sized mammals than does the effect of top predators.

It is evident that the effect of jaguars and other top predators on the control of mid-sized terrestrial mammals, and therefore, on forest regeneration, needs to be further investigated. However, even the study of coexisting species of Neotropical felids, and the effect of the interaction among species in a given area, is poorly understood. In sum, factors influencing distribution, local abundance, and relationship between overlap in resource utilization and competition among Neotropical felids remain unclear.

Conclusion

In order to fulfill our goals of understanding depredation of livestock by large Neotropical carnivores and its consequences for the conservation of keystone species, one of the most important tasks for wildlife managers is the preservation of keystone species and their original habitats. Although several studies have recently investigated patterns of livestock depredation by large Neotropical carnivores (HOOGESTEIJN et al., 1993; MAZZOLLI et al., 2002; POLISAR et al., 2002; CONFORTI; AZEVEDO, 2003), information on a possible link between depredation of livestock and the conservation of keystone species is still lacking.

The idea of top predators functioning as keystone species and therefore acting as important species for conservation biology has been widely proposed. The alteration of the whole balance of Neotropical ecosystems has been suggested after the extermination of top predators. Terborgh and Winter (1980) suggested that the extinction of top predators could alter the balance of evolved predator-prey relationships, therefore causing secondary extinctions. It has been demonstrated that some bird species can be extinct through predation by generalist mammals in communities where top predators have been wiped out (TERBORGH; WINTER, 1980; KARR, 1982). The absence of large carnivores can also set off a cascade of events, affecting populations of bird species as part of changes at multiple trophic levels, as empirically demonstrated by Berger et al. (2001).

As discussed by Estes (1996), the keystone species concept has the potential to contribute to conservation issues due to the linkage between keystone species and ecosystems. By understanding and gathering empirical data on both components, we substantially increase our capacity to better understand patterns of large carnivores predation on livestock and the consequences for the conservation of important carnivore species. Neotropical environments have been rapidly exterminated at ever-increasing rates. If we loose our capacity to investigate and understand how large carnivores might function within their ecosystems we may also loose our capacity to understand patterns of depredation on livestock, therefore precluding our ability in defining practical guidelines for minimizing predation on livestock.

Predators comprise most of keystone species (POWER et al., 1996), and therefore are vital to the integrity of several ecosystems (ESTES, 1996). If we understand how predators might function on their natural habitats, we may better understand their ecosystem dynamics and predict some problems that might lead to depredation on livestock. We should therefore turn our attention and efforts to preserve large Neotropical carnivores together with their original habitat. In order to reach this difficult goal, we should seek for multidisciplinary methods encompassing issues beyond the study of the impact of large carnivores predation on livestock, including the predator-prey relationship, local human perception towards large wild carnivores, management of keystone species and finally, management of domestic stock in close contact with wild carnivores. Those are difficult tasks that we are called upon if we wish to promote large carnivore conservation.

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Capítulo 11
Local perceptions toward large carnivores in livestock raising areas

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Introduction

Destruction of natural areas contributes to human-predator conflicts by increasing the proximity between human-occupied areas and carnivore habitats. In addition, deforestation reduces natural prey availability. Consequently, livestock losses from wild carnivore depredation tend to increase. Conflict between ranchers and jaguars (*Panthera onca*) due to cattle losses is thought to increase as a consequence of deforestation, which forces jaguars into marginal forest areas (HOOGESTEIJN et al., 1993). Moreover, when natural prey availability is significantly reduced, jaguars attack livestock as an alternative source of prey (RABINOWITZ, 1995).

Carnivore conservation efforts have tested the feasibility of preventive methods against livestock depredation, such as the use of electric fences and night-time corrals. However, there are many limiting factors to the success of such methods. First, ranchers often resist changing their husbandry practices (OLI et al., 1994). Second, in many regions where extensive livestock husbandry is practiced, those methods are simply unfeasible. In the Pantanal of Brazil, large herds of cattle are raised in pastures that average thousands of hectares.

Reimbursement for livestock depredation is another alternative that has been implemented in some regions as a means of minimizing human-carnivore conflict (THOMPSON, 1993). In the short term, compensation schemes may provide acceptable financial help for producers and reduce the killing of predators in retaliation for livestock losses. But in the long term, it may contribute to perpetuate the problem by discouraging ranchers to improve their husbandry practices in order to prevent carnivore attacks (CONFORTI; AZEVEDO, 2003). A compensation scheme must be accompanied by improvement in livestock management. Otherwise, not only would it fail to address the causes of depredation but also it could represent a heavy economic burden for the agency responsible for it (JACKSON et al., 1994). Moreover, a fair monetary compensation program should reimburse the producer for the loss of the animal as well as the time he looses from finding the carcass to receiving the payment (THOMPSON, 1993).

Despite all efforts to promote a peaceful coexistence between livestock producers and carnivores, it seems that a definitive solution is far from being found. As part of this multidisciplinary effort, social science has contributed to a more comprehensive view of the problem by including people's perceptions of carnivores into the big mosaic of human-carnivore interactions. But why is it important to assess perceptions toward carnivores? The way people perceive a species may dictate their attitudes toward it. If people hold strong negative perceptions about an animal, they will likely oppose conservation efforts to protect it or to promote reintroduction programs.

Understanding the relationship between local people and protected areas (including wildlife) is important to the success of conservation efforts. In addition, local people should be involved in planning and managing protected areas (NEWMARK et al., 1994). But incorporating human dimensions into natural resource management is a relatively new concept. Today, it is accepted that success in any major environmental project depends upon an effective work on social assessment and public participation (THOMPSON, 1993). In Europe, for example, success of any wolf (*Canis lupus*) restoration program depends on public acceptance and support (ZIMEN, 1981).

Perceptions of people who live in areas where certain species occur must be known considering that those are the people directly affected by their existence and whose attitudes are more likely to determine the fate of individual animals. Ironically, many surveys on public perceptions toward predators do not include local people's opinions because they represent small segments of society (ERICSSON; HEBERLEIN, 2003). However, in any country, rural communities control large surface areas so that their impact on wildlife must be considered (CONOVER, 1994).

Perceptions toward a species are shaped by a combination of factors, such as knowledge, experience (including damage caused by the species – e.g., livestock depredation and its economic impact), as well as educational level, occupation, and age. As one should expect, people living in livestock raising areas where depredation is a reality tend to hold different perceptions toward large carnivores compared to the general public (ERICSSON; HEBERLEIN, 2003).

Knowledge about the species

It is generally accepted that knowledge plays an important role in leading perceptions toward a more positive direction. A study on perceptions of wolves among residents in the northeast of the U.S. showed a positive relationship between knowledge and support for wolf conservation (KELLERT, 1985). However, knowledge and perceptions are not always positively correlated given that the latter may be influenced by other factors. A survey with Minnesota residents revealed that knowledge and support for wolves did not have any relationship (KELLERT, 1999). In a recent study in Sweden, ERICSSON; HEBERLEIN (2003) found that hunters had the most accurate knowledge on wolves compared to non-hunting groups, yet hunters were the less favorable group toward the species. In this case, their occupation influenced perceptions toward wolves but it is important to mention that within each group of respondents, knowledge and support for wolves had a positive relationship.

One should expect that knowledge would lead people to understand the importance of a given species in the balance of an ecosystem so that people would more likely support conservation efforts. Low knowledge, in turn, could lead to false concepts that could contribute to negative attitudes. For instance, a species that usually does not represent a threat to humans may be perceived as dangerous and that may influence negatively attitudes toward it. Not only have wolves been perceived as a threat to livestock but also to personal safety (DUNLAP, 1988). As a consequence, this species was hunted to extirpation in large areas of Europe and North America (BJERKE et al., 1998).

Specific knowledge about an individual population can dictate attitudes toward it. In Norway, Bjerke et al. (1998) observed a relationship between the perceived size of a wolf population (number of wolves people thought there were in the area) and the preferred size (number of wolves people thought there should be). The proportion of people wanting the eradication or reduction of the wolf population increased as the size of the perceived population increased.

Education should be considered a valuable tool in changing attitudes; however, education programs alone may not be enough to transform attitudes. It is questionable if providing more information about a species and its importance would lead people's perceptions into a more positive direction when attitudes are strongly negative.

Experience

Seeing or hearing an animal are kinds of experience that one could consider either positive or negative, depending on the circumstances. In other words, trekkers may find it exciting to hear the howl of a wolf in the wilderness, while the same howl may cause ranchers to worry about their livestock.

It is thought that direct experience leads to stronger attitudes (PETTY et al., 1992). However, seeing or hearing a wolf did not influence attitudes of people living in wolf areas in Sweden (ERICSSON; HEBERLEIN, 2003).

Attitudes can be measured by asking people what they think that should be done to an animal in face of certain situations or experiences. Zinn et al. (1998) assessed the acceptability of 4 management actions toward mountain lions (*Puma concolor*): 1) monitor the animal, 2) capture and relocate the animal, 3) frighten the animal away with fireworks or rubber bullets, or 4) destroy the animal. Respondents were asked which management action would be more appropriate after each of the following mountain lion-human interactions: A mountain lion enters your residential area and: 1) is seen by someone, 2) kills a pet, 3) injures a human, or 4) kills a human. Results show that monitoring the mountain lion was acceptable only in the case of seeing the animal in the area. Relocating the animal was acceptable in all cases but human death. Frightening the animal was not acceptable in any situation. Destroying the mountain lion was acceptable in the two most extreme situations: injury or death of a human. The study showed that acceptability of management actions depends on the severity of the experience with the animal. This assessment is more objective and accurate than simply asking people about their attitudes toward a species. Thus, assessing the acceptability of management actions in face of specific situations may be a valuable tool in predicting public response to wildlife management (ZINN et al., 1998).

Livestock depredation and its economic impact

As one should expect, great economic impact from livestock depredation leads to strong negative attitudes toward carnivores. Oli et al. (1994) conducted a survey in the Annapurna Conservation Area, Nepal, and found that most people living in snow leopard (*Panthera uncia*) habitat wished for total eradication of this predator. In that study, the authors monitored local depredation incidence themselves and could assess the real dimension of the problem, concluding that livestock losses from snow leopard depredation had a high economic impact. Kumar and Rahmani (1995) reported that all surveyed farmers and shepherds living in the surrounding areas of a sanctuary in India were against conserving wolves. In that case, despite the low depredation rates from wolves, the economic impact was high because of the local people's low income.

Obviously, livestock depredation is a negative experience. But in some cases, depredation experience with a carnivore species does not seem to be significant to influence perceptions. In Iguaçu National Park area, South Brazil, ranchers with and without experience with livestock depredation from jaguars were surveyed. However, results show no relationship between perceptions toward jaguars and depredation experience suggesting that the economic impact of depredation was not significant to influence local ranchers' perceptions about the species (CONFORTI; AZEVEDO, 2003).

Educational level

Another important factor to consider when assessing perceptions is educational level. A study on attitudes toward wolves revealed that educational level and attitudes were positively correlated (ERICSSON; HEBERLEIN, 2003). Bjerke et al. (1998) observed that people with elementary school education only had a more utilitarian and negativistic view of wolves compared to people of higher education.

Not surprisingly, the way people absorb new information is also influenced by their educational level. In Iguaçu National Park, Brazil, TV reports about research on wildlife showed radio-collared jaguars being handled and released by researchers in the Park. The programs mentioned that the animals were native to the Park and had been captured with live traps. But a survey revealed that some of the local people misunderstood the programs believing that the collared-jaguars had been brought from other places by the researchers to be released into the Park (CONFORTI; AZEVEDO, 2003). As a result of this misunderstanding, the majority of people living in the surrounding rural communities started believing that local authorities had released jaguars into the Park. Additionally, many local people saw another TV program about the financial difficulty faced by a local zoo to maintain jaguars in captivity. There was no relationship between the zoo and the research project on free-ranging jaguars. However, the survey curiously revealed that many people combined these unrelated subjects and started believing that the collared-jaguars were former captive animals that were released into the National Park because the local zoo could no longer afford to maintain them in captivity. It was suggested that the low educational level of the respondents might have influenced their ability to understand new information given that a considerable proportion of them had not completed elementary school, despite the fact that all respondents were adults. Thus, for education purposes, it is important to assess educational levels so that a suitable approach can be chosen for each target group.

Age, occupation, and area of residence

Older people usually hold more negative perceptions of carnivores than younger people (BJERKE et al., 1998), which is rather a result of growing up in times of general less favorable attitudes toward carnivores than a consequence of aging (ERICSSON; HEBERLEIN, 2003).

Occupation can also influence the way people perceive carnivores. In a wolf study in Norway, people with different occupations were interviewed. Results show a relationship between occupation and attitudes, where students were the most favorable group, while pensioners held the most negativistic view about wolves (BJERKE et al., 1998). Another study revealed that hunters were less favorable toward wolves than non-hunters (ERICSSON; HEBERLEIN, 2003).

Different studies have demonstrated that the place where people grew up or lived influenced their perceptions toward carnivores. Bjerke et al. (1998) observed that people who grew up in livestock-producing farms held a more negativistic view of wolves compared to other people. Ericsson and Heberlein (2003) found that people who grew up in urban areas held more positive attitudes toward wolves; hunters and non-hunters living in wolf areas were less favorable to wolves than the general public, even after controlling for knowledge and interactions with the species (e.g., depredation). As suggested by those authors and other studies (DUDA et al., 1998; ENCK; BROWN, 2000), rural populations may perceive conservation efforts, such as wolf recovery programs, as a symbol of dominance that the urban society exerts over rural communities.

Changing attitudes for a possible human-carnivore coexistence

Attitudes may be changed into a more positive direction through education, when people start accepting carnivores as an important part of any ecosystem where they occur. But the economic impact of livestock damage may represent an obstacle to this acceptance. In such cases, when education is not enough to transform attitudes, alternative ways to provide financial support for ranchers should be considered. Government, non-governmental organizations, and private companies that earn profit from ecotourism in protected areas should combine efforts to provide financial support for local livestock producers. Then, preventive methods to protect livestock against carnivore attacks should be implemented. In cases where prevention efforts do not preclude losses, compensation schemes could be used. Additionally, more people from rural communities adjacent to protected areas should be employed in profitable ecotourism activities related to carnivore conservation. Moreover, involvement of local people in planning and managing wildlife could make carnivore conservation efforts more acceptable. Ideally, people should protect carnivores for their ecological importance and right to exist. But in real life, where depredation can become an unbearable economic burden for ranchers, financial support for livestock producers may become imperative if carnivores are to be preserved.

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Capítulo 12
Depredation management techniques for coyotes and wolves in North America: lessons learned and possible application to Brazilian carnivores

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Introduction

Many carnivore populations throughout the world are declining due to expansion of human populations, habitat loss and fragmentation, illegal poaching, competition with other predators, legal hunting, introduction of exotic species, disease, declines in native prey, and increased competition with livestock and other human land uses (SCHALLER, 1996). In Brazil, detailed knowledge of carnivore-human interactions and conflicts involving livestock is an emerging, but necessary, element for the conservation of many species. A major obstacle facing conservation efforts, reintroduction programs, and recovery plans for many carnivore species throughout the world is the continual issue of depredations by carnivores on agricultural interests (MECH, 1996). In the United States, efforts to reintroduce and/or recover wolves (*Canis lupus*) and grizzly bears (*Ursus arctos*) in the northern Rocky Mountains has been met with much opposition by the livestock industry with depredations on livestock cited as the main reason for resistence. Gaining local support for carnivore conservation and swiftly dealing with depredation problems will always be an issue for biologists and managers as human populations continue to expand into and reduce carnivore habitat, and conflicts between humans and carnivores increase (MECH, 1996).

Predation on domestic livestock and poultry by carnivores is a historical and continuing problem faced by agricultural producers throughout the world (HARRIS; SAUNDERS, 1993). In the United States alone, producers lost 273,000 sheep and lambs valued at $16.5 million to predators in 1999 (U.S. DEPARTMENT OF AGRICULTURE, 2000). These losses to predators represented 36.7% of total losses to all causes. In 1999, depredations on sheep and lambs were principally caused by coyotes, *Canis latrans* (61%), dogs (15%), mountain lions, *Puma concolor* (6%), and bobcats, *Lynx rufus* (5%). Losses of sheep and lambs due to specific predators varie geographically (Table 1). Cattle and calf losses to predators in the U.S. totaled 147,000 head during 2000 with an estimated loss of $51.6 million (U.S. Department of Agriculture, 2001). Coyotes caused 64.6% of predator losses on cattle and calves, followed by dogs (18%), and mountain lions and bobcats (7% combined). The loss of goats to all predators was estimated to be about $3-4 million annually. While losses of poultry to predators are not well documented, they are considered to be substantial.

The coyote is a generalist carnivore that adapts to landscape modifications and is actually doing better today (in terms of population size and distribution) than when North America was first settled by Europeans. Wolves are increasing in the northern Rockies and Great Lakes region due to federal protection and reintroduction programs. Wolves are doing so well in parts of the U.S. they were downlisted from Endangered to Threatened status in 2003 (FEDERAL REGISTER, v. 68, n.. 62, April 1, 2003). As stated previously, coyotes are a leading cause of depredations on domestic livestock in North America. As such, the coyote has received considerable attention and persecution (current estimate: >100,000 coyotes removed annually in the U.S.) in an attempt to reduce depredation losses (Wagner, 1988). Due to public pressure and increasingly fragmented ranch/farm operations, large-scale population reduction programs are becoming less pronounced (WAGNER, 1988, KNOWLTON et al., 1999). In contrast, techniques that are more benign and focus on solving the actual depredation problem are receiving more attention. Non-lethal techniques are becoming more popular and are readily accepted by the general public (ARTHUR, 1981; REITER et al., 1999). However, after >40 years of research on methods to reduce predation (FALL; MASON, 2002), it is quite clear that protecting livestock from carnivores is a complex endeavor with each depredation event and management situation requiring an assessment of the legal, social, economic, biological, ethical, and technical aspects (KNOWLTON et al., 1999). No one technique will solve the problem in all circumstances. Successful resolution of conflicts with predators involves an analysis of the efficacy, selectivity, humaneness, and efficiency of all the various management scenarios available (CLUFF; MURRAY, 1995; KNOWLTON et al., 1999).

Control techniques may be considered either corrective (after a depredation event) or preventive (before the event). Techniques can also be classed as lethal or non-lethal. Some techniques can be further classed as either selective or non-selective. Selectivity of the technique is extremely important when attempting to actually solve the depredation problem. General population reduction through lethal means may not solve the depredation problem (CONNOR et al., 1998). Techniques that selectively remove the offending individual (SACKS et al., 1999a, b; BLEJWAS et al., 2002) are preferred over non-selective techniques that the killers may avoid. However, identifying the “problem” animal can be very difficult (LINNELL et al., 1999). Methods that are more selective for the target species are also preferred (KNOWLTON et al., 1999).

The purpose of this paper is to present the various techniques that were developed to reduce or prevent depredations on livestock by coyotes and wolves in North America. These techniques are the result of decades of research, evaluation, and funding (FALL; MASON, 2002). While the techniques were developed for coyotes and wolves, depredation problems for many carnivore species in Brazil may also be controlled in similar situations. Most of these techniques have direct application to carnivores in Brazil of similar body size (Table 2) and behavioral characteristics, and would be useful for depredation problems involving many of the different species of felids and canids in Brazil.

Determination of predation

One of the first priorities when dealing with carnivore-livestock conflicts is determining or verifying the species responsible for the predation event (FRITTS, 1982). Examination of the carcass and surrounding kill site requires careful observation (WADE; BOWNS, 1984: ACORN; DORRANCE, 1998). Determining the cause of death is best done when the carcass is fresh (WADE; BOWNS, 1984; DOLBEER et al., 1994). Skinning out the carcass, particularly around the head, throat, neck, and flanks, will generally reveal hemorrhaging in the tissue if the victim was killed by a predator. Animals that die and then are fed on by a carnivore (but not killed by a predator), will not show hemorrhaging. For animals that are considered to have been depredated, the location of the attack site, presence of blood, trampled vegetation, size and spacing on canine punctures, claw marks causing hemorrhaging under the skin, presence of scats and tracks, and even the behavior of the herd (alert or nrevous livestock, injured stock, females calling or searching for young), will assist in determining if predation occurred and who the culprit may have been (O'GARA, 1978; WADE; BOWNS, 1984; DOLBEER et al., 1994; ACORN; DORRANCE, 1998). Many carnivores will scavenge carcasses and should not be confused with predation.

Maintaining records of depredation events in a centralized location will allow agencies to develop databases on the magnitude of the depredation problem. In the U.S., the National Agricultural Statistics Service (USDA) maintains and compiles the livestock losses due to predators. This database then provides an avenue for examining the severity of the problem, geographical distribution, the predatory species responsible, the vulnerability of particular type and ages of livestock, the monetary value of the losses, and where management actions may be warranted in the future. This database also compiles the efforts by livestock producers in terms of what techniques they employ to prevent or reduce depredations (particularly non-lethal methods), the costs of employing those methods, and the frequency of such efforts. This database is summarized annually and published (U.S. DEPARTMENT OF AGRICULTURE, 2001).

Predator management in the U.S.

The various techniques for managing predation discussed below are the result of decades of changing attitudes and ideas. The history of predator management in the U.S. is certainly one of shifting paradigms (WAGNER, 1988). Over 300 years ago and up to about the 1930's, the main belief in predator management was "kill them all." Protection of livestock and big game herds was the principle motivation for the mass killing of predators throughout North America. Much of this attitude was a carryover of European beliefs in wildlife management (WAGNER, 1988) and ideals on providing protection for stocks (both domestic and native). In the late 1930's, some biologists and ecologists began to question these beliefs and proposed that predators had a place in the environment and a role to play in maintaining healthy and robust game populations (WAGNER, 1988; CLARKSON, 1995). At this time the general public was apathetic toward predators and protection of livestock was still viewed largely as justification to kill predators.

With the coming of the environmental movement in the 1960's and passage of the Endangered Species Act in the early 1970's, there began to be an understanding and demand by the general public to begin to question and rethink the past perceptions of predators (LEOPOLD, 1964; HORNOCKER, 1972; WAGNER, 1988, CLARKSON, 1995). As the general populace shifted from rural to urban, public opinion of predators became more favorable, and reintroduction, management, and preservation of large carnivores became a controversial issue in many states (WAGNER, 1988; CLARKSON, 1995; MECH, 1996). With these changing attitudes towards conservation of predators, demand for more humane techniques and non-lethal methods grew (ARTHUR, 1981; REITER et al., 1999). Where once agencies removed predators with mass population reduction programs, these same agencies were now being told to be selective and work on a smaller scale. One of the primary aspects of lethal control is that it generally must be reapplied each year. Selective (site or individual specific), yet lethal, removal is now preferred over wide-spread removal programs (FRITTS et al., 1992; JAEGER et al., 2001). In contrast, it is hoped that some non-lethal techniques will prevent the depredation problem from beginning, possibly last several years, and not need application yearly (e.g., reproductive interference).

Solving the actual depredation problem without removing the predator lead to the development of more non-lethal techniques. While lethal techniques are still employed and will be discussed, focus and demand is shifting to non-lethal techniques (REITER et al., 1999; FALL; MASON, 2002). It should be emphasized that for coyotes, depredation control is a management issue, not a conservation issue (KNOWLTON et al., 1999). In contrast, depredation management for wolves is principally a conservation issue to reduce conflicts with livestock and the rural community (MECH, 1996).

Lethal techniques

The concept of lethal control of predators was indeed the first and sometimes only, consideration when Europeans settled North America. The land was big, untamed, and predator populations seemed endless. As such, most lethal techniques have been around a long time (e.g., steel traps). Today, many lethal techniques require special training, certification, or licensing in order to use. Several methods are best left to professional specialists trained in wildlife damage management. Some techniques are available for use by livestock producers, but local regulations need to be checked before implementing any of these lethal techniques. Lethal techniques are viewed less favorably by the general public to control predators than non-lethal methods (ARTHUR, 1981).

Livestock protection collar – Livestock protection collars (LPC's) consist of rubber pouches or bladders filled with Compound 1080 attached around the throat of lambs and kid goats (ACORN; DORRANCE, 1998). The LPC is designed to kill predators when they puncture the bladders during an attack on a lamb or kid. The main advantage of LPC's is that they kill the problem animal and frequently kill individual predators that have evaded other control techniques (CONNOLLY; BURNS, 1990; BURNS et al., 1996; BLEJWAS et al., 2002). The LPC comes in two sizes, large and small, with the larger LPC working effectively on larger lambs. The major disadvantages of LPC's are the initial purchase costs and labor required to place the collars of the lambs or kids, the collar being punctured by thorns, wire, or snags, anticipating which lambs or kids are most likely to be attacked (use of a sacrificial herd has been tried with limited success), and the required training and accountability of the collars (ACORN; DORRANCE, 1998; KNOWLTON et al., 1999). Because of the use of compound 1.080 in these collars, generally their application is limited and may require assistance or training from agency personnel.

M-44 – M-44 is a mechanical device that ejects sodium cyanide into an animal's mouth after they pull on the device (CONNOLLY, 1988; ACORN; DORRANCE, 1998). Because of the use of cyanide as the poisoning agent, application of this technique in the U.S. generally requires certified agency personnel. M-44 consists of a holder wrapped with cloth, fur, wool, or steel wool; a plastic capsule or case that holds the cyanide; and a spring-loaded unit that ejects the cyanide. When assembled, the components are encased in a tube driven into the ground and baited with fetid meat, a lure, or tallow (DOLBEER et al., 1994). When an animal tries to pick up the bait with its teeth, the cyanide is ejected into its mouth. Non-target species are sometimes attracted to the bait used on M-44s; however, species specificity can be enhanced by proper site and lure selection (DOLBEER et al., 1994). A study on coyotes in California found that M-44 was not a selective technique in targeting or removing the breeding animals involved in sheep depredations (SACKS et al., 1999b; JAEGER et al., 2001).

Aerial hunting – Aerial hunting is a commonly used method for reducing predator numbers (WAGNER, 1988; CLUFF; MURRAY, 1995). Different types of fixed- and rotary-wing aircraft have been used to control wolves, coyotes, bobcats, and foxes in North America. A 12-gauge semiautomatic shotgun is most commonly used with number 4 buck-shot, BB, or number 2 shot. Aerial hunting can be more efficient if a ground crew works with the aircraft. The ground crew induces coyotes to howl by using a horn, siren, voice, or recorded howl. When animals respond, the aircraft is directed to the area by two-way radios. Early morning and late afternoon appear to be the most productive times for aerial hunting. In the U.S., federal law requires each state to issue aerial hunting permits; some states also require low-level flying waivers. This technique is usually performed by trained agency personnel and pilots.

Denning – Denning was an effective method to reduce wolf numbers in Canada (CLUFF; MURRAY, 1995), but is no longer practiced due to the public viewpoint that it kills the innocent. In the intermountain west of the U.S., the removal of pups from the den to reduce depredations by coyotes is still practiced (WAGNER, 1988). Increased depredations of livestock (mainly lambs) during the spring and summer by coyotes may indicate that a pair of adults is provisioning a litter of pups nearby (TILL; KNOWLTON 1983). Removal of only the pups and leaving the adults in place was equally effective in reducing depredations as removing both the pups and adults (TILL; KNOWLTON, 1983). Den hunting is difficult and time-consuming, particularly on hard ground and in heavy cover (DOLBEER et al., 1994). Some people use a dog to locate the den. Caution should be taken while digging out dens because of cave-ins. Use of a chemical smoke cartridge is often employed to remove the pups. An alternative to denning is the use of sterilization (see *Reproductive Interference*) which worked effectively without the requirement of finding the den every year and the effects lasted several years (BROMLEY; GESE, 2001a,b).

Box traps – Trapping the problem animal is a technique that producers can often do themselves. Regulations should be consulted as there may be restrictions of the type of trap that can be used. Box traps are available from several companies in various sizes, materials, and configurations to capture various sizes of predators. Generally, most large predators are difficult to capture in box traps because of their caution and reluctance to enter the confined area of a trap, but can work effectively with smaller carnivore species (DOLBEER et al., 1994).

Leg-hold traps – Steel leg-hold traps have been used for centuries to remove problem carnivores (WAGNER, 1988, CLUFF; MURRAY, 1995). Setting of leg-hold traps does require a bit more experience than setting box traps, but is still a technique that producers can do themselves. Local trappers will often offer instruction in the proper use and setting of traps. In the U.S., regulations vary among states on the types of traps, baits, sets, and trap visitation schedule allowed. In the U.S., some states no longer allow the use of leg-hold traps. Leg-hold traps are manufactured in various sizes for capture of different carnivore species (DOLBEER et al., 1994). Modification of traps (e.g., padded jaws) and attachment of a trap tranquilizer device can diminish injuries to the animal (SAHR; KNOWLTON, 2000). Tension devices should be used to exclude non-target species (PHILLIPS; GRUVER, 1996). Selectively removing the offending animal causing the depredations with a trap can be difficult (SACKS et al., 1999b; JAEGER et al., 2001). Success in trapping really depends on the placement of the trap (along travel routes such as dirt roads and trails). The trap can be set unbaited on a trail, or set off the trail and baited with a lure, bait or natural substance (scat or urine). The type of lure and trap location are very important in selectively targeting the intended species (DOLBEER et al., 1994). When placed beside a carcass, a trap can catch non-target animals (e.g., vultures, eagles, badgers). In the U.S., many states no longer allow trapping in the vicinity of a carcass.

Calling and shooting – Calling and shooting can be used as a means to control certain predators (COOLAHAN, 1990). Calling and shooting, with or without the help of lure dogs, can be a selective means of removing the offending animals that kill livestock, particularly during the denning and pup-rearing seasons (SACKS et al., 1999b). Commercial calls and recorded calls are available from various manufacturers. Predator or duck calls that imitate the sound of a rabbit in distress work well, but require some practice. Some individual predators can become wise to the call. Conversely, the call may be an effective method to remove a trap-wise animal. Some recommendations when trying to call in a predator: (1) Ensure that the area being called is upwind to prevent the predator from detecting the caller's scent. (2) Have a full view of the area so that the predator will be unable to approach unseen. (3) Avoid being seen by wearing camouflage clothing and hiding in vegetation. (4) Most effective times to call predators are early morning and late afternoon (DOLBEER et al., 1994).

Hunting dogs – The expense of hunting dogs often precludes the use of this technique for most producers, but a local houndsman may be employed to remedy a predation problem. Two types of dogs can be used (DOLBEER et al., 1994). Dogs that hunt by sight, such as greyhounds, which are kept in a box or cage until the predator is seen, then released to catch and kill the animal (effective only in open terrain). The other type of dog is the trail hound, which follows an animal by its scent. Trail hounds hunt on bare ground; however, heavy dew can make trailing easier. Hot, dry weather makes trailing difficult; therefore, early morning is the most effective time. Several breeds such as bluetick, black and tan, Walker, and redbone, in packs of 2-5 dogs are used as trail hounds. Trained trail hounds are used to catch and "tree" predators (e.g., raccoons, opossums, bears, and cougars). Often these dogs are able to track the offending animal from a kill, thus making this control method highly selective.

Snares – Similar to trapping, snaring is a technique that can be implemented by producers themselves, but also requires some level of expertise to be successful and not educate the problem animal by being inexperienced setting a proper snare (DOLBEER et al., 1994). Snares are made of varying lengths and sizes of wire or cable looped through a locking device that allows the snare to tighten. There are generally two types of snares: body and foot. The body snare is used primarily on coyotes and foxes. This snare is set where the animals crawl under a fence, at a den entrance, or in some other narrow passageway. The foot snare has been used to capture large predators and is spring-activated (LOGAN et al., 1999). When the animal steps on the trigger the spring is released, lifting the noose and tightening it around the foot. Deer and livestock can be prevented from interfering with the snare with a pole or branch placed across the trail (0.9 m above the ground). The selectivity of the foot snare may be improved by placing sticks under the trigger that break only under the weight of the heavier animals. Open-cell foam pads can be placed under the trigger pan to prevent unintentional triggering of the snare by small mammals (LOGAN et al., 1999). Foot snares have advantages over large traps because they are lighter, easier to carry, and less dangerous to humans and non-target animals (DOLBEER et al., 1994).

Sport hunting – Sport hunting or public harvest of large carnivores as a management technique is practiced in the U.S. and Canada (BOERTJE et al., 1995). In several African countries, sport hunting of large cats provides a financial incentive for ranchers to keep predators that may otherwise cause unacceptable livestock problems in their area. Rather than losing money to these predators, the rancher can profit from their presence. Setting up lodging and guide services can provide ranchers with increased revenue and make these large predators an asset rather than a liability. Permits and harvest quotas would need to be closely regulated to maintain a harvestable predator population. These hunts can also provide funds for conservation of these predators in areas where recovery is still an issue. Returning some of the profits of these hunts back to the local community can also increase tolerance of these large predators by local farmers and producers.

Non-lethal techniques

Most non-lethal procedures fall within the operational purview of the agricultural producer. Most livestock producers (83%) utilize at least one non-lethal method to prevent or reduce predation (Table 2). During 1999, producers spent $8.8 million on non-lethal methods to protect sheep and lambs, and $184.9 million to protect cattle and calves (U.S. Department of Agriculture, 2000). While there are reports of success with some non-lethal methods, failures are common, few have been subjected to critical evaluation or testing, and none have proven universally successful (KNOWLTON et al., 1999).

Livestock husbandry practices – One of the first lines of defense against depredations that a livestock producer can enact themselves is examining, and perhaps modifying, their animal husbandry practices (ROBEL et al., 1981; FRITTS, 1982; WAGNER, 1988; ACORN; DORRANCE, 1998). Several livestock management practices have been suggested as a means of reducing depredation losses. As a general rule, the more time you spend with your livestock, the less likely a predation event will occur. Several recommendations follow: (1) Using herders is a time-tested tradition that can alleviate predation. (2) Dead livestock can attract coyotes and other predators. Thus, removal or burial of carrion will not encourage predators to remain in the area and perhaps kill livestock (FRITTS, 1982). Taking carcasses to a rendering plant can also be useful, although rendering plants generally will not accept sheep carcasses because the wool fouls the rendering equipment. (3) Confining or concentrating flocks during periods of vulnerability (for example, at night or during lambing) can decrease depredation problems. Calves and lambs are very vulnerable after birth, as well as ewes or cows following a difficult birth. Removing the afterbirth or stillborn lambs and calves can also reduce attractiveness of the area following a birth. Lambs that are weak or light-weight are especially vulnerable to predators and confining them for 1-2 weeks will reduce their potential to be killed. (4) Shed lambing, synchronizing birthing, and keeping young animals in areas with little cover and in close proximity to human activity will also reduce the risk of predation. The largest drawback of these procedures is that they generally require additional resources and effort, and may only delay the onset of predation (FRITTS et al., 1992, KNOWLTON et al., 1999). For these methods to be effective, producers must develop strategies that will work for their own situations.

Guard dogs – The use of guard dogs to deter coyotes and wolves from livestock has been a traditional use by many livestock producers, particularly in fenced pastures, and is gaining increased acceptance and use throughout the livestock industry (CLUFF; MURRAY, 1995; COPPINGER; COPPINGER, 1995; ACORN; DORRANCE, 1998). In Colorado, 11 sheep producers estimated that their guard dogs saved them an average of $3,216 of sheep annually and reduced their need for other predator control techniques (ANDELT, 1992). Several key points should be made with regards to guard dogs: (1) The dog breeds most commonly used as livestock guardians include the Great Pyrenees, the Komondor, the Akbash, the Anatolian shepherd, the Shar Planinetz, the Kuvasz, and the Maremma. While there does not appear to be one breed of dog that is most effective, livestock producers rated the Akbash as more effective at deterring predation because it is more aggressive, active, intelligent, and faster (ANDELT, 1999). The Great Pyrenees is the most common guard dog breed used to protect flocks of sheep in Alberta (ACORN; DORRANCE, 1998). (2) Studies investigating the effectiveness of guard dogs have shown the dogs to be effective in some situations and ineffective in others (LINHART et al., 1979; COPPINGER et al., 1983; GREEN et al.., 1984; GREEN; WOODRUFF, 1987; COPPINGER; COPPINGER, 1995; ANDELT;

HOPPER, 2000). This disparity may be due to the inherent difficulty of guard dogs to effectively protect large flocks that are dispersed over rough terrain and in areas where thick cover conceals approaching predators. Thus, the effectiveness of guard dogs can be enhanced by confining flocks to more open pastures which allow a good view of the area. (3) Training and close supervision of the dogs seem important for this technique to be successful. Introducing the dogs to the flock at an early age (a pup at 7-8 weeks of age) seems to increase their effectiveness by bonding the dog to the sheep. (4) Check for reputable breeders when purchasing a pup. Some breeders will certify their dogs to be free from hip dysplasia and some will even guarantee replacement of a dog if it fails to perform properly.

Some poorly trained or supervised guard dogs have killed sheep and lambs, harassed or killed wildlife, and threatened people that intrude into their area. As compared to guard llamas, a main drawback of guard dogs is the need to feed and water the dog in the area containing the sheep and the possible bonding of the dog to humans if the flock is near human habitation. Another disadvantage is that the use of guard dogs precludes the use of other control devices (e.g., traps, snares, M-44's) and techniques (e.g., calling and shooting). Dogs can be killed or injured by poisons, snares, and traps used for predator control. In recent tests using 4 guard dogs together to protect calves from wolves in Montana, the wolves (about 50-60 kg body weight) eventually killed all 4 dogs in the pasture and continued to depredate calves.

Guard llamas – The use of llamas for protecting livestock from predators is growing in popularity. Studies have found llamas to be a practical and effective technique to deter predators from depredating livestock (FRANKLIN; POWELL, 1994; MEADOWS; KNOWLTON, 2000). The llamas behavioral trait of chasing predators out of pastures is likely a result of its evolution with native predators in South America. A major advantage of guard llamas is that they can be kept in fenced pastures with sheep or goats, do not require any special feeding program, are relatively easy to handle, and live longer than guard dogs (KNOWLTON et al., 1999). Several recommendations have been made when using llamas as livestock guardians: (1) Do not use an intact male as they may kill or injure ewes when attempting to breed with them. Female llamas also do not appear to work well and may be aggressive towards the stock they are supposed to be protecting. (2) Using 2 or more llamas in single or adjacent pastures is also discouraged as they will bond with one another and ignore the sheep. (3) Traits that may be useful in selecting a llama for use as a livestock guardian include leadership, alertness, and weight of the llama (CAVALCANTI; KNOWLTON, 1998). (4) Finding a reputable breeder is a good precaution when looking to purchase a guard. (5) Flocks in pastures with heavy cover may reduce their effectiveness similarly to guard dogs. Open pastures with good visibility are the best situations for guard animals to effectively operate. Attempts to use llamas to protect calves from wolves have been met with limited success with wolves reducing visitation in some pastures, while in other cases the wolves killed the guard llama. This technique would probably not be useful for jaguars (*Panthera onca*) due to their innate predatory abilities (i.e., they would probably kill the guard animal).

Guard donkeys – Similar to guard llamas, donkeys have also been used as livestock guardians (GREEN, 1989; ACORN; DORRANCE, 1998). The protective behavior displayed by donkeys apparently stems from their apparent dislike of dogs. A donkey will bray, bare its teeth, chase and try to kick and bite any canid (including ranch dogs). Recommendations on the use of donkeys as livestock guardians include: (1) Use only a jenny or gelded jack (intact jacks are too aggressive towards livestock). (2) Use one donkey per flock or group and keep other donkeys or horses away or the animal will bond with them. (3) The donkey should be introduced to the livestock about 4 to 6 weeks prior to the onset of predation to properly bond with the group. (4) Donkeys are most effective in small, fenced pastures. (5) Check with a reputable breeder when shopping around for a donkey. Similar to guard llamas, donkeys do not require special feeding; can be kept penned with the sheep, and live longer than guard dogs.

Supplemental feeding – Supplemental or diversionary feeding as a non-lethal technique to divert a predatory species away from a vulnerable commodity for a period of time has received some attention (BOERTJE et al., 1995; CLUFF; MURRAY, 1995), but has not been tested to prevent predation on livestock. Many predators will readily consume food provisioned by humans. In the northwest U.S., black bear (*Ursus americanus*) damage to coniferous trees (they feed on the sapwood during the spring) could be reduced with supplemental feeding (COLLINS, 1999; PARTRIDGE et al., 2001; NOLTE et al., 2002). Supplemental feeding should only be used for the duration of protection of the resource that is required, as continued feeding could actually increase the number of predators in an area by increased reproduction and emigration (i.e., a numerical response).

Fencing and barriers – Livestock and poultry may sometimes be protected from predators with a properly constructed and placed barrier, such as a predator exclosure, electrical fencing, screening, or even a moat (de CALESTA; CROPSEY, 1978; GATES et al., 1978; LINHART et al., 1982, NASS; THEADE, 1988, ACORN; DORRANCE, 1998). Some recommendations suggested for predator fencing include: (1) Ordinary fencing will not keep most predators from entering areas as they learn to jump over or dig under the fencing. (2) Many large predators may be deterred or excluded by adding an electrified single-wire strand charged by a commercial fence charger along a wire mesh fence. The electrified wire needs to be placed 20 cm out from the fence and 20 cm above the ground. A fence 1.5 m high with 9 to 12 alternating ground and charged wires spaced 10-15 cm apart is an effective barrier against coyotes (GATES et al., 1978; DOLBEER et al., 1994; ACORN; DORRANCE, 1998). (3) A wire mesh fence can also be used and is more versatile, longer lasting, and can be stretched tighter than a conventional farm mesh wire (DOLBEER et al., 1994). (4) Smaller carnivores may be deterred by use of a 0.9-m wire-netting fence placed 0.6-m above ground and 0.3 m below the surface; a 15-cm length of the fence below the ground is bent outward at a right angle and buried 15 cm deep (DOLBEER et al., 1994). Fencing gives the additional advantage of increased efficiency during herd management, not often realized by producers. The costs of materials, installation, and maintenance usually preclude the use of fences for protecting livestock in large pastures or under range conditions (KNOWLTON et al., 1999).

Frightening devices – Devices such as lights, distress calls, loud noises, scarecrows, plastic streamers, propane exploders, aluminum pie pans, and lanterns have been used to frighten away predators (ACORN; DORRANCE, 1998). Most testing has been with devices that periodically emit bursts of light or sound to try to deter coyotes from sheep in fenced pastures and open-range situations (LINHART. 1984; LINHART et al., 1992), but the benefits are often short-lived (BOMFORD; O'BRIEN, 1990; KOEHLER et al., 1990). While all of these devices can provide some level of temporary relief in reducing damage or deterring predators, habituation by the predator to the device is common. The usefulness of the device can be prolonged by frequently changing the location of the devices, changing the pattern of the stimuli, or combining several techniques (LINHART et al., 1992; KNOWLTON, et al., 1999). Using a combination of warbling-type sirens and strobe lights reduced coyote predation on lambs by 44% (LINHART, 1984). These battery-operated devices were activated in the evening by a photocell set on a schedule of 10-second bursts at 7- to 13-minute intervals. The use of propane exploders delayed or prevented lamb losses to coyotes for a period of time (PFEIFER; GOOS, 1982).

A recent development used to deter wolf predation is the Radio Activated Guard (RAG) box (SHIVIK; MARTIN, 2001; BRECK et al., 2002). This device is activated only when a radio-collared wolf is in the vicinity and its radio-collar activates the device, preventing habituation of the animal to the lights and siren. This has application only in areas with radioed animals, but can deter endangered predators from causing problems to livestock producers (BRECK et al., 2002). The use of frightening devices is not widespread, mainly because the use of sirens and strobe lights at night near people is generally not acceptable (KNOWLTON et al., 1999).

Repellents and learned aversions – Presently, there are no commercially available repellents that effectively deter the act of predation (KNOWLTON et al., 1999). Several noxious compounds have been tested (e.g., thiabendazole, pulegone, cinnamaldehyde, allyl sulfide) with a few of these reducing food consumption among predators. There are some areas where chemicals apparently have repelled animals from certain objects. Quinine hydrochloride and capsaicin appeared to discourage coyotes from chewing on irrigation hoses (WERNER et al., 1997), but these repellents do not deter predation. Thiabendazole has been used to condition black bears to avoid beehives (POLSON, 1983). Probably one technique that received much heated debate and attention in the past couple of decades was the use of conditioned taste aversion using lithium chloride to reduce coyote predation on sheep. The main problem was that results of studies were mixed. Some researchers reported success (GUSTAVSON et al., 1974, 1982; FORTHMAN-QUICK et al., 1985a,b), while others were either unable to replicate those findings or found it to be ineffective in field situations (BURNS, 1980, BOURNE; DORRANCE, 1982; BURNS, 1983; BURNS; CONNOLLY, 1985). While lithium chloride indeed does reduce prey consumption, it apparently does not deter the act of predation. Ten years after extensive field trials using lithium chloride, a survey of the same sheep producers revealed that only one producer still used it (CONOVER; KESSLER, 1994). Current available evidence suggests that conditioned taste aversions are either ineffective or unreliable for deterring predation, but may limit food consumption (KNOWLTON et al., 1999).

Electronic training collar – A new device receiving some attention as a non-lethal method to deter predation on livestock is the use of an electronic training (shock) collar usually used for training dogs (ANDELT et al., 1999, SHIVIK; MARTIN 2001). Using captive coyotes, researchers reported that the training sequence with the electronic collar stopped all attempted attacks on lambs, decreased the probability of an attempted attack, eliminated successive chases, and even caused avoidance of lambs (ANDELT et al., 1999). Application may be limited under field conditions because the predator must be captured and the training collar attached (batteries would need to be occasionally changed), but does suggest avenues of future research on response-contingent aversive stimuli that changes the behavior of the predator during the attack phase of a predatory sequence (SHIVIK; MARTIN, 2001).

Reproductive interference – In the 1960's there was interest in the use of chemical sterilants to influence the reproductive rate of coyotes (BALSER, 1964). This interest was based upon the assumption that reduced reproduction would reduce population levels and that fewer coyotes would result in fewer depredations on livestock. Trials with diethylstilbesterol indicated that reproduction among coyotes could be curtailed (BALSER, 1964; LINHART et al., 1968), but depredation rates were not measured, timing was critical, the approach was impractical without effective delivery systems, and research on this substance eventually ceased (KNOWLTON et al., 1999). Currently there is renewed interest in reproductive inhibition using either chemical or immunocontraceptive agents (DeLIBERTO et al., 1998), mainly as a means of changing the predatory behavior of coyotes. Surgical sterilization (tubal ligation and vasectomy) of coyotes was effective in reducing predation rates on domestic lambs without affecting social behavior and territory maintenance (BROMLEY; GESE, 2001a,b). Among wolves, vasectomies of males have been proposed as a method of population control (HAIGHT; MECH, 1997). However, at the present time there are no substances available for fertility control among predators that is species specific. Species specificity may have to be achieved through appropriately designed delivery systems.

Relocation of problem animals – Translocation of individual predators that cause problems has been successful with grizzly bears (BRANNON, 1987), but has proven less useful for wolves that depredate livestock (BANGS et al., 1995, CLUFF; MURRAY, 1995). All to often wolves return to the capture site, or move to areas with livestock and start depredating cattle again (BANGS et al., 1995). Those that kill livestock again are removed from the population (BANGS et al. 1995). Relocation is expensive and time consuming (CLUFF; MURRAY, 1995), but is often considered necessary when dealing with recovery or reintroduction of a valuable endangered predator.

Financial incentives – Certain financial incentives have been used to mitigate livestock losses and temper resistence to carnivore recovery in the U.S. Compensation for livestock deaths due to wolves is practiced in parts of the U.S. (FRITTS, 1982; FRITTS et al., 1992) and Canada (GUNSON, 1983), either through government funds or private donations (e.g., Defender's of Wildlife's Wolf Compensation Fund). Problems with these programs are that producers feel they do not receive full market value, compensation is only for verified losses (does not include missing animals), and that payment for losses does not encourage producers to correct poor management practices or try non-lethal techniques (FRITTS et al., 1992). A more recent incentive has been the production of "predator friendly" products in which consumers pay more for goods (e.g., wool, meat) that comes from ranches that do not kill predators. Some producers will also allow their public grazing allotments to be bought out by some non-governmental organization as an incentive to move their livestock to another area with less risk of predation. For example, ranchers in western Montana are removing cattle from areas occupied by endangered species (grizzly bears) and placing them elsewhere with financial assistance from an NGO. Another financial incentive gaining a foothold in the private sector is ecotourism. Some ranchers in the U.S. sell trips to the public for viewing of wolves on their ranches and set up lodging and guiding services to recoup financial losses that may incur from livestock depredations. Similar operations are now being established in Brazil, particularly in the Pantanal region for viewing of jaguars and other wildlife species.

In closing, many different techniques exist to reduce or deter depredations by carnivores. Selectivity, efficiency, and compatibility of the technique should be carefully evaluated prior to implementation. Surveys indicate that non-lethal techniques are readily accepted by the general public (ARTHUR, 1981; REITER et al., 1999). Surprisingly, compensation programs to ranchers are less acceptable to the public than other non-lethal techniques (ARTHUR, 1981; REITER et al., 1999). Among lethal techniques, those methods that are considered cruel and inhumane, or are not selective to the target species, are generally unacceptable to the public (STUBY et al., 1979, ARTHUR 1981, REITER et al., 1999). It cannot be stressed enough that no one technique will solve all depredation problems in all situations. Using various techniques in combination will allow one to be able to adjust to the behavior of the target animal and environmental conditions. In areas where carnivore conservation is an issue or endangered/threatened species occur, non-lethal techniques should be considered first, with lethal control only if non-lethal methods fail or are impractical in that current situation. There is the perception that as long as you respond, listen, and are doing "something" to solve their depredation problem, livestock producers will appreciate your attempts to help and can lead to acceptance of carnivores in their area (FRITTS et al., 1992). Doing nothing or not responding to their requests for assistance generally leads to the 3 S's: "shoot, shovel, and shut-up." Being out in the field, responding quickly (usually within 24 hours); (FRITTS et al., 1992), and showing that you care about their problem will lead to increased tolerance of carnivores among livestock producers and local communities.

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Table 1 – Percent of depredated lambs lost to specific predators for six states in the Rocky Mountain region during 1999 (Source: U.S. Department of Agriculture 2000).

Predator	Arizona	Colorado	Idaho	Montana	Utah	Wyoming
Coyote	60.0	71.1	82.4	79.4	64.2	77.3
Bobcat	–	–	–	–	2.7	–
Eagles	–	–	–	7.1	1.6	10.0
Dogs	26.7	12.2	5.4	1.6	6.4	1.8
Foxes	–	2.2	–	4.8	1.1	4.5
Cougar	–	3.3	5.4	1.6	15.5	4.1
Bears	–	7.8	4.1	1.6	8.0	2.3
Other[a]	–	–	–	3.2	0.5	–

[a] Other predators include wolves, ravens, vultures and other animals.

Table 2 – Species, common name, and body size of various Brazilian carnivores for which depredation management techniques developed in North America may have application. Source: Ginsberg e Macdonald (1990), Eisenberg e Redford (1999).

Species	Commmon name	Body size (kg)
Panthera onca	Jaguar	61-120
Puma concolor	Puma	24-50
Chrysocyon brachyurus	Maned wolf	23-25
Felis pardalis	Ocelot	7-9
Atelocynus microtis	Short-eared dog	6.5-7.5
Cerdodyon thous	Crab-eating fox	5-6
Pseudalopex gymnocercus	Pampas fox	4-6

Table 3 – Percent of non-lethal methods used by livestock producers to reduce predator losses of sheep and lambs for six states in the Rocky Mountain region during 1999 (Source: U.S. Department of Agriculture, 2000).

Method	Arizona	Colorado	Idaho	Montana	Utah	Wyoming
Fencing	21.7	31.3	46.4	36.0	53.6	27.0
Guard dogs	23.2	23.0	55.2	27.5	28.5	36.0
Llamas	60.9	9.1	9.9	22.7	7.4	20.0
Donkeys	6.0	3.4	2.5	15.1	2.3	7.9
Shed lambing	23.8	66.6	45.5	65.6	46.5	55.7
Herding	8.7	7.1	11.3	12.9	11.9	13.4
Night penning	20.4	79.4	50.2	44.4	34.4	53.5
Fright devices	6.3	5.6	7.3	3.3	5.8	9.2

Capítulo 13
Aspects of livestock depredation by jaguars in the southern Pantanal, Brazil

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Introduction

Due primarily to land-use changes and consequent habitat degradation, jaguars *(Panthera onca)* have been restricted to a fraction of their former ranges. According to SANDERSON et al. (2002), only 46% of their historic range is currently occupied by jaguars.

In the savannas and gallery forests of central-west Brazil, cattle ranching has been a traditional activity with thousands of cattle being extensively grazed in habitats used by jaguars and their natural prey. Cattle ranching in Neotropical savannas with seasonally flooded natural pastures such as in Pantanal is relatively less destructive to the environment than large scale agricultural fields. The Pantanal has been described as an important site for jaguar conservation in the long term (SANDERSON et al., 2002).

Although jaguars are opportunistic and prey on a variety of native species, they also prey on livestock. Although cattle management is rudimentary and mortality due to other causes is high, the killing of livestock by jaguars is the major source of conflict with humans, and these carnivores are subsequently killed on a regular basis to prevent further losses.

More than 95% of the Pantanal region is comprised of vast tracts private land. Therefore, the protection of species such as jaguars largely depends on the goodwill of ranchers in the region. However, the lack of information about the predation problem and the factors that influence it creates an impasse that brings ranchers to resolve it in their own ways (not always legal) causing unnecessary killing of jaguars (CAVALCANTI, pers. obs.; HOOGESTEIJN et al., 1993). Local residents have developed a cultural tendency to exert a persistent and systematic control of jaguars in the region (CRAWSHAW, 2002). The problem is aggravated when animals without depredation behavior are erroneously killed in place of “problem animals”; the killed animal is not always the one causing damage. In some areas, jaguars are hunted and killed in a preventive way before they cause damage to livestock (RABINOWITZ 1986; HOOGESTEIJN et al., 1993). This practice does not solve the predation problem (since it does not necessarily kill the culprit), but can aggravate it. Some animals are injured or maimed by shotgun pellets or rifle bullets, diminishing their ability to hunt, upon which the animal may then switch to killing livestock (RABINOWITZ, 1986; HOOGESTEIJN et al., 1993).

Despite being illegal, many ranchers continue to kill jaguars on their property in an effort to reduce livestock depredations. In 1999 alone, 16 jaguars were killed in a small area in the southern Pantanal (RONDON, pers. commun.). The killing of jaguars for depredation control is done without any scientific evidence of which cats are actually killing livestock. The fact that a few individuals do kill livestock does not justify the indiscriminate killing of other individuals in the population. Furthermore, lethal control measures undertaken in other countries for different carnivore species have proven ineffective (SHAW et al., 1988, CUNNINGHAM et al., 1995, BJORGE; GUNSON, 1985).

The high mortality of jaguars and the increasing number of complaints about livestock depredations requires a search for alternatives to these conflicts. The use of non-lethal methods of management (i.e., frightening devices, aversive conditioning, electric fences), or a compensation program through a state or federal agency or a cooperative system among cattle owners, to be investigated. However, a search for solutions will not be possible without first understanding the depredation dynamics in affected areas. If we can identify the factors that influence jaguar depredation on cattle, as well as depredation patterns they utilize, we may be able to apply alternative, non-lethal preventive measures in a more efficient way.

Prior research studies on jaguars have focused on their ecology, home range, and activity patterns (SCHALLER et al., 1976, SCHALLER; VASCONCELOS, 1978; SCHALLER; CRAWSHAW, 1980, CRAWSHAW; QUIGLEY, 1984; CRAWSHAW; QUIGLEY, 1991; RABINOWITZ; NOTTINGHAM, 1986; QUIGLEY, 1987; EMMONS, 1989; QUIGLEY; CRAWSHAW, 1992; SCOGNAMILLO et al., 2002). Some information regarding the killing of livestock was provided from these studies. However, depredation was not their main focus; hence data was not obtained consistently. Few studies have concentrated on the problem of livestock depredation (RABINOWITZ, 1986; HOOGESTEIJN et al., 1993,).

The present study examinies interactions between jaguars and livestock in the Pantanal of Mato Grosso do Sul, Brazil. Specifically, we are examining which factors (environmental and biological) may influence cattle depredation events by these large cats. We are collecting information on jaguar movements and habitat use in relation to livestock, and predation rates and patterns. Although these carnivores kill livestock, their perceived damage is usually exaggerated (CAVALCANTI, prelim.; RABINOWITZ, 1986; FARRELL, 1999). We are also assessing the magnitude of livestock predation in relation to other mortality factors.

Depending upon our results, we will examine whether alternative measures of depredation prevention and control may be useful in the context of our situation. Investigations into non-lethal methods to reduce livestock depredations will be necessary for the long-term survival of these species.

Predation

Although jaguars are extremely powerful and able to subdue large prey species such as tapir and peccaries, they are opportunistic and prey on a variety of species, including small prey. In the Pantanal, their natural prey species include capybaras *(Hydrochaeris hydrochaeris)*, caiman *(Caiman crocodilus)*, collared and white-lipped peccaries *(tayassu tajacu* and *tayassu pecari,* respectively), marsh deer *(Blastocerus dichotomus)*, armadillos *(Dasipus* sp.*)*, tapirs *(Tapirus terrestris)*, tortoises *(Geochelone carbonaria)*, howler monkeys *(Allouata caraya)*, giant anteaters *(Myrmecophaga tridactyla)*, lesser anteaters *(Tamandua tetradactyla)*, chicks of storks, herons, and other wading birds, and anacondas *(Eunectes murinus).* Descriptions of their feeding ecology have been published elsewhere (SCHALLER; VASCONCELOS, 1978; EMMONS, 1987, 1989; NUNEZ et al., 2000; CHAWSHAW; QUIGLEY, 2002; DALPONTE, 2002).

In addition, to wild prey, jaguars prey on livestock. Cattle depredations by jaguars have been described as early as 1883 by in thurn, in British Guiana. In 1914, Theodore Roosevelt described a region in Brazil where natural prey was plentiful and jaguars killed only the occasional calf. However, in other areas there were reports of jaguars preying almost exclusively on cattle and horses. However, the perceived damage is usually exaggerated (CAVALCANTI, prelim. data). Quigley and Crawshaw (1992) stated that “misconceptions and surrounding circumstances need clarification if the [depredation] problem is to be dealt with realistically.” Farrell (1999) reported a discrepancy of 27% between kills reported by ranch hands and kills verified in the field. Perovic (2002) reported a ranch in Argentina where only 18% of alledged kills were verified as jaguar depredations. Hoogesteijn et al. (1993) reported that in a ranch in the llanos of Venezuela, losses of calves to cats represented 15% of the total mortality (40 calves a year), and although considerable, this represented only 20% of the number estimated by ranch managers. The same authors stated that thefts by rustlers were often blamed on these cats. Furthermore, evidence that jaguars eat cattle is not evidence that by doing so they are a pest or that they have in fact killed the animal. Jaguars, like other opportunistic predators, do scavenge on occasion (CAVALCANTI, pers. obs.).

According to Hoogesteijn et al. (1993), the main factors that predispose cattle to depredation by jaguars in South America are loss of habitat, poaching of the large cats and their prey, and rudimentary herd management. although cattle ranching has been a traditional activity for many decades in the Pantanal, as well as in other parts of South America such as Venezuela, ranches are extensive and the management of livestock rudimentary. Cattle range freely over large tracts of land, and where fences exist, they surround pastures that average 40 km^2. Herds are exposed to floods, drought, epidemics, parasites, and malnourishment.

The annual flooding pattern in the Pantanal dictates cattle management. Cattle are moved to higher and drier grounds in the wet season and moved back into lower areas as flood waters recede. If the wet season begins early, manpower is limited, thus the moving of herds is initiated too late and many cattle remain in wet areas for the season. Small patches of forested land quickly become devoid of palatable vegetation and malnutrition follows (SCHALLER, 1979). A similar situation occurs in the Venezuelan llanos (MONDOLFI; HOOGESTEIJN, 1986) where wet conditions favor predation by jaguars as cattle become malnourished and vulnerable. Despite of basic profilatic programs on occasional round-up and basic care to newborn calves, very little management is conducted in the Pantanal. Some animals remain in the field after round-up, and miscounts are common (CAVALCANTI, pers. obs.). Schaller (1979) noted that in the Pantanal, only 1 in 5 cows successfully raised her calf.

According to Hoogesteijn et al. (1993) jaguars generally attack young cattle (weaned calves and heifers 1-2 years of age) more often than adults, although they can take mature bulls on occasion. in Venezuela, pumas often killed small or new-born calves or small juveniles (HOOGESTEIJN, 1993; FARRELL, 1999), and the vast majority (69%) of cattle kills were comprised of young calves less than 2 months old (SCOGNAMILLO et al., 2002). in the northeast of Argentina, young animals between 1-3 years comprise the majority of jaguar kills (PEROVIC, 2002). In the Pantanal, Crawshaw and Quigley (2002) observed a distinct segregation of the age classes for cattle killed by jaguars and pumas. adult cows comprised 57% of jaguar kills and calves comprised 92% of puma kills. Cunningham et al. (1995) reported that nearly all the calves found or reported killed by mountain lions in Arizona were < 185 kg, with the exception of 1 adult cow killed at the same time as her calf.

The relative contribution of domestic cattle to the diet of jaguars

Although jaguars kill a variety of native prey species, domestic cattle can comprise a substantial part of their diet. In the northern Pantanal, domestic cattle were the most important prey in terms of available biomass (SCHALLER; CRAWSHAW, 1980). Dalponte (2002) indicated that domestic cattle and capybaras represent the base diet of jaguars in northern pantanal, representing 63% of items encountered in jaguar scats. Crawshaw and Quigley (2002) found that cattle comprised 46% of jaguar kills and 35.3% of puma kills in the southern Pantanal, although their data was likely biased (i.e., kills were reported by ranch hands, who are generally more aware of cattle kills than wild animal kills). Native prey is generally smaller, are killed and consumed in secluded sites making kills more difficult to find, thus they are generally under-represented. Schaller (1983) noted the difficulty in assigning relative importance to potential prey species in a jaguar's diet for the same reasons, plus the difficulty of finding jaguar and puma scats in the field. Crawshaw and Quigley (2002) examined 17 prey items killed by radio-collared jaguars and found that only 29% were cattle, while 41% were white-lipped peccaries. Nunez et al. (2000) documented the diet of jaguars and pumas in Mexico and found that their study animals did not kill domestic stock, jaguars never killed animals smaller than an armadillo, and suggested that if large natural prey were to decline, these cats may begin to kill livestock.

Problem animals

Rabinowitz (1986) and Hoogesteijn et al. (1993) suggested that only a few individuals kill livestock, and the destruction or removal of a problem animal may end the depredation problem. Rabinowitz (1986) tracked 2 jaguars that travelled regularly near cattle pastures without causing problems. In addition, he found jaguar tracks within 90 m of a camp site, but those individuals always circumvented the area.

Some studies have found the majority of livestock killers are animals that had been wounded, and therefore incapable of normal hunting behavior (RABINOWITZ, 1986, Hoogesteijn et al. 1993). In Belize, Rabinowitz (1986) examined skulls of problem animals that had been shot. The majority of the skulls examined (10 of 13) showed old injuries either to the head or body. Examination of skulls and carcasses of nonproblem animals showed no evidence of former injuries. In 2 studies in Venezuela, the majority of the cats (75% and 53%) killed for depredation control had previously sustained severe wounds that precluded them from hunting normally (HOOGESTEIJN et al., 1993). However, seemingly healthy animals also kill domestic stock (CAVALCANTI, pers. Observation; SCHALLER; CRAWSHAW, 1980; HOPKINS, 1990; SÁENZ; CARRILLO, 2002). Some suggest that females with young may teach their young to kill cattle (CRAWSHAW, pers. commun.; RABINOWITZ, 1986; HOOGESTEIJN; MONDOLFI, 1996), but there is no direct evidence that these are "problem animals." Schaller and Crawshaw (1980) once recorded when a female jaguar captured a domestic calf in the company of her presumably independent female offspring, but there was no direct evidence that the two shared the carcass.

Some studies indicate that livestock-depredating cats are more likely to be males (SUMINSKI, 1982; RABINOWITZ, 1986; STANDER, 1990; CHELLAM; JOHNSINGH, 1993; CUNNINGHAM et al., 1995; SÁENZ; CARRILLO, 2002), and more likely to be sub-adults than adults (ANDERSON, 1981; RABINOWITZ, 1986; STANDER, 1990; SABERWAL et al., 1994). Other studies, however, have found that adults of both sexes are more likely to kill cattle than younger animals (BOWNS, 1985).

The impact of livestock depredation by jaguars in the context of other causes of livestock mortality

There is no question that some jaguars kill livestock. However, other causes of mortality can be more important economically than jaguar depredations (HOOGESTEIJN et al., 1993). Snake bites are an important mortality factor in the Pantanal. During 1999 in our study site, snake bites were responsible for 27 mortalities, while predation by cats were responsible for 19 (CAVALCANTI, prelim. data). Other sources of mortality include trampling, breaking legs in cattle guards, predation by vultures and hawks, botfly wounds, infections, and toxic plants. Hoogesteijn et al. (1993) noted that in Venezuela the lack of veterinary health care and low production rates were more damaging to livestock operations than cat depredation; pregnancy rates reached only 40–50% and weaning was about 30–40%. Diseases (foot-and-mouth, brucellosis, and leptospirosis), the lack of systematic stock selection, floods and droughts all restrict cattle production. In the Pantanal, few ranchers adopt what is called a “breeding season”. Different age classes (bulls, adult cows, heifers, young calves) are kept in the same large pastures all year long.

Study area

The study area is located in the southern Pantanal, an inundable plain of 140,000 km^2 in the Central-West part of Brazil, on the border with Paraguay and Bolivia. It is characterized by low areas subject to annual floods, a typical Pantanal pattern. The altitude ranges between 89 m and 120 m above sea level. The climate is seasonal, with a rainy season between October and March and a dry season between April and September (CRAWSHAW; QUIGLEY, 1984). The concentration of rains influences the level of the rivers which flood large areas in the wet season. The hot and cold seasons coincide with the rainy and dry seasons, respectively. The vegetation has been described as a mosaic complex, with influence from different vegetation types (biomes) such as cerrado in central Brazil, the Paraguayan Chaco, and the Amazon forest (PRANCE; SCHALLER, 1982). Open fields are interspersed with isolated islands of secondary forest, which are an important refuge for both predators and prey species. Gallery forests border temporary and permanent rivers and provide long corridors for wildlife. White-lipped peccaries *(Tayassu pecari),* an important prey species for jaguars, are abundant in the area, as well as caiman *(Caiman crocodilus yacare)*, deer *(Ozotocerus bezoarticus)*, collared peccaries *(Tayassu tajacu),* marsh deer *(Blastocerus dichotomus)*, and armadillos *(Euphractus sexcinctus).* With more than 200 years of occupation, the Pantanal remains relatively unchanged due to environmental factors like its typical flooding regime and the consequent difficult access. Extensive beef cattle management has been a traditional activity since the beginning of the eighteenth century, and it is the main economic activity in the region.

Methods

Due to the secretive nature of jaguars and pumas, information is obtained through the analysis of radio-telemetry data. Animals were captured with the help of trained hound dogs and an experienced local hunter (ROOSEVELT, 1914; ALMEIDA, 1976). Treed cats were immobilized with a combination of telazol and ketaset (Fort Dodge do Brasil). individuals were examined for general body condition, sexed, aged, measured, weighed, and fitted with a motion-sensitive traditional telemetry radio-collar (telonics, USA) or a GPS-based collar (televilt, Sweden) and released. Age was estimated on the basis of presence of milk or permanent dentition, and tooth color and wear, in addition to other features (ASHMAN et al., 1983).

Home range size, habitat use, and movement patterns in relation to cattle is being documented using conventional radio-telemetry or GPS technology. Radio locations are being obtained from the ground, through triangulation from locations with known universal transverse mercators (WHITE; GARROT, 1990). Due to the extent of the study area and limited access to the entire ranch from the ground, a cessna aircraft is used for aerial locations as well.

In addition to traditional radio-telemetry, individual animals were also fitted with gps-based telemetry collars. These GPS collars have provided coverage over large areas with a high degree of accuracy and precision and they operate 24hours a day throughout the year recording several fixes/day fixes at preset schedules. A receiver that remotely downloads information from the collars (rx-900, televilt) is used every 3 weeks to retrieve data. This large amount of individual locations provides information on animal movements continuously, independent of weather, time of day, or season.

The use of GPS collars allow for the simultaneous location of several individuals (or at least within minutes of each other depending on satellite orbits), which will provide an estimate of space use of each individual during depredation events at night. With GPS and traditional telemetry collars, we are collecting information on jaguar movements, space use, and examining depredation patterns. Predation sites are identified by data provided by the GPS collars. When 2 or more consecutive locations are found within <200 m from each other, sites are searched for a kill (ANDERSON, pers. commun.). In addition, simultaneous animal locations allow for examination of social behaviors, such as courtship and territoriality.

Preliminary results

Between September and December 2000, we captured 8 individual cats, 4 jaguars (1 male, 3 females) and 4 pumas (2 males, 2 females) and equipped them with traditional telemetry radio-collars. Between October and November 2001, we recaptured 2 of our collared female jaguars and replaced their traditional collars with GPS collars. In addition, a new male was captured and fitted with a GPS collar.

Study animals have been monitored for a cumulative total of 114 months: 79 months for 5 jaguars and 35 months for 4 pumas. Male puma #1 was monitored intermittently for 6 months but we have lost his radio signal since April 2001. Similarly, in June 2001 we lost contact with male puma # 2, after monitoring him for only 6 months. Female puma #1 was poached at the end of April after being monitored for only 5 months. Five jaguars (2 adult males, 3 adult females) and 1 puma (subadult female) were located a total of 1809 times until may 2002. From these locations, 460 (25.4%) were obtained with ground triangulation, 98 (5.4%) with aerial flights, and 1251 (69.2%) with GPS collars.

Preliminary estimates of our study animals' home ranges were made using the 95% minimum convex polygon (MOHR, 1947). The home range of male jaguar #2 – in 6 months of monitoring, and during the wet season only – has been estimated to be 296 km^2. This is a much larger figure than ever estimated before. Male jaguar # 1 had his home range estimated at 180 km^2. These figures could lead to a belief that our study area has a low density of jaguars. however, as opposed to previously thought, our study males had an overlap area of 132 km^2, the equivalent of 45% of the home range of male #2 and 73% of the home range for male #1. Indirect monitoring has indicated the presence of additional individuals in the area. At least 3 other males (2 adults and 1 subadult) and 1 female (adult, pregnant) have been identified in the area through camera trap photographs. The wet season home ranges of the females have been estimated at 52 km^2 (female #2) and 39 km^2 (female #3). The area of overlap between these females is about 4 km^2, or 7.6% of the estimated home range for female # 2 and 10% of the estimated home range for female #3.

Predation

Between November 13, 2001 and June 30, 2002, (30 weeks of monitoring), we have been able to record 67 clusters of locations for female jaguar #2. from these, 64 were checked on site (95,5%) and 3 remain unchecked (4,47%) due to inaccessibility of the area by car, horse, or even on foot. we found prey remains on 39 instances (60,9%). from these, 19 (48,7%) were comprised of native prey species and 20 (51.3%) were domestic cattle.

We recorded 125 clusters of locations for female jaguar #3. From these, we were able to inspect 114 (91,2%). Prey remains were found on 63 instances (55,3%). From these, 37 (58,7%) were comprised of native prey species and 26 (41,3%) were domestic cattle.

While female #2 killed only calves less than 1 year old, female #3 killed 57,7% adult cows and 42,3% calves less than 1 year old. From all domestic cattle male #2 killed, the majority were calves than 1 year old (84,6%). Other age classes were adult cows (7,7%) and heifers older than 1 year old (7,7%).

The GPS collar deployed on male jaguar #2 allowed us to record 64 clusters of locations during 30 weeks of monitoring. from these, we were able to inspect only 42 (65.6%), due to the large size of the area occupied by this male and the locations of several clusters in areas we could not reach during the wet season. from the clusters we were able to check, we found prey remains on 26 instances (61,9%). From these, 13 (50%) were comprised of native species and 13 (50%) were domestic cattle.

From all remains of native prey, we encountered for the 3 cats equipped with GPS collars, caiman, white-lipped peccaries and feral hogs were the most significant, representing 41,4%, 20%, and 12,8% respectively of the cumulative totals. Although female #3 killed a variety of prey species, it showed a preference for caiman, which comprised the majority of all native prey killed by this cat (64,8%). Male #2 also killed several species, but within native species, its diet was comprised mostly of feral hogs (46,1%). female #2 had the most varied diet of all cats, preferring white-lipped peccaries (26,3%), caiman (21%), and giant anteaters (15,8%) over other native prey species.

Discussion

The first time we collected data on a cluster of locations on female jaguar #2, we used traditional equipment and were unable to find any carcasses, even after intensively monitoring the cat for 4 consecutive days, from a vehicle 250 m away from it. Although at the time we searched we did not find anything, we were not convinced that there were no carcasses at the site. We recently were able to find an old carcass of an adult peccary in an area 250 m away from were this female remained for those 4 consecutive days. This example illustrates the limitations of using traditional equipment and the difficulties inherent to this kind of work.

Although 50% of what our male jaguar #2 killed was domestic cattle, this figure is likely to be exaggerated due to a potential bias in our sampling logistics. Most clusters of this male that we were not able to check were at unreachable places that did not have cattle in the wet season. Likely, there were several native prey remains that despite our efforts we were not able to find.

Crawshaw and Quigley (2002) observed a distinct segregation of the age classes for cattle killed by jaguars and pumas in the Pantanal. Adult cows comprised 57% of jaguar kills and calves comprised 92% of puma kills. In our study, there was a marked difference among individual jaguars. While female #3 killed 57,7% adult cows and 42,3% calves less than 1 year old, female #2 did not kill a single adult cow and killed only calves less than 1 year old.

Some authors suggest that old and injured or maimed animals are more likely to predate on domestic stock than healthy individuals. In our study, male jaguar #2, despite his old age and missing canine, has been successful in killing dangerous prey such as feral hogs. These animals possess large teeth that can inflict serious wounds on a jaguar. In comparison, male jaguar

#1, a healthy individual on his prime and likely with prime hunting capabilities, has been killing domestic livestock as well as native species.

Data presented here is preliminary. We will assess other environmental factors that could potentially affect or are related to livestock depredation such as vegetation cover and proximity to specific areas/sites with water, range core areas, etc, seasonality of depredation events, cattle management practices, precipitation. We may find slightly different results as we gather additional data.

We will need data from at least 2 years to accurately access the impact of cat depredation on livestock. The new GPS technology has allowed us to find carcasses of animals killed by jaguars, either livestock or native species. We are able to track individual cat predation patterns. Therefore, we should be able to estimate how much does it cost to the rancher to maintain a healthy cat on his property. By extrapolating predation patterns we observe to the whole population, we will be able to estimate with good confidence the economic damage jaguars pose to producers in the region. The results will be presented in the context of other sources of mortality and management in general. Our objective is to investigate the losses due to depredation when they are set in the context of total losses from all causes. We can then examine whether losses to jaguars pose a serious economic liability or they can be tolerated on a livestock ranch in the Pantanal.

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Parte V
Banco genômico e manejo em cativeiro

Capítulo 14
Importância do banco de amostras biológicas e do manejo em cativeiro na conservação de carnívoros neotropicais

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Introdução

O crescimento desenfreado e os hábitos consumistas dos países desenvolvidos estão gerando um aumento na demanda por recursos naturais renováveis e não renováveis. De maneira similar, guerras, exploração dos recursos naturais e expansão das atividades agropecuárias nos países em desenvolvimento têm aumentado a destruição e a fragmentação ambiental. Como conseqüência, a taxa de extinção de animais e plantas é alarmante e, estudos têm mostrado que cerca de um quarto de todas as espécies de mamíferos estão ameaçadas de extinção (ENTWISLE; DUNSTONE, 2000).

No Brasil, 68 espécies de mamíferos estão na lista oficial de espécies ameaçadas de extinção (MACHADO et al., 2005), incluindo dez espécies da ordem Carnivora. Entretanto, a falta de conhecimento acerca da distribuição, história natural e ecologia da maioria das espécies de mamíferos da ordem Carnivora têm impedido a formulação mais acurada dessa lista. Adicionalmente, essa lista não considera as diferentes situações para as espécies em diferentes biomas, podendo então mascarar situações críticas em âmbito local. A validade desse método é ainda comprometida pela falta da integração entre os países sul-americanos. Muitas espécies de mamíferos da ordem Carnivora são comuns a vários países de nosso continente, e variações nos métodos de manejo e fiscalização de um país podem afetar populações de países vizinhos.

Estratégias para a conservação de mamíferos da ordem Carnivora têm sido extensamente debatidas (GITTLEMAN et al., 2001). Entretanto, pouca atenção tem sido dispensada para entender tais aspectos na conservação das espécies neotropicais. Possivelmente, as estratégias adotadas para mamíferos da ordem Carnivora em outras regiões do globo podem fornecer subsídios para uma ação concentrada nos neotrópicos, obviamente considerando-se as particularidades locais. Por exemplo, programas de criação em cativeiro, bancos de amostras biológicas e técnicas de reprodução assistida têm sido considerados importantes ferramentas para a conservação (WILDT, 1989; BALLOU; FOOSE, 1996; WILDT; WEMMER, 1999), e é possível que sejam aplicadas com sucesso nas espécies de carnívoros neotropicais (MORATO; BARNABE, 2002).

Especificamente, as populações em cativeiro podem atuar como fonte de pesquisas permitindo o aumento do conhecimento sobre as espécies de interesse; atuam como um seguro contra eventos de extinção de larga escala ou devastação de populações em vida livre; e atuam como embaixadores para as espécies de interesse por meio de atividades educacionais (WILDT, 2001). Por outro lado, bancos de amostras biológicas e técnicas de reprodução assistida podem contribuir para a preservação de informação genética e posterior retorno desta à natureza, respectivamente. Deve-se levar em consideração que há pouco conhecimento sobre aspectos da criopreservação de gametas e fisiologia reprodutiva de carnívoros neotropicais o que tem limitado a utilização dessas ferramentas conservacionistas. Além disso, os recursos técnicos e financeiros são elevados trazendo o seguinte questionamento: devemos realmente incluir tais ferramentas como prioridades para a conservação dos carnívoros neotropicais?

Neste capítulo, será discutido o papel dos programas de criação em cativeiro, banco de amostras biológicas e técnicas de reprodução assistida para a conservação de carnívoros neotropicais, incluindo recentes avanços e, finalmente, como tais ferramentas podem contribuir para a conservação das espécies em questão.

Aplicação do banco de amostras biológicas e criação em cativeiro

Segundo Holt (2001), os bancos de amostras biológicas podem ser estabelecidos com dois objetivos: 1) desenvolver uma coleção de tecidos somáticos, linhas celulares, DNA e soro de uma variedade de espécies, primariamente para estudos taxonômicos, demográficos e médicos, e; 2) desenvolver uma coleção de gametas e embriões que serão utilizados por meio de reprodução assistida. Considerando que muitos animais mantidos em zoológicos brasileiros estão com idade avançada e, conseqüentemente, com reduzido potencial reprodutivo, torna-se urgente a preservação de informação genética desses indivíduos. Os bancos de amostras biológicas oferecem a condição ideal para estocagem dessas amostras que podem ser mantidas por várias gerações, estando disponível para uso futuro. Em combinação com as técnicas de reprodução assistida como inseminação artificial, fecundação in vitro, injeção intracitoplasmática de espermatozóides, clonagem e transferência de embriões, outras oportunidades surgem para o manejo genético, principalmente, evitando-se encontros potencialmente agressivos e por meio de movimentação de material genético entre populações cativas e em vida livre, aumentando a viabilidade da população (HARNAL et al., 2002).

Dessa forma, a criopreservação de amostras biológicas pode atuar como um seguro contra a extinção (WILDT, 2001). Informações sobre fisiologia reprodutiva são essenciais para o planejamento e implementação do BAB e subseqüente incorporação por meio de ART (Brown et al., 1994). Entretanto, a falta de conhecimento dos aspectos reprodutivos tem limitado a aplicação de ART em várias espécies, incluindo os carnívoros neotropicais (MORATO, 2001).

A criação em cativeiro tem sido considerada uma importante ferramenta para a conservação (SOULÉ, 1991) haja vista que os zoológicos podem manter e propagar animais vivos para educação, pesquisa e reintrodução de animais vivos ou mesmo material genético. Está claro que em muitos casos a educação e a pesquisa têm sido aplicados com sucesso em zoológicos europeus e norte-americanos, entretanto para os países da América Central e do Sul esses conceitos são novos e a maioria dos zoológicos ainda não usa os animais para fins educacionais ou de pesquisa. Outro aspecto importante é que para a propagação de indivíduos há necessidade de um manejo reprodutivo ordenado a fim de que se mantenha a diversidade genética da população fundadora (BALLOU; FOOSE, 1996), porém, grande parte da população de mamíferos da ordem Carnivora em zoológicos da América Latina é de origem desconhecida não havendo registro genealógico adequado.

Considerando a importância da criação em cativeiro em programas de reintrodução deve-se ressaltar que se trata de um procedimento complexo e de alto custo. Adicionalmente, há poucos relatos de sucesso em procedimentos de reintrodução de mamíferos carnívoros (BREITENMOSER et al., 2001). Barreiras primárias incluem transferência de doenças, comportamento inapropriado no novo ambiente e interação com a população residente. É possível que no futuro ART seja uma alternativa para a reintrodução de um animal vivo, evitando-se as barreiras supracitadas.

Papel da criação em cativeiro e do banco de amostras biológicas em genética da conservação

O principal objetivo dos biologistas da conservação é a preservação dos ecossistemas com todas as espécies inseridas nestes e, obviamente, a diversidade genética de cada uma destas espécies (CRANDALL et al., 2000). Entretanto, devido à escassez de recursos técnicos e financeiros, para alcançarmos tal objetivo é necessário decidirmos, em um primeiro momento, o que preservar e como (JOHNSON et al., 2001). Nesse sentido, a biologia molecular tem fornecido importantes subsídios para responder tais questões. Além disso, as ferramentas moleculares têm contribuído para a integração de diversas disciplinas incluindo a biologia da reprodução, fisiologia, imunologia, demografia, virologia, comportamento, ecologia e medicina da conservação

(JOHNSON et al., 2001). De maneira geral, a genética da conservação pode contribuir na definição de unidades demográficas (espécies e unidades intra-específicas), quantificação e interpretação de variabilidade genética, parentesco, cruzamentos extrapar, estrutura e relação entre e dentro de grupos sociais, identificação de indivíduos e caracterização de patógenos em populações naturais (JOHNSON et al., 2001; TARBELET et al., 2001).

Apesar da aplicabilidade dessas ferramentas para a conservação, poucos estudos têm caracterizado o estado genético dos carnívoros neotropicais e as informações atuais estão restritas a algumas espécies de felinos. É possível que a falta de informações seja resultado do número limitado de amostras biológicas e, certamente, o acúmulo de amostras de diferentes espécies e de diferentes regiões permitiria a obtenção de dados necessários para a elaboração de planos de manejo para cada espécie de interesse (EIZIRIK et al., 2001; JOHNSON et al., 2001).

Nesse sentido, os BRB e a criação em cativeiro podem auxiliar no acúmulo de amostras biológicas que serão utilizadas em análises genéticas. Isso é particularmente importante se conseguirmos desenvolver uma rede de trabalho integrado a todos os países da América que possuem fauna, de mamíferos da ordem Carnivora, em comum. Obviamente, tal estratégia englobaria amostras dos diversos biomas subsidiando a elaboração de um amplo plano de manejo e, mesmo, auxiliando na definição de prioridades de preservação de amostras biológicas, com especial referência aos gametas e embriões.

Papel do banco de amostras biológicas e criação em cativeiro em medicina da conservação

Recentemente, o estudo de doenças tem emergido como tema central na conservação de mamíferos da ordem Carnivora (FUNK; FIORELLO, 2001). Doenças infecciosas podem afetar os animais alterando a dinâmica e viabilidade das populações onde ela ocorre e/ou o bem-estar e a qualidade de vida dos indivíduos acometidos (KIRKWOOD; COLENBRANDER, 2001).

É importante considerar que agentes infecciosos e parasitários são elementos naturais na cadeia alimentar e exercem papel importante no processo de seleção natural, no entanto, a introdução de organismos exóticos, incluindo agentes virais e bacterianos, nos ecossistemas, têm sido uma das maiores causas de extinção e perda de biodiversidade nos últimos tempos (PIMM, 1991) e devemos considerar que tais agentes podem ser introduzidos acidentalmente nas populações naturais por translocação, reintrodução e movimentação de animais vivos, e transferência de gametas e embriões. O risco de transmissão de doenças associados com movimentação de gametas e embriões é geralmente menor que aqueles associados aos processos de translocação e reintrodução (HOWARD, 1999), entretanto, doenças transmitidas via sêmen e transferência de embriões têm sido reportadas em muitas espécies de mamíferos, incluindo carnívoros (KIRKWOOD; COLENBRANDER, 2001). Tais riscos podem ser minimizados pelo adequado processamento e armazenamento das amostras. Por exemplo, a lavagem e retirada do plasma seminal antes da inseminação artificial elimina a incidência de piometra em fêmeas receptoras (HOWARD, 1993). Adicionalmente, como mencionado anteriormente, amostras estocadas podem ser úteis para a caracterização dos patógenos presentes em populações naturais e, antes de realizarmos qualquer técnica reprodutiva para a reintrodução de informação genética em uma população natural, as amostras podem ser varridas para possíveis doenças que podem afetar a espécie em questão.

Papel da biologia da reprodução no banco de amostras biológicas e criação em cativeiro

Considerando que a manutenção de diversidade genética é dependente da reprodução (WILDT et al., 1993) e que o objetivo da conservação de carnívoros é reverter o declínio populacional e assegurar a sobrevivência das populações remanescentes (SILVA et al., 2004), as biotécnicas reprodutivas são ferramentas auxiliares na conservação de espécies da ordem Carnivora de nossa fauna. Para maior eficiência na aplicação das biotécnicas, o conhecimento da fisiologia reprodutiva das espécies de interesse é necessário. Atualmente tais estudos estão concentrados em espécies de felinos e canídeos (VELLOSO et al., 1998; MOREIRA et al., 2001; MORAIS et al., 2002; VIAU, 2003; MORATO et al., 2004) havendo lacuna de conhecimento para mustelídeos e procionídeos.

Quando consideramos a aplicação de biotécnicas reprodutivas no manejo genético de populações ameaçadas, a inseminação artificial e a fecundação in vitro estão dependentes da colheita e armazenamento adequados de gametas.

A criopreservação de sêmen permite o armazenamento, no longo prazo, de amostras genéticas. Além disso, pode facilitar a transferência de informação genética entre diferentes populações, sem os custos e o estresse inerentes ao processo de transporte animal (WILDT; WEMMER, 1999). Outra vantagem é a possibilidade de manter “indivíduos” sem o requerimento de recintos e os cuidados de manutenção de animais vivos. No entanto, ainda temos limitações, para algumas espécies, no procedimento de colheita e/ou criopreservação.

Amostras de sêmen podem ser obtidas por eletroejaculação em animais previamente anestesiados (SILVA et al., 2004). Amostras de sêmen já foram obtidas, por meio dessa técnica, em mais de 28 espécies de carnívoros selvagens (HOWARD et al., 1984), incluindo carnívoros neotropicais. A partir desses estudos, pesquisadores brasileiros e americanos já obtiveram amostras de pequenos e grandes felinos neotropicais em cativeiro (MORATO et al., 1998; SWANSON et al. 2003), grandes felinos neotropicais em vida livre (MORATO et al., 2001) e canídeos neotropicais em cativeiro (SONGSASEN, comunicação pessoal). Como mencionado anteriormente, não há informações para mustelídeos e procioníddeos neotropicais. Amostras também podem ser obtidas de animais mortos, para tanto, a maceração da cauda do epidídimo e lavagem das células obtidas permitem a obtenção de espermatozóides viáveis (JEWGENOW et al., 1997; MORATO et al., 1999).

As amostras de sêmen podem ser criopreservadas, no entanto, fatores espécie-específicos tais como; tolerância a substâncias crioprotetoras (ROTH et al., 1996) ou sensibilidade ao choque térmico (QUEIROZ, 2003) exercem influência no sucesso da preservação espermática. Por exemplo, o resfriamento lento (4°C/2h) mostrou-se menos agressivo em relação a danos causados no acrossoma em gatos domésticos (PUKAZHENTI et al., 1999; BAUDI, 2005) e *Leopardus tigrinus* (BAUDI, 2005). Por outro lado, o resfriamento rápido (4°C/30 min.) forneceu melhores resultados para *Leopardus pardalis* (BAUDI, 2005), apesar de já ocorrer danos significativos ao acrossoma a 9°C (QUEIROZ, 2003). O meio de diluição para o resfriamento e/ou lavagem do sêmen também parece influenciar a viabilidade da célula espermática. Em estudo recente, Morato et al (2003) verificaram que o meio TEST gema de ovo tinha maior efeito protetor ao acrossoma quando comparado ao meio HAM F 10. Além das alterações observadas após o resfriamento, a congelação e a descogelação do sêmen também são responsáveis por danos na célula espermática. Estudos buscam melhor compreensão da concentração ideal de crioprotetor (HEWITT; ENGLAND, 2001), assim como o método de congelação (controlado, vapor de nitrogênio, dry shipper) mais adequado (PUKAZHENTI et al., 1999; MORATO et al., 2003).

Além do esforço para a otimização do sêmen criopreservado para a utilização em inseminação artificial, há necessidade de estudos para sincronização de fêmeas receptoras. A sincronização e a indução da ovulação são difíceis e possuem grande especificidade, sendo necessário entendimento básico da fisiologia reprodutiva da fêmea para que possamos manipular adequadamente o ciclo. Variabilidade entre as espécies inclui fatores como ovulação induzida ou espontânea e sensibilidade a hormônios exógenos (BROWN et al., 1994).

Apesar dessas limitações, o objetivo de transferência de informação genética entre populações e, consequentemente a manutenção de diversidade genética, pode ser mais facilmente alcançado pela criopreservação e transporte de sêmen. A recuperação e a preservação de oócitos é igualmente importante como ferramenta de manejo genético, mas certamente há maiores barreiras a serem transpostas. Enquanto os efeitos fisiológicos de criopreservação são similares para a desidratação celular, alteração de volume celular e efeitos de membrana, os oócitos sofrem danos maiores quando comparados à célula espermática, e os oócitos em estágios de desenvolvimento mais tardios são mais susceptíveis a danos cromossômicos (KOLA et al., 1988) devido à sensibilidade do fuso meiótico da metáfase II às baixas temperaturas.

A criopreservação de folículos pré-antrais é uma alternativa para a estocagem de um grande número de oócitos imaturos recuperados de um animal. Oócitos inclusos em folículos pré-antrais parecem ser menos sensíveis aos danos causados pelas baixas temperaturas, principalmente porque eles são menores, não possuem zona pelúcida e grânulos corticais e possuem menos lipídios intracitoplasmáticos sensíveis a baixas temperaturas (GOSDEN et al., 1994; SHAW et al., 2000). Além disso, o fuso meiótico, o qual é sensível a baixas temperaturas, ainda não está formado nesses oócitos, reduzindo os riscos citogenéticos, relacionados às divisões subseqüentes, após a descongelação da célula (OKTAY et al., 1998).

Considerando-se a baixa taxa de concepção e o potencial de danos cromossomais no processo de criopreservação dos oócitos, a preservação destes gametas em tecido ovariano pode ser uma alternativa viável. Parênquima ovariano pode ser obtido de indivíduos recém-mortos e congelados em temperatura controlada (SZTEIN et al., 1998), descongelados e transplantados para indivíduos da mesma espécie (SZTEIN et al., 1998) ou mesmo para indivíduos de espécies diferentes (GUNASENA et al., 1997). Oócitos de parênquimas ovarianos transplantados podem completar a maturação quando o indivíduo receptor é submetido a um tratamento com gonadotrofinas exógenas. Apesar disso, ainda é difícil obter gestação por meio desta técnica (JEREMIAS et al., 2003). Talvez o melhor método de obter indivíduos viáveis por meio da recuperação de oócitos post-mortem seja a maturação in vitro, seguido da fecundação in vitro e cultivo embrionário.

A maturação meiótica de telófase I para metáfase II é relativamente fácil de se obter in vitro, exceto para canídeos cujos mecanismos envolvidos na maturação oocitária ainda são pouco conhecidos (SONGSASEN et al., 2002). Mesmo que tenha ocorrido a maturação nuclear in vitro, os resultados de fertilização e desenvolvimento são inferiores aos alcançados in vivo. Possivelmente isso ocorra em função de uma assincronia entre a maturação nuclear e a citoplasmática nos procedimentos in vitro (SUNSTROM; NILSSON, 1988; SCHRAMM et al., 1994; GOUDET et al., 1997; KITO; BAVISTER, 1997; SINGH et al., 1997; SPINDLER; WILDT, 1999). Além das barreiras supramencionadas, a fertilização também depende de espermatozóides maduros e funcionais.

Em muitas pesquisas têm sido realizadas para determinar melhores protocolos de preparo de amostras de sêmen para a fecundação in vitro, mesmo de espermatozóides recuperados diretamente do testículo (FRANÇA et al., 2000; HONARAMOOZ et al., 2002) ou epidídimo (MORATO et al., 1999). Avaliações primárias referem-se à motilidade espermática (DROBNIS et al., 1988), morfologia espermática (PUKAZHENTHI et al., 1996), integridade do acrossoma (SUAREZ et al., 1984; TALBOT, 1985; CUMMINS E YANAGIMACHI, 1986), habilidade de sofrer capacitação (YANAGIMACHI, 1994), reação acrossômica (YANAGIMACHI, 1994) e descondensação da cromatina (PERREAULT, 1990). As condições in vitro que satisfaçam tais requerimentos variam entre as espécies, razão pela qual, a fertilização in vitro e o desenvolvimento embrionário têm sido relatado a partir de oócitos e espermatozóides imaturos, em várias espécies de mamíferos.

Em casos em que há baixo número de espermatozóides, possivelmente após recuperação, post-mortem, de espermatozóides do testículo ou epidídimo ou mesmo seguindo a castração, métodos alternativos de fecundação, como a injeção intracitoplasmática de espermatozóides (ICSI), podem ser requeridos. A ICSI é rotineiramente aplicada, em reprodução humana, com grande sucesso. Entretanto, os efeitos dessa técnica sobre a saúde dos nascidos ainda estão sob investigação e isso deve ser levado em consideração também quando utilizarmos esta técnica em espécies raras ou ameaçadas de extinção.

De forma semelhante, o cultivo embrionário apresenta resultados variáveis entre espécies e laboratórios assim como em relação às diferentes técnicas utilizadas. A barreira inicial, de bloqueio do desenvolvimento embrionário e ativação do genoma maternal (SESHAGIRI et al., 1992), já foi transposta e embriões podem se desenvolver até o estágio de blastocisto. Obviamente há variações no sucesso de desenvolvimento embrionário de acordo com a espécie, laboratório e técnica utilizada.

O momento ideal da transferência de embriões também tem sido largamente investigado nas diferentes espécies. O ambiente maternal adequado, em relação às necessidades do embrião transferido, pode ser alcançado com a administração de hormônios exógenos, entretanto, há necessidade de conhecimento da fisiologia reprodutiva da espécie de interesse. Certamente não se trata de uma barreira intransponível, e de fato, para algumas espécies, já se obteve nascimentos de indivíduos viáveis. Sendo assim, podemos observar que a combinação da maturação in vitro, a fertilização in vitro, o cultivo embrionário e a transferência de embriões têm se mostrado uma valiosa ferramenta para a produção de novos indivíduos, haja vista o sucesso obtido em animais domésticos e humanos.

Outra ferramenta de interesse na reprodução artificial é a transferência nuclear. Se considerarmos os recentes sucessos na clonagem de mamíferos com células somáticas, há grande expectativa da aplicação dessa técnica para a produção de indivíduos (HOLT, 2001). Esta técnica pode beneficiar pesquisas em doenças por meio da propagação de um indivíduo com (ou sem) um gene específico, assim como pode ser utilizada como parte de um programa de modificação genética com a finalidade de aumentar a produção agroindustrial. No entanto, deve-se considerar se é viável a aplicação dessa técnica, para a produção de indivíduos idênticos, no manejo e conservação de espécies raras e/ou ameaçadas de extinção. Ressalta-se que o objetivo das técnicas de reprodução artificial é auxiliar na manutenção de diversidade genética, e a clonagem, se mal aplicada, pode contribuir para a perda de variabilidade genética. Além disso, devem-se considerar as dificuldades e a baixa taxa de sucesso da técnica em animais domésticos, o que torna esta opção pouco viável, no momento, para espécies raras e/ou ameaçadas de extinção (HOLT, 2001).

Por outro lado, o desenvolvimento de técnicas de criopreservação e descongelação de tecidos somáticos permitiria o estoque de informação genética que, em futuro próximo, poderia ser utilizado na transferência nuclear. Os tecidos somáticos são menos susceptíveis aos danos da criopreservação e, além disso, são facilmente coletados e estocados em condições de campo. Na medida que haja progresso na aplicação da técnica é possível que a comunidade zoológica e conservacionista a utilize para a propagação de informação genética em um programa de manejo, para a conservação da espécie de interesse.

Aspectos éticos da criação em cativeiro e banco de amostras biológicas na conservação de espécies raras e/ou ameaçadas de extinção

Devemos ter em mente que nenhuma dessas estratégias, isoladamente, é suficiente para solucionar os problemas de ameaça dos mamíferos da ordem Carnivora. A produção de novos indivíduos, por exemplo, pouco pode representar se não houver atenção quanto à saúde de cada indivíduo assim como da população como um todo. Além disso, atenção especial deve ser dada à preservação de habitat uma vez que o desaparecimento deste inviabiliza qualquer estratégia que busque o retorno de animais à natureza. As técnicas de reprodução assistida precisam ser integradas ao conceito de manejo metapopulacional de forma que possam ser efetivas como estratégias de conservação (Figura 1). Finalmente, a aplicação destas técnicas deve levar em conta aspectos legais, sociais, econômicos, ecológicos e de saúde. Sabemos que os recursos a serem aplicados em conservação são escassos e a escolha da metodologia de manejo deve seguir uma ampla discussão entre comunidade científica, sociedade civil organizada e governo.

Considerações finais

A população em cativeiro pode atuar como reserva de informação genética in vivo, em paralelo, os bancos de amostras biológicas podem manter informação genética, in vitro, de um indivíduo já morto, por muitas gerações. Grandes populações de uma dada espécie podem ser mantidas dentro de um banco de amostras biológicas, e amostras armazenadas podem ser usadas no sentido de facilitar o manejo de metapopulações estabelecendo-se o fluxo gênico entre populações isoladas. Da mesma forma, pode auxiliar na identificação de patógenos, contribuindo para o estabelecimento de estratégias que diminuam os riscos de catástrofes desta ordem. É importante salientar que a manutenção da informação genética in vivo ou in vitro depende de infra-estrutura adequada, suporte financeiro e acadêmico. Tanto a criação em cativeiro quanto o Banco de Amostras Biológicas só podem atingir seus objetivos se incorporados a um plano integrado de conservação, incluindo: desenvolvimento de técnicas de reprodução assistida, manejo genético e sanitário, estudos de comportamento e história natural, análise de viabilidade de habitat e preservação de habitat.

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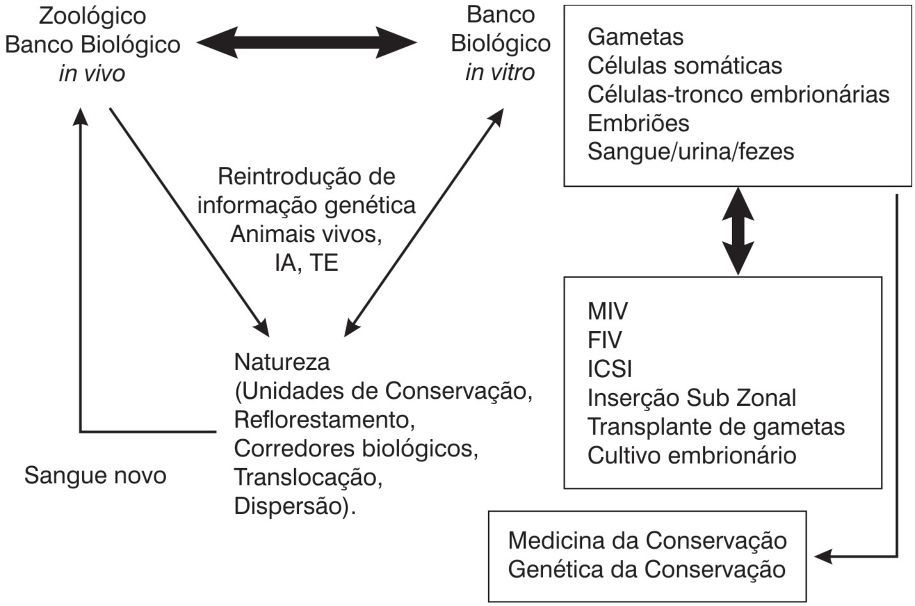
Figura 1 – Integração do banco de amostras biológicas e criação em cativeiro ao conceito de manejo metapopulacional.



Capítulo 15
Developing a functional genome resource bank for wild neotropical carnivores

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Introduction

In the recent past, the Earth possessed a thriving abundance of carnivores, a group of species that has captivated human attention for millions of years, initially because to ignore them was to risk becoming their dinner and later because of their 'baffling' tendency to consume the livestock that ranchers placed into the ranges of their natural prey species (JACKSON; NOWELL, 1996). Carnivores comprise seven major families (Canidae, Felidae, Ursidae, Mustelidae, Viverridae, Hyaenidae, Procyonidae) containing upwards of 271 species, and, despite the best efforts of mankind, continue to play a key and defining role in their ecosystems. Across the globe, humans have destroyed their habitat, decimated their prey base and viciously hunted, poisoned and literally driven over countless individuals, pushing some populations to the brink of extinction. And, yet, most carnivore species still survive (PURVIS et al., 2001). As habitat loss, disruption of gene flow and increased inbreeding slowly erode remaining levels of genetic variation and began spiraling the survivors into extinction, it may be time to consider taking more drastic ameliorative action. A full repertory of conservation tools including habitat preservation for wild populations, captive propagation of zoo populations and mechanisms for creating in situ-ex situ linkage will be necessary to improve survival probabilities, especially in the context of maintaining adequate genetic variability across a maximal number of carnivore species.

As a component of this conservation repertory, genome resource banks (GRBs), consisting of frozen gametes, embryos, blood products and tissues, hold tremendous potential in conserving and managing extant genetic diversity, but must be functional in design and usage to be of real value. Maintaining a few frozen sperm samples in a liquid nitrogen tank in an isolated laboratory does not equate to creating a fully functional GRB. To be functional, GRBs must be established based on a fundamental knowledge of cryobiology for a diverse range of biological samples, from spermatozoa to oocytes to embryos to somatic cell lines. Their utility is limited by the efficacy of related technologies, such as in vitro fertilization (IVF), artificial insemination

AI, and embryo transfer (ET), for producing offspring and dependent on proper donor selection, sample identification, storage security and integration into organized population management programs. Their development and use also must engage and excite the human psyche in order to garner necessary public and financial support.

Genome resource banking – theoretical benefits and realistic expectations

The potential conservation benefits of genome resource banking have been discussed extensively for at least 15 years and are still valid from a theoretical perspective. To summarize salient points made by others (WILDT et al., 1992, 1997; WILDT, 2001; HOLT et al., 1996; HOLT et al., 2003), GRBs offer a number of short-term and long-term benefits for conservation (Table 1). In the short-term (months to years), GRBs represent valuable tools for actively managing captive populations, provided that effective techniques for AI or ET have been established. Banks of frozen germ plasm can facilitate genetic management programs (such as the Species Survival Plans or SSPs of the American Zoo and Aquarium Association) by providing a means to breed pairings that are optimal from a genetic perspective (i.e., based on mean kinship values) but are either behaviorally or physically incompatible. Clouded leopards might represent an ideal species for this GRB application. Despite fairly robust numbers (~120 animals), the Clouded Leopard SSP population suffers from extremely low levels of genetic variation (<75%), primarily due to the incompatibility and aggression of males when paired with unfamiliar females. In this species, territorial defense overrides the breeding instinct and clouded leopard males have earned a deserved reputation for killing potential mates. Artificial insemination with frozen spermatozoa could offer an alternative to population managers who wish to avoid the inherent risks association with pairing and natural breeding of this species.

GRBs also would be useful as a means to move genetic material between individuals, populations and countries without transporting living animals. Many carnivores, especially smaller-sized species that are both predators and prey in the wild, can be subjected to extreme psychological and physical stress when confined in small crates and transported over long distances via ground or air transportation. Especially with international transport, animals may be in transit for days as they are moved thousands of miles with intermittent handling and holding periods at connecting airports. Provisions must be made to provide water and food for these animals during transportation, and despite the best of planning, occasional shipments go awry and animals are lost, injured or die in transit. Transporting of live animals stresses both the animals as well as the population managers responsible for arranging shipment of typically valuable individuals. Moving frozen gametes or embryos would be a preferable option to avoid this situation. As one example, the Ocelot SSP, through an international program termed the Brazilian Ocelot

Consortium, is attempting to establish a Brazilian ocelot population in U.S. zoos with the importation of 20 captive-born ocelot founders from Brazil over a five year period (SWANSON, in press). To augment these traditional methods of establishing a new population, research studies in Brazil have allowed generation and cryopreservation of ~80 ocelot IVF embryos representing 15 potential founders to the U.S. population. A portion of these frozen embryos are being imported into the U.S. in a liquid nitrogen dry shipper for transfer into generic ocelots in U.S. zoos, allowing production of Brazilian ocelot founders while reducing the number of live animals subjected to transport stress.

As a corollary to moving genetic material among captive populations, GRBs also can offer a mechanism to move genes between wild and captive populations. Rather than capturing wild animals as new founders for a captive population, it may be possible to collect and freeze spermatozoa from wild males in their natural range. Frozen sperm may be used for AI or IVF with captive individuals while the gamete donor remains in the wild. As one example, this approach has been used successfully to obtain new founders for the Cheetah SSP, using frozen spermatozoa collected from wild Namibian cheetahs to inseminate and produce offspring in captive cheetahs in U.S. zoos (HOWARD et al., 1997). A similar approach is being attempted with wild jaguars in Brazil and wild ocelots in Mexico, where reproductive physiologists are collecting and freezing semen from wild cats in collaboration with field biologists conducting radiotelemetry studies (MORATO et al., 2001; SWANSON, unpublished).

Yet another potential benefit of GRBs is the ability to extend the generation interval for reproduction, an invaluable attribute when managing small populations and species with naturally short generation intervals (BALLOU, 1992). With short generation intervals, genetic drift occurs rapidly through natural segregation of alleles. As a consequence, genetic variation is lost quickly unless the population size is expanded or generation interval is increased. GRBs can be used to artificially extend the generation interval by delaying reproduction of an individual for years, even until after the animal has died. As an example, black-footed ferrets have a very short generation interval, with females only breeding from one to three years of age before becoming non-reproductive. Males are not mature until two years of age but can breed for their lifetime (~5 years) (HOWARD et al., 2003). Delaying reproduction in females is not feasible because of their short period of fecundity but, by collecting and freezing ferret spermatozoa for later AI, the male's generation interval may be extended for years. Similarly, GRBs can be valuable in expanding the theoretical population size as another means to counter drift, by maintaining genetic variation as frozen germ plasm rather than living individuals. Exhibit and holding space in zoos is limited, restricting the total number of species and individuals of a given species that can be maintained in captivity. One calculation estimates that all of the world's zoos, acting in concert, can only maintain a few hundred species in genetically viable populations (CONWAY, 1986). With effective use of GRBs, the number of individuals needed for each species might be reduced by 30-50%, opening up holding space for twice the number of species in the world's zoos. For example, in the GRB Action Plan for tigers (WILDT et al., 1993), it has been estimated that 90% of existing genetic variation can be maintained for 100 years with a captive population size of just 120 animals, provided that 30-50% of offspring are produced by AI using frozen spermatozoa from a managed GRB.

GRBs also represent important collections for research purposes if DNA, whole blood, serum, plasma, tissues and cell lines are included in addition to germ plasm. Archived biological samples are useful for multi-disciplinary research applications, including epidemiology, molecular and population genetics, endocrinology, immunology, nutritional assessments, and possibly even somatic cell nuclear transfer (i.e., cloning). Storage of these biological samples, in contrast to germ plasm cryopreservation, is fairly straight-forward for most specimens, with the possible exception of certain cell lines in which post-thaw cell viability is critical. One example of this GRB application is retrospective evaluation of viral antibodies in banked serum samples collected routinely from anesthetized nondomestic cats, providing data on the prevalence of feline immunodeficiency virus in dispersed global populations and the potential infectivity of stored germ plasm (BROWN et al., 1993).

Long-term benefits of GRBs are primarily related to securing genetic material as insurance against species extinction and preventing more subtle genetic drift over longer periods of time. For small populations with restricted geographic or institutional distribution, preserving the genetic make-up as insurance against catastrophic or stochastic events is of great importance. For example, ocelots in south Texas and northern Mexico represent a defined subspecies (Leopardus pardalis albescens) existing in a shrinking habitat with a total wild population size estimated at as few as 300 animals (TEWES; EVERETT, 1987). Efforts have been initiated in Mexico to collect and freeze spermatozoa from males captured for ongoing radiotelemetry studies to begin building a frozen repository of genetic material as insurance against drastic population reduction and loss of potential founders.

Becoming functional

In theory, GRBs could revolutionize how we manage wildlife populations in captivity and in situ. Moving from the potential value of GRBs to actual application and benefit to conservation involves transforming GRBs from a theoretical to functional conservation tool. For a GRB to be truly functional, several criteria must be met. First, techniques for collecting and preserving biological resources must be established and shown to have adequate efficacy in allowing post-thaw recovery of viable gametes, embryos or cell lines. This, in turn, requires extensive basic and applied research on the individual, species or family level. Proven protocols for collecting and cryopreserving spermatozoa, oocytes and embryos only exist for a handful of carnivore species, with pronounced interspecies variability being the norm. Second, the use of frozen-thawed gametes or embryos for wildlife propagation requires the development of efficient procedures for artificial insemination (AI) and embryo transfer (ET), techniques which are nonexistent for all but a very few carnivores. Third, from a logistical perspective, prioritization of species and individual animals within populations for banking often is required to avoid unnecessary use of limited resources. Frozen biological samples also must be adequately labeled, cataloged and traceable within a collection database, and be stored securely in monitored holding tanks, preferably in two separate locations. Finally, GRBs and related technology must be readily applicable and integrated into population management programs, with defined goals and adequate personnel and resources to implement recommendations.

Basic cryobiology research in carnivores

Development of a functional GRB must begin with basic research studies to investigate the cryobiological properties of samples selected for preservation. Even in the absence of proven protocols for AI or ET, it is possible to initiate cryobanking of germ plasm or other samples from threatened wildlife populations, but only if appropriate cryopreservation procedures have been identified. As with any application of reproductive sciences, it cannot be assumed that protocols that are effective in one species will produce equivalent results when extrapolated to another species, no matter the closeness of genetic relatedness. Species differences in reproductive characteristics and function (including cryopreservation of spermatozoa and embryos) should be expected as the norm rather than the exception (HOLT, 2000; THURSTON et al., 2002).

Basic cryobiological research with carnivores has focused primarily on sperm cryopreservation, with limited studies of embryo, oocyte and ovarian tissue cryopreservation. Most of the basic research has centered on the domestic dog and cat with some extrapolation to nondomestic canid and felid species. One reason for the preponderance of sperm research is the relative ease in obtaining sperm samples from living domestic felids and canids as compared to recovering oocytes or embryos. With domestic species, semen collection via electroejaculation (cats) or artificial vagina (AV) (dogs and cats) is easily performed. Male reproductive tracts also are readily available from spay and neuter clinics. In addition, the physiological properties of spermatozoa (small size, high permeability, low water content) are more conducive to developing effective protocols for cryopreservation as compared to oocytes, for example (RALL, 2001).

In canids, commercial interests and a robust market for AI among purebred dog owners have provided impetus for sperm freezing research, with the unfortunate consequence that some findings are proprietary and unpublished. However, several recent studies have investigated the impact of various extenders, cryoprotectants, and cooling, freezing and thawing rates on dog sperm cryopreservation with high post-thaw motility and viability now routinely obtained (FARSTAD, 1996; WILSON, 1993). Function assays, such as zona binding, also have been incorporated to provide more objective data on the effect of freezing without requiring extensive AI trials (HAY et al., 1997). With the exception of the blue fox and the wolf (FARSTAD, 1996; GOODROWE et al., 1998; 2001), detailed sperm freezing studies have not been reported among nondomestic canids, possibly due to difficulties in training nondomestic canids for AV use and inability to collect semen routinely from most canids via electroejaculation.

In felids, the traditional sperm freezing technique (pelleting on dry ice) was derived from studies conducted in the late 1970's (PLATZ et al., 1978) with little modification until recently. While sperm pelleting allows recovery of viable sperm with adequate motility, acrosome damage can be severe (50-70%), compromising the sperm's ability to bind to the zona pellucida and penetrate salt-stored oocytes in sperm function assays (WOOD et al., 1993; HAY; GOODROWE, 1993). In nondomestic felids, acrosome damage from sperm pelleting also has been severe, with some apparent species differences (SWANSON et al., 1996a). This sperm pelleting method has been used routinely for the past 20 years with nondomestic cats and, despite causing pronounced acrosome damage, can provide adequate post-thaw viability for AI or IVF procedures. Recent refinements based on systematic research studies have included altering the initial cooling rates and dilution rate with cryoprotectants which substantially improves acrosomal and plasma membrane status, especially for samples with high percentages of pleiomorphic spermatozoa (PUKAZHENTHIA et al., 1999; 2002). Slow cooling combined with freezing samples in plastic straws in two steps over liquid nitrogen vapor have improved the percentage of intact acrosomes substantially (PUKAZHENTHI, personnal communication). In other carnivore species such as ferrets, sperm cryopreservation studies also have demonstrated pronounced acrosome damage with pelleting or straw freezing but sperm viability has been acceptable for AI procedures (HOWARD et al., 1991; 2003).

Cryopreservation of the female's gamete, the oocyte, is more problematic than sperm freezing because of the oocyte's large size, low surface-to-volume ratio, and the delicacy of cytoskeletal structures (RALL, 2001). Among carnivore species, cryopreservation of oocytes has been reported in only one species, the domestic cat (LUVONI; PELLIZZARI, 2000). Mature oocytes survived cryopreservation more readily than immature oocytes but cleavage and embryo development rates were low, and in vivo viability has not been demonstrated. In carnivore species, cryopreservation of embryos remains the primary method available to preserve the female's genetic contribution. Most basic embryo freezing research in carnivores has been conducted in the domestic cat, with limited extrapolation to nondomestic cat species. One advantage for domestic cat studies is that ovaries are readily obtainable from spay clinics and protocols have been established for in vitro maturation of oocytes and embryo culture (JOHNSTON et al., 1989; POPE et al., 1997). Techniques also are available for hormonal treatment of females and laparoscopic recovery of in vivo matured oocytes for IVF (GOODROWE et al., 1988). Several cryopreservation studies have been conducted with in vivo-generated cat embryos and IVF embryos produced using in vivo and in vitro matured oocytes (DRESSER et al., 1988; POPE ET AL., 1994; POPE et al., 1997; SWANSON et al., 1999). In general, domestic cat embryos present no special obstacles to cryopreservation, with protocols developed in other species routinely allowing high embryo survival (>50%) post-thaw. Embryo cryopreservation studies in nondomestic felids have focused primariliy on in vivo viability after ET (POPE et al., 2000; SWANSON, 2001). Interestingly, pronounced species differences were observed in a recent study in tigers (CRICHTON et al., 2003). Two freezing protocols used successfully to freeze domestic cat, ocelot and wildcat embryos were totally ineffective in tigers, with none of the frozen-thawed tiger embryos developing in culture. In contrast, embryo vitrification (ultra-rapid cooling to prevent ice crystal formation) permitted almost 50% of tiger embryos to survive after thawing.

A final method for germ plasm cryopreservation in carnivores involves freezing of ovarian tissue. Primoridal follicles have high resistance to cryoinjury, and might be suitable for GRBs provided that these follicles can be matured adequately post-thaw either in vitro or in vivo (RALL, 2001). Freezing of domestic cat ovarian follicles has been reported (JEWGENOW et al., 1998) with 10-20% of follicles surviving post-thaw. Complete maturation of primordial follicles in vitro to permit fertilization of oocytes has not been possible but transplantation of nonfrozen and frozen-thawed cat ovarian tissue to immunodeficient mice has allowed development of antral follicles (GOSDEN et al., 1994; BOSCH et al., 2002). Lastly, cryopreservation of somatic tissue cells may have value beyond serving as voucher specimens for molecular genetic studies. Recent advances in nuclear transplantation using adult fibroblasts (WILMUT et al., 1997) to produce viable offspring in sheep and other livestock species has generated interest in some conservationists in using this approach with wildlife species (RYDER; BENIRSCHKE, 1997). Among carnivore species, somatic cell nuclear transfer has been used to produce a single kitten in the domestic cat (SHIN et al., 2002) with unsuccessful attempts reported in the domestic dog (WESTHUSIN et al., 2001). Given the primitive nature and limited success of current cloning technology in domestic animals and livestock, attempted extrapolation to nondomestic carnivores at the present time should be considered premature and a waste of limited research resources. As cloning technology evolves, the potential for application to wildlife species also may change. For this reason, it would be beneficial to expand cryobanking of somatic cells such as fibroblasts from genetically-valuable individuals for inclusion in a GRB.

Developing assisted reproductive techniques for GRB application

GRBs can only be functional if effective protocols have been established to use the frozen resources, whether sperm, oocytes or embryos. A review of published reports reveals that AI or ET with frozen-thawed germ plasm has been used successfully to produce viable offspring in just a handful of carnivore species (Table 2). With the exception of AI in the domestic dog and AI and ET in the domestic cat, efficacy of this technology using frozen germ plasm has not been established based on consistent results in multiple research trials and therefore cannot be considered proven for routine usage in nondomestic carnivore species. Although lack of established protocols does not preclude developing GRBs for these carnivore species, it does illustrate the substantial amount of research that will be required, on a family, species and individual basis, to transform static GRBs into functional conservation tools. A review of progress in developing assisted reproductive techniques in felid species might be informative as to the nature and extent of research needed for each carnivore taxon that is to be included in a GRB (WILDT; ROTH, 1997).

To date, AI with fresh or thawed sperm has been used to produce offspring in ten felid species domestic: cat, leopard, puma, leopard cat, cheetah, tiger, clouded leopard, snow leopard, ocelot, tigrina (HOWARD et al., 1992A; DRESSER et al., 1982; MOORE et al., 1981; BARONE et al., 1994; WILDT et al., 1992; HOWARD et al., 1992B; DONOGHUE et al., 1993; HOWARD et al., 1996; ROTH et al., 1997; SWANSON et al., 1996b; MORAES et al., 1997), with frozen-thawed spermatozoa used successfully in three species: leopard cat, ocelot, cheetah (WILDT et al., 1992; SWANSON et al., 1996b; HOWARD et al., 1997). Improved success with AI in felids corresponded with determination of effective hormonal treatments for induced ovarian stimulation and development of a laparoscopic insemination technique that permitted spermatozoa to be deposited in the distal uterine horns near the oviducts (HOWARD et al,, 1992a; WILDT; ROTH, 1997; HOWARD, 1999). Similarly, laparoscopic AI with nonfrozen and frozen-thawed spermatozoa in ferrets has proven useful for producing offspring (HOWARD et al., 1991, 2003). Although cross-species applicability of laparoscopic AI has been demonstrated in nine cat species over the past ten years, pregnancy percentages have been low in most species and further refinement through studies of down-regulation of ovarian function prior to stimulation and/or alteration of hormonal stimulation regimens is being evaluated.

Systematic research studies with IVF in domestic and nondomestic cats over the past 15 years have investigated the basic reproductive biology of cats, including ovarian stimulation, oocyte retrieval, in vitro maturation, sperm capacitation, sperm-oocyte interaction, in vitro culture conditions and embryo development. These studies have culminated in the ability to produce IVF-derived embryos in at least 15 species: domestic cat, wildcat, leopard cat, fishing cat, caracal, clouded leopard, cheetah, tiger, jaguar, puma, ocelot, tigrina, Geoffroy's cat and jaguarundi (POPE, 2000; MORATO et al., 2000; SWANSON, 2001; SWANSON et al., 2002; unpublished data], using fresh or thawed sperm for insemination. However, successful transfer of IVF embryos has been successful in just five species: domestic cat, tiger, wildcat, caracal, ocelot (GOODROWE et al., 1988; DONOGHUE et al., 1991; POPE et al., 1989; POPE et al., 2001; SWANSON, 2001) with individuals from three species becoming pregnant after transfer of frozen-thawed IVF embryos (domestic cat, wildcat, ocelot) (POPE et al., 1994; POPE et al., 2000; SWANSON, 2001). Across most cat species, standard IVF methods now permit fairly routine generation of embryos but the efficiency of embryo transfer (based on embryo survival and pregnancy percentages) has been highly variable. Systematic research investigating normative reproductive traits associated with early pregnancy and comparative recipient synchronization protocols likely holds one of the keys to improving pregnancy success and embryo survival after transfer of frozen-thawed embryos (SWANSON, 2001).

The logistical challenges of GRBs

Creating an infrastructure for a GRB presents a variety of organizational and logistical challenges that must be addressed. Several recent publications have discussed these various issues in great detail for developing GRB action plans and will be summarized briefly here (WILDT et al., 1993; WILDT, 1997b; GRB ADVISORY GROUP, 1998). It is recommended that individuals considering developing a GRB should consult these earlier documents. Before initiating a carnivore GRB, criteria must be established to determine which species and individuals must be prioritized for inclusion. Depending on the scope of the program and resources available, preserving germ plasm and other biological samples from every individual of every species is not feasible or warranted in most cases. Factors to consider in selecting species for inclusion include the current degree of endangerment in the wild and the threats to future survival, presence of a captive breeding or in situ conservation programs, and existence of prerequisite knowledge of basic reproductive biology, cryobiology and assisted reproduction for the species. Information from the IUCN Red List may be used as a starting point to assess endangerment risk for a species, combined with more precise regional or local information on subspecies or populations, if available. The existence of a captive breeding program is advantageous in providing access to genetically-valuable individuals for cryobanking as well as supplying study subjects for basic and applied reproductive research to improve the utility of the GRB. As discussed in the next section, managed captive populations are essential for integrating reproductive sciences into conservation and making GRBs fully functional. Similarly, the existence of an organized in situ program is important for facilitating collection of biological samples from wild individuals but also for creating in situ-ex situ linkages through the GRB. Lastly, the extent of available scientific knowledge about the natural history and reproductive biology of a species, including information on cryobiology and assisted reproduction, will determine if inclusion of the species in the GRB will have any applied conservation benefit.

Once species have been selected, attention must be directed to organization of the GRB collection. First, each sample must be easily identifiable with clearly distinguishable labels on vials or straws within the liquid nitrogen tanks. If disease concerns exist, pre-screening of sample donors for exposure to infectious diseases and storage of frozen samples in sealed containers should be considered. Each sample in the GRB must be traceable within a central inventory database that contains, at a minimum, an ID number (either assigned by the GRB or based on other inventory systems such as ISIS or international studbooks), the type of sample, the institution where collected, the tank storage location, and the date of collection. Other information, such as various reproductive parameters, may be included in an expanded inventory database or kept cross-indexed in a separate database. Commercially-available computer software programs, such as word-processing applications or data spreadsheets, may be used for inventories or more specialized GRB management programs (such as programs based on ISIS software) may be employed. Liquid nitrogen tanks and/or freezers containing GRB samples must be maintained in a secure location with appropriate alarm systems for monitoring the function of storage systems. Preferably, GRB samples will be divided between primary and secondary storage sites to protect against catastrophic loss at any one location.

Incorporation of GRBs into genetic management programs

As static repositories of biological material, GRBs do have some value, primarily for preserving existing genetic variation in threatened populations as insurance against extinction. However, for GRBs to have a significant conservation impact, they must be actively used and integrated into genetic management programs (HOLT et al., 2003). At present, there is not a single GRB established for a wildlife species that is used on a routine basis to meet significant genetic management goals. This deficit is a by-product of the time scale required to develop GRBs and related technology needed for its application, as well as a reflection of the difficulties inherent to organizing and funding large-scale GRB programs. Among carnivore species, in only two, the black-footed ferret and cheetah have captive populations benefited genetically from use of assisted reproductive procedures with frozen-thawed germ plasm (Howard et al., 2003; Howard et al., 1997). In ferrets, laparoscopic AI with frozen-thawed spermatozoa has helped to equalize founder representation in the remnant captive population and produce additional offspring for reintroduction purposes. In this program, almost 100 black-footed ferret kits have been generated via AI, although most have been produced using non-frozen spermatozoa. In cheetahs, spermatozoa collected and frozen from wild males in Namibia were used to inseminate captive cheetahs in U.S zoos, producing three pregnancies and a single surviving offspring. This female cub was a partial founder to the U.S population since the sire, the sperm donor, was not represented genetically in U.S. zoos.

Of importance, both black-footed ferrets and cheetahs are managed within Species Survival Plans (SSPs) within accredited zoos of the American Zoo and Aquarium Association. SSPs can provide critical direction, support and structure for the development and application of GRBs. Within SSPs, population managers evaluate the genetic composition of the targeted population by using computer programs to analyze pedigree relationships derived from studbook data (Ballou & Foose, 1992). In combination with demographic information, these genetic assessments are used to determine the most valuable individuals within the population and suggest pairings for natural breeding. This prioritization of genetically valuable individuals for reproduction is the same information needed to build functional GRBs, to target males for sperm collection and females for AI or IVF. In addition, SSP coordinators are responsible for developing master plans to manage species over several years, a process that could benefit greatly from integrated GRBs and assisted reproduction. Lastly, SSP coordinators are charged with building in situ connections to assist in conserving their species in the wild, providing another avenue for developing and incorporating functional GRBs. Within the U.S., it would seem logical that GRBs be established and integrated within the SSPs on a species by species basis. In other regions, such as Europe, similar management programs (EEPs) are in place, but in many developing countries, no genetic management programs yet exist. Without genetic management programs to provide support and direction, the danger is that development and application of GRBs will be sporadic and inconsistent, producing 'one-time' successes from AI or ET using frozen germ plasm that have little or no conservation relevance.

A final concern is related to the scale of GRB development and integration. Attempts to create broad-based GRBs that cover multiple species and vast geographic regions are proportionally that much more difficult to fund, staff and manage than are smaller GRB programs and also are much more likely to fail (HOLT et al., 2003). Large programs are more realistic if the goal is to just cryopreserve as much biomaterial as possible from threatened populations (provided that proven cryopreservation protocols exist across species). But if the goal is to create a functional GRB, then a species-based approach is the most logical and feasible in most regions, given limitations in funding and other necessary resources.

The human factor in GRB development

There exists another formidable barrier to establishing GRBs as conservation tools - the human psyche - in particular, two human psychological traits. The first trait may be termed 'temporal myopia', the tendency of humans to focus short-sightedly on the near-term - the next day, the next week, the next year - and lack the long-term perspective to appreciate the accumulative impact of small ecological changes over time. Hence, addressing global warming, habitat destruction, and other incremental progressive changes are never a priority, and degradation of the environment consistently fails to register as a major concern in public opinion polls. The second defining trait has been called 'exemptionism', the belief that the governing laws of nature don't apply to humans because our intelligence and technology can overcome any problems that we may create. The 'technological fix' is part of our lexicon, and combined with temporal myopia, creates a deadly combination when trying to overcome inertia among the lay public in raising environmental consciousness and promoting substantive action.

Within the professional conservation community, most individuals do have a long-term ecological perspective but may see little conservation value in reproductive biotechnology, including GRBs; possibly a valid view at present but also notably short-sighted. GRBs currently play a negligible or minimal role in any conservation program but the current status should not be construed as being predicative of future utility. The challenge is finding a balance between the over-exuberance for technological solutions expressed by the public (and a few scientists) and the resistance and debasement of technology-based intervention evident among some in the conservation community. One solution is to ensure that GRBs have defined short-term goals and immediate quantifiable benefits when incorporated into conservation programs. Meaningful application of GRBs in shorter time frames will help to capture the public's myopic attention and garner necessary institutional, financial and societal support while demonstrating to the conservation community that these approaches do have real merit. The potential conservation value of GRBs still exists but continually selling (or over-selling in some cases, as with cloning) that promise with no demonstrable benefits eventually will be perceived as subterfuge by both the public and other conservationists. The challenges inherent to establishing a functional GRB for carnivores should not be compounded by a loss of confidence in our ability to achieve that goal.

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Table 1 – Successful artificial insemination with frozen-thawed spermatozoa and embryo transfer of frozen-thawed embryos for production of viable offspring in carnivore species.

Taxon	Species	AI	ET	Reference(s)
Canidae	Domestic dog	Yes	No	Seager, 1969
	Grey wolf	Yes	No	Seager et al., 1975
	Blue fox	Yes	No	Fougner et al., 1973
Felidae	Domestic cat	Yes	Yes	Platz et al., 1978 Dresser et al., 1988
	Cheetah	Yes	No	Howard et al., 1997
	Ocelot	Yes	Yes	Swanson et al., 1996 Swanson, 2001
	Leopard cat	Yes	No	Wildt et al., 1992
	African wildcat	No	Yes	Pope et al., 2000
Mustelidae	Domestic ferret	Yes	No	Howard et al., 1991
	Siberian polecat	Yes	No	Howard et al., 1996
	Black-footed ferret	Yes	No	Howard et al., 1996
Ursidae	Giant panda	Yes	No	Hu and Wei, 1990

Table 2 – Potential benefits of a functional genome resource bank.

1) Resolve behavioral incompatibilities or physical defects that prevent natural breeding
   *eg., AI with frozen-thawed spermatozoa in incompatibile, aggressive clouded leopards
2) Transport frozen germ plasm as alternative to moving living animals among captive populations
   eg., international transport of frozen Brazilian ocelot embryos for transfer to generic ocelots in U.S. zoos
3) Introduce new founder genes into captive populations using frozen germ plasm collected from wild individuals
   eg., semen collection and freezing in wild Namibian cheetahs with subsequent use for AI in captive populations
4) Extend generation intervals to reduce genetic drift within small populations
   eg., AI using frozen-thawed spermatozoa from black-footed ferrets to produce offspring beyond their normal 2-5 year generation interval
5) Increase number of exhibit/holding spaces for living individuals and species in captive populations
   eg., maintain 30-50% of genetic variation for tigers as frozen gametes and/or embryos
6) Overcome infectious diseases in genetically valuable individuals
   eg., in vitro fertilization using spermatozoa from herpes-infected Pallas' cat founders with subsequent embryo cryopreservation and transfer
7) Assess exposure to infectious agents or evaluate genetic heterozygosity in captive and wild populations
   eg., serological survey (immunoglobulins, antigens, DNA) for infectious viral diseases among cat populations in zoos
8) Preserve extant genetic variation as insurance against catastrophic loss
   eg., establish repositories of frozen germ plasm from threatened wild populations of Texas ocelots or Brazilian jaguars

* Examples represent applications of GRBs that have been used successfully, are currently in progress or have been recently proposed.

Capítulo 16
Planos de manejo da fauna em cativeiro

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Introdução

A explosão demográfica humana e o progresso tecnológico vêm danificando os ecossistemas. Atualmente muitos deles se encontram seriamente ameaçados, outros infelizmente já extintos e, conseqüentemente, com eles a fauna. Diante dessa realidade, os animais cativos são um grande patrimônio genético, de suma importância para a conservação das espécies. O tratamento correto desses animais, seja na manutenção seja no manejo demográfico e genético, é prioritário para manter populações viáveis. Para que isso aconteça, além das leis gerais e específicas para cada tipo de instituição, são necessários planos de manejos para as espécies, com seus programas coordenados, principalmente, para as espécies que estão ameaçadas, em risco eminente de extinção ou ainda para aquelas consideradas insuficientemente conhecidas. Os planos de manejo podem, assim, assegurar que todas as instituições tenham os mesmos procedimentos de manejo para cada espécie, garantindo a qualidade de vida dos animais. Para tanto, é preciso que os dados sobre esses animais sejam confiáveis e atualizados, sendo os registros genealógicos importantíssimos para subsidiar os pareamentos e consequentemente a movimentação do plantel em cativeiro.

Registro genealógico (Studbook)

Um plano de manejo, para uma determinada espécie, tem como base o Studbook, banco de dados que contém todas as informações dos antecedentes e descendentes de cada animal. É importantíssimo conhecer a população que será manejada, a procedência dos animais, a idade, e se estão ou não em idade reprodutiva. Com esses dados é possível analisar as características de cada população, podendo ainda detectar os possíveis problemas em relação ao coeficiente de consangüinidade, mortalidade dos filhotes, e se os problemas são da população ou de um único indivíduo. Via de regra, o Studbook Internacional engloba os animais cativos no mundo todo para uma determinada espécie e, em alguns casos, também existem os studbooks regionais, que englobam a população de uma espécie de um determinado país ou região . Ambos utilizam os mesmos padrões para o lançamento dos dados e o Studbook regional se utiliza do número do Studbook Internacional do animal para identificá-lo.

Os dados encontrados no Studbook geralmente são:

1 – Número de identificação

2 – Histórico dos animais: desde os primeiros dados lançados até a atualidade, com o número de identificação, data de nascimento, filiação (número de identificação dos pais), local onde se encontra, data de óbito e destino da carcaça;

Programas para lançar os dados do Studbook: Rede Global de Informação para a Conservação de Espécies

O ISIS (International Species Information System) é um sistema de informação por computador desenvolvido para controle das espécies de animais selvagens em cativeiro no mundo. Esse grande banco de dados possui informações sobre 1,65 milhões de animais de zoológicos em mais de 550 instituições, de 73 países em seis continentes (www.isis.org). Na América do Norte e Austrália já é amplamente utilizado, seguido da Europa, onde está se expandindo. Ainda está sendo iniciado na América do Sul e África.

A maioria das instituições afiliadas ao ISIS são parques, zoológicos, aquários e centros de pesquisa (www.isis.org).

O ISIS inclui programas computacionais de pesquisa de gerenciamento de plantéis (ARKS, SPARKS, MedARKS, REGASP). Todas estas ferramentas necessitam de informações biológicas básicas: idade, sexo, procedência, marcação, tipos de entrada e saída, etc.

Por meio do programa ARKS (Animal Records Keeping System), os zoológicos gerenciam seus animais individualmente, por espécime e por instituição. Com esses dados o ISIS analisa os animais e pode gerar diversos tipos de relatórios sobre a população de cada instituição. Estas informações são disponibilizadas em forma impressa apenas para os zoológicos membros, e a partir de 1996 se tornaram disponíveis para qualquer pessoa ou instituição interessada, por meio do utilitário web http://www.worldzoo.org. O programa de pesquisa MedARKS possui a função de gerenciar os registros médicos dos indivíduos de uma instituição. Já o SPARKS objetiva gerenciar as informações sobre as populações de uma espécie em cativeiro nas diversas instituições. O SPARKS é fundamental para trabalhos de conservação e sobrevivência das espécies em risco de extinção.

É cada vez mais importante e necessária a utilização desses programas para os trabalhos de conservação em cativeiro, pois a cada dia continuam chegando aos zoológicos brasileiros um número absurdo de animais oriundos da natureza (www.aza.org). Essa rede global de informações interfere diretamente nas decisões que os técnicos das instituições precisam tomar, como po exemplo na formação de novos casais para a reprodução de uma determinada espécie. Outro fator, tão importante quanto a chegada das espécies ao cativeiro, é a destruição de seus ambientes naturais por ações humanas. Esses animais "avaliados" pelo programa serão utilizados, no futuro, como portadores de gens naturais da espécie.

Genética das populações cativas

Devido ao declínio populacional dos carnívoros, o desenvolvimento de metodologias adequadas de manejo genético e reprodutivo das populações em cativeiro torna-se essencial, não só pela manutenção dos espécimes, mas, sobretudo, pela possibilidade de preservação de alelos do estoque genético original das populações naturais. O ponto inicial de um programa efetivo de manutenção de uma população viável ex situ deve ser a definição exata do que se pretende conservar (espécie, subespécie, população) (DUARTE et al., 2001).

Uma das premissas para que os animais cativos possam ser utilizados nos programas de conservação, por meio de ações de manejo (por exemplo, translocação e reintrodução) é que representem o material genético das populações que outrora ocupavam as áreas para onde estão retornando. Entretanto, a manutenção da estrutura genética original em uma população cativa é quase impossível de ser atingida, diante dos inúmeros fatores que afetam, decisivamente, as freqüências alélicas, tornando-as muitas vezes bastante distintas da população original.

Populações de espécies ameaçadas em cativeiro geralmente são fundadas por um pequeno número de indivíduos, o que torna a perda de variabilidade genética quase inevitável. Uma forma de minimizar a ocorrência desta perda consiste num manejo baseado em aumento do número efetivo da população, equalização da representatividade da população fundadora, escolha de fundadores saudáveis e cruzamento selecionado por meio da análise do pedigree (TEMPLETON; READ, 1984; FRANKHAM, 1995).

Desde a captura até a chegada dos fundadores de uma população ao zoológico ou criadouro existe um caminho tortuoso, estimando-se que 90% dos animais venham a óbito. Essa pressão seletiva é implacável, pois como se sabe, a seleção é uma das principais causas de perda de diversidade genética nas populações. Portanto, podemos imaginar que mesmo os animais fundadores, provavelmente, não retenham as freqüências alélicas das populações originais. Esse é o primeiro efeito do cativeiro na população, impedindo que ela represente a bagagem genética das populações naturais. Esse é um fator incontrolável e teremos sempre que aceitar que os animais fundadores sempre representarão a base genética natural. Mesmo sabendo dessas restrições, deveremos partir dessa premissa para que o trabalho possa ser iniciado.

Um exemplo muito claro disso foi o que ocorreu com o programa de conservação ex situ do cervo-do-pantanal (*Blastocerus dichotomus*). Esse programa foi iniciado a partir de um resgate de animais que seriam afetados pela Usina Hidrelétrica Sérgio Motta (Porto Primavera), portanto seria uma possibilidade de dar início a um programa com uma amostra aleatória dos animais da população. Foi organizada uma estrutura para a captura e quarentena dos animais. O sistema de captura, transporte e manejo dos animais na quarentena foi praticamente o mesmo. Houve uma mortalidade média de 35% dos animais desde a sua captura até o destino à instituição mantenedora dos animais em cativeiro (DUARTE, 2001a; DUARTE, 2001b). Apesar da homogeneidade de todos os procedimentos, houve diferenças significativas nas respostas ao ambiente cativo, caracterizando uma pressão seletiva importante. Podemos imaginar que aqueles animais mais reativos ao manejo em cativeiro possam ter morrido e alelos ligados a esta reatividade possam ter sido perdidos. Eles nunca mais serão recuperados e podem influenciar na sobrevivência das futuras gerações após o retorno à natureza.

Apesar de não termos controle sobre a seleção ocorrida antes da chegada às instituições signatárias dos programas de conservação ex situ, após a entrada desses animais no programa isso se inverte. Temos a partir daí condições de evitar a seleção que é o mais importante fator de perda de variabilidade, pois é direcional (FRANKHAM et al., 1986; ARNOLD, 1995). A deriva genética, também importante como fator de perda de diversidade, não é tão efetiva, pois afeta principalmente alelos raros e se torna importante somente após muitas gerações com populações pequenas (BALLOU; FOOSE, 1997).

A única forma de evitar a seleção no plantel cativo é dar a todos os animais a mesma capacidade de se reproduzir. Essa técnica, denominada contribuição igualitária de fundadores, permite que alguns alelos, mesmo que deletérios, permaneçam na população. É importante entendermos que um alelo deletério hoje pode ser o responsável pela adaptação da espécie a uma nova situação. Sendo assim, o responsável pelo livro de registro genealógico e os técnicos em geral devem entender que todos os animais fundadores têm o mesmo valor para o programa de conservação, independentemente do aspecto fenotípico do animal. Isso deve, conseqüentemente, refletir nos descendentes deles.

Muitas pessoas crêem que a biologia molecular ou os testes de DNA são as chaves para a definição de cruzamentos em um programa, mas isso não é verdadeiro. Os marcadores moleculares são utilizados como fontes de informação acerca do animal, mas temos que entender que eles são geralmente 4, 5 ou, no máximo, 10 marcadores, num universo de milhares de genes. Não podemos, por exemplo, indicar que um animal seja padreador prioritário de uma geração só porque ele tem um alelo ou haplótipo raro. O restante do genoma do animal não está sendo analisado e não se sabe se ele tem todos os milhares de alelos restantes como alelos comuns ou freqüentes na população. Por outro lado, a biologia molecular pode auxiliar muito nos programas de cativeiro, definindo, por exemplo, a paternidade em casos em que há dúvidas ou ainda auxiliando na definição de acasalamentos menos aparentados quando não se conhece a origem dos animais.

Como pode ser visto, a contribuição igualitária de fundadores deve ser a base do processo de gerenciamento dos acasalamentos. Para que isso seja possível, é imprescindível que se conheça a genealogia. Sem ela, fica impossível implantar esta metodologia de trabalho.

O cálculo da contribuição dos fundadores é simples. Uma vez que a mãe e o pai passam aos seus filhos 50% de material genético de cada um, é possível calcular quanto de cada fundador permaneceu na população, mesmo que estejamos falando da décima ou vigésima geração (Figura 1).

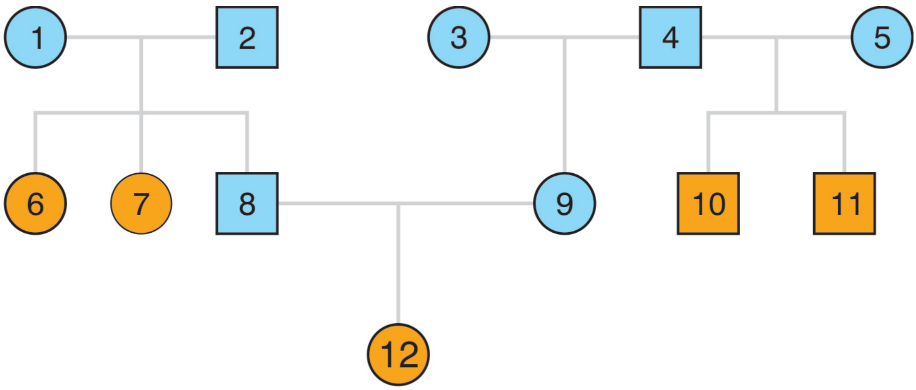

Figura 1 – Árvore genealógica de uma população hipotética, em que: os círculos representam as fêmeas e os quadrados, os machos; cor roxa indica animais atualmente mortos e cor laranja indica animais atualmente vivos.

Pela Figura 1 podemos identificar os animais fundadores, que são aqueles que vieram da natureza e tiveram descendentes, neste caso os animais 1, 2, 3, 4 e 5. Os que adentram no plantel e morrem sem ter descendentes não são considerados fundadores.

O cálculo da contribuição dos fundadores leva em consideração somente os animais atualmente vivos, ou seja, os animais 6, 7, 10, 11 e 12. Os animais 6 e 7 são filhos dos animais 1 e 2, portanto têm 50% de cada um desses fundadores. Dessa forma teríamos: 6 (50% de 1 e 50% de 2), 7 (50% de 1 e 50% de 2), 10 (50% de 4 e 50% de 5), 11 (50% de 4 e 50% de 5) e 12 (25% de 1, 25% de 2, 25% de 3 e 25% de 4). A contribuição dos fundadores se dá pela somatória das percentagens da contribuição desse fundador no plantel dividido pelo número de animais do plantel. Assim, a contribuição do animal 1 na população atual seria 50% (vinda do animal 6) + 50% (vinda do animal 7) + 25% (vinda do animal 12), dividida por 5 que é o número total de animais do plantel que estão vivos, gerando 25%, ou seja, 25% da população possui material genético do animal 1. Teríamos então: 25% de 1, 25% de 2, 5% de 3, 25% de 4 e 20% de 5.

Logicamente essa conta se complica quanto mais gerações tivermos para analisar.

Dessa maneira, o que temos que fazer logo que assumimos o manejo de uma população é tentar corrigir os desvios da representatividade de cada fundador. Nem sempre isso é possível, como no exemplo real da população brasileira do cachorro-vinagre (*Speothos venaticus*) (Figura 2).

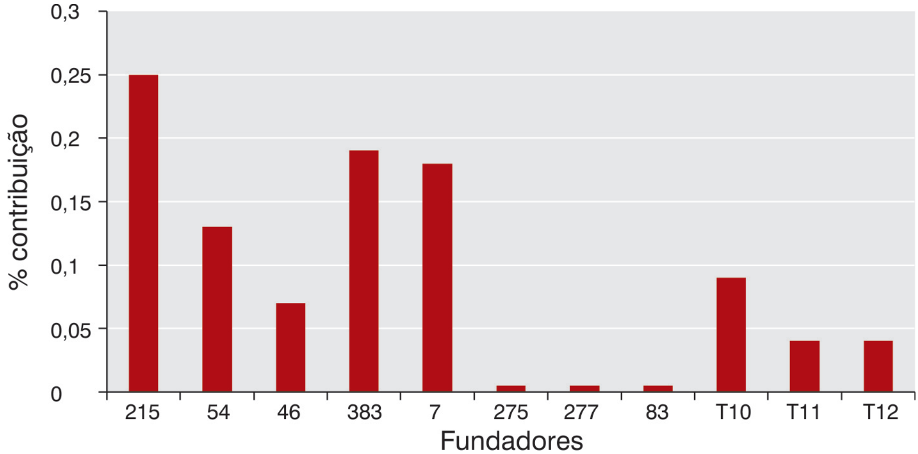

Figura 2. Gráfico demonstrando o percentual de contribuição dos 11 fundadores da população cativa de cachorros-vinagre (*Speothos venaticus*) do Brasil.

Pela Figura 2 podemos observar que os fundadores 275, 277 e 83 estão pouquísimo representados na população atual e deveria ser tentada uma mudança no panorama. Entretanto, como pode ser observado na Tabela 1, somente um animal tem representação dessa linhagem no plantel, tornando impossível a retomada da representação desses fundadores. Nesse caso, recomenda-se assumir a perda desses três fundadores e trabalhar com os restantes, que estão mais equilibrados.

Tabela 1 – Percentual de contribuição dos animais fundadores, em amarelo, nos animais atuais do plantel, em vermelho.

Animais atuais	Fundadores										
	215	054	046	383	007	275	277	083	T10	T11	T12
770	0,25	0,12	0,06	0,28	0,28	0	0	0	0	0	0
907, 906	0,37	0,19	0,09	0,17	0,017	0	0	0	0	0	0
832	0	0,03	0,23	0,18	0,18	0,13	0,13	0,13	0	0	0
1087, 1088, 2470, 2471, 2589, 2590, 2591, 1117, 1118, 1119, 1179, 1180, 1181, 1185, 1184, ISA, Dora	0,31	0,16	0,08	0,23	0,23	0	0	0	0	0	0
T11	0	0	0	0	0	0	0	0	1	0	0
T10	0	0	0	0	0	0	0	0	0	1	0
T12	0	0	0	0	0	0	0	0	0	0	1
M1, F1, F2	0,13	0,06	0,03	0,14	0,14	0	0	0	0,5	0	0

Outro fator implacável sobre a população cativa é a deriva genética, que significa a perda de alelos por força do acaso. Logicamente, isso só ocorre de maneira importante nas populações pequenas. Populações com 500 indivíduos em fase reprodutiva mantêm o seu potencial adaptativo, com pequena perda de alelos (FRANKLIN, 1980). Isso significa que deveríamos ter populações cativas nessa ordem de grandeza, se quisermos manter a diversidade genética com o passar das gerações. Entretanto, isso seria utópico, uma vez que as vagas disponíveis em cativeiro são limitadas e, praticamente, em nenhuma espécie teríamos condições de manter uma população tão grande. Dessa maneira, a regra para a conservação ex situ de populações é que ela seja a maior possível, porém não desprezaremos uma população de uma espécie ameaçada só porque está limitada a 20 ou 30 animais em cativeiro. Muita coisa pode ser feita com ela, especialmente com as possibilidades advindas das técnicas de reprodução assistida.

Deve ser evidenciado o que chamamos de “efeito fundador”, ou seja, o efeito do número e qualidade dos animais que iniciam uma população. Se uma população emerge de dois ou três fundadores, podemos imaginar que em pouquíssimas gerações teremos uma consangüinidade muito alta, conseqüentemente grande homozigose e perda de diversidade genética. Sabe-se que a heterozigose em populações cativas pode ser maximizada se constituída por 20 a 30 fundadores não aparentados, desde que estes representem grande parte dos alelos da população (FRANKHAM, 1995).

Um decréscimo na diversidade genética pode ocasionar baixo desempenho reprodutivo, menor resistência contra doenças infecciosas e parasitárias e menor flexibilidade adaptativa a um determinado ambiente (LACY, 1997).

A consangüinidade incrementa substancialmente a possibilidade de expressão de alelos deletérios, que podem causar uma maior taxa de mortalidade infantil. A endogamia deve ser evitada, através do controle do pedigree e troca de indivíduos e/ou germoplasma entre instituições. Cruzamentos consangüíneos podem eventualmente ser recomendados em um Sistema de Contribuição Igualitária de Fundadores, que recomendará a restrição da reprodução dos fundadores pouco representados e o cruzamento preferencial de seus descendentes.

Em suma, devemos ter em mente que tanto a diversidade alélica quanto a heterozigozidade são desejáveis em populações cativas. A diversidade alélica é importante para a habilidade de a população adaptar-se no longo prazo, enquanto que a heterozigozidade é importante para adaptações imediatas (ALLENDORF, 1986).

Todas essas preocupações para manter a população estável em cativeiro, com a variabilidade genética e a capacidade reprodutiva inalteradas em relação à população original, de nada servirão se não houver uma união simultânea de esforços no sentido de preservar os ecossistemas aos quais essas espécies pertencem, já que dentro de algumas décadas poderemos ter excelentes populações mantidas em cativeiro, porém, sem a menor possibilidade de encontrá-las na natureza, o que tornaria todos os esforços despendidos em vão (DUARTE et al., 2001).

Plano de manejo

Tem por objetivo principal recomendar como manter corretamente a espécie em cativeiro. A partir do levantamento e caracterização da população por meio da análise do studbook, é elaborado um programa para manejar essa população, que envolve a manutenção dos indivíduos em cativeiro, programas de reprodução , movimentação da população e estímulo à pesquisa científica para melhor conhecimento da espécie. Este programa pode ser regional ou mundial.

As recomendações tendem a ser amplas e suprir todos os campos para a manutenção da população. Em alguns casos, como no Brasil, as recomendações do plano de manejo são publicadas em um documento denominado protocolo de manejo e distribuídas às instituições, com revisões realizadas geralmente a cada três anos.

O coordenador do plano de manejo tem como função assegurar que as recomendações do protocolo de manejo sejam seguidas pelas instituições detentoras dos espécimes, articular e fomentar a promoção das pesquisas, assim como manter intercâmbio com os pesquisadores de campo que desenvolvem trabalhos com a população in situ. Não é sempre que há um coordenador para um determinado plano, porém aqueles que o possuem fluem melhor, facilitando o entrosamento entre as instituições envolvidas e as ações propostas.

O Protocolo

1. Biologia do animal

Contém informações gerais sobre a biologia do animal in situ e ex situ, como anatomia e fisiologia, habitat, dados comportamentais, alimentação, reprodução, estimativa de tempo de vida, etc.

2. Alimentação

Recomendações para uma dieta básica, precauções e formas de oferecimento do alimento.

3. Estrutura social

Recomendações quanto à estrutura social da espécie, se social ou solitária, e como deve ser mantida. Dados sobre conflitos entre os indivíduos, introdução de outro indivíduo, etc.

4. Reprodução

Recomendações para o acasalamento: maturidade sexual, períodos de acasalamento, gestação, nascimento e cuidados com os filhotes.

5. Estrutura física

Além de seguir a legislação vigente em cada país, tanto as recomendações dos protocolos de manejo internacionais quanto os protocolos de manejo regionais, muitas vezes, incluem recomendações para melhorar a estrutura física (recinto) onde é mantido o animal, de acordo com as experiências acumuladas pelos técnicos das instituições que mantém os espécimes. Contém recomendações sobre tipos de barreiras que podem ser utilizadas, substratos e tamanho mínimo do recinto. Ambientações e adequações dos recintos para o melhor manejo, como tipo de portas, necessidade de cambiamentos e áreas de segurança.

6. Aspectos veterinários

Recomendações sobre tipos de exames, forma de coleta de material, vacinação e contenção química, suplementações nutricionais, etc.

Identificação

Orienta formas de marcação do animal para identificação

Desenvolvimento de um plano de manejo

1. População a ser manejada

Determinação da população que será trabalhada no plano de manejo. Caracterizá-la genética e demograficamente, por meio do histórico dos animais, sua genealogia, os fundadores identificados, a correta identificação dos indivíduos e onde eles estão, a idade média dessa população e os coeficientes de consangüinidade.

2. Participantes

Quem está envolvido com o plano de manejo? Geralmente as instituições detentoras dos animais que fazem parte da população a ser manejada e que concordaram em seguir as recomendações propostas. É importante que em cada instituição exista um representante do plano de manejo, salvaguardando que tudo o que for recomendado e tudo o que acontecer a cada animal seja reportado imediatamente ao coordenador e que todos os questionários e documentos sobre a espécie sejam respondidos. Muitos planos de manejo possuem consultores, que são especialistas de várias áreas, para auxiliar na análise de dados e para dar suporte às propostas de ações.

3. Objetivos

Detalhar os objetivos do plano de manejo é essencial para nortear as ações.

4. Recomendações

Periodicamente os dados genéticos e demográficos da população são analisados e a partir dos resultados são emitidas as recomendações para o manejo de cada espécime, como pareamento, separação de casais e se a população deve ou não continuar se reproduzindo naquele período.

5. Pesquisa

No decorrer das ações do plano de manejo é que se detecta em quais áreas de conhecimento sobre a população em questão é necessário o desenvolvimento de pesquisas, que auxiliarão na manutenção da população e conservação da espécie.

Conservar uma espécie que não conhecemos é um desafio árduo e a pesquisa vem ao encontro dessa questão, tentando solucionar alguns problemas e embasar ações de manejo, para que possamos obter o resultado necessário no sentido de reproduzir a espécie em cativeiro.

Os zoológicos sul-americanos geralmente têm deficiência de pessoal técnico, impossibilitando o desvio de suas ações para o desenvolvimento de pesquisas. Por outro lado, temos a universidade, que dispõe de enorme "mão-de-obra científica", por meio de seus docentes, alunos de graduação e pós-graduação. O material de estudo, tão almejado pelos pesquisadores, está disponível nos zoológicos. Assim sendo, fica claro o potencial benefício gerado pela associação dessas duas forças nos programas de reprodução em cativeiro.

Muitas vezes, os interesses entre esses dois segmentos se tornam divergentes, ou talvez não totalmente convergentes. O técnico do zoológico tende sempre a pensar no bem-estar dos indivíduos mantidos por ele, enquanto o pesquisador tende a dar importância para a informação científica, mesmo que para isso haja necessidade de submeter o animal a uma situação estressante ou de risco. Em geral, o pesquisador está pensando na conservação da espécie no sentido mais amplo e o técnico do zoológico tende a valorizar os "indivíduos" que possui dentro do contexto da conservação. O que é importante é que os dois estão convergindo no básico, ou seja, estão buscando a conservação. Dessa maneira, deve-se encontrar um meio termo entre esses dois segmentos; não são aceitas pesquisas muito invasivas, mas também não será aceito que ela não seja feita.

Há reclamações constantes desses dois segmentos. Os pesquisadores reclamam, em geral, sobre a falta de espaço fornecido pelos zoológicos, com suas exigências burocráticas e éticas. Já os técnicos reclamam dos pesquisadores, por não retornarem as informações coletadas durante os trabalhos realizados nas instituições.

Para que essas diferenças sejam diminuídas, os dois segmentos devem entender alguns pontos básicos. O pesquisador deve considerar que o técnico do zoológico possui uma relação afetiva com os animais do plantel e não aceita que alguns procedimentos que causem problemas a eles possam ser realizados. É o próprio emprego do técnico que está em jogo, já que os animais do plantel fazem parte do patrimônio da instituição. Talvez para o pesquisador aquele indivíduo tenha pouca ou nenhuma importância para a conservação da espécie, mas o técnico vê o animal como importante para a instituição.

Por outro lado, o técnico deve entender que pesquisas demoram muito a ter resultados concretos. Muitas vezes, o material colhido em um zoológico pode permanecer em estudo por anos. Além do resultado obtido na prática, o pesquisador deve publicá-lo e isso pode demorar muito (em geral dois anos após a submissão). Antes de publicar, o pesquisador tem receio de divulgar os resultados, vislumbrando sempre a possibilidade de plágio de seu estudo.

A essência está no técnico do zoológico que deve fornecer padrões mínimos de pesquisa e regras claras para o desenvolvimento dos estudos, tendo sempre em mente que a atração do pesquisador sempre será uma boa tática. Do pesquisador espera-se bom senso nas propostas de pesquisa e da criação de um canal de comunicação com o zoológico, passando-lhe os resultados de suas pesquisas assim que concluí-las. Uma cópia do relatório, geralmente enviado às entidades financiadoras, já deve ser o suficiente, sem trabalho a mais.

Aparando as arestas existentes para a convivência entre pesquisadores e técnicos de zoológicos, essa união tem potencial para ser a grande força motriz necessária para a implantação dos programas de conservação ex situ, tornando-os peça importante para a conservação in situ das espécies ameaçadas.

Um pouco de história

Os planos de manejo para espécies ameaçadas de extinção tiveram início no Brasil ao final da década de 1980, após a administração de um curso pioneiro, para os profissionais que atuavam no manejo de fauna, por meio da Sociedade de Zoológicos do Brasil, da qual participaram também membros de universidades e ONGs.

No ano de 1989, como resultado do Curso Manejo Integrado: Cativeiro e Natureza, foram criados os planos de manejo para o lobo-guará (*Chrysocyon brachyurus*), ararajuba (*Guaruba guarouba*) e jacaré-de-papo-amarelo (*Caiman latirostris*). Porém, efetivamente, o primeiro plano de manejo em cativeiro e, posteriormente, em natureza para um mamífero carnívoro foi o do lobo-guará.

O segundo mamífero carnívoro alvo de um plano de manejo foi a jaguatirica *Leopardus pardalis* (1990), seguido pelo cachorro-do-mato-vinagre *Speothos venaticus* (1990).

Durante a década de 1990 os planos de manejo da jaguatirica, lobo-guará e cachorro-do-mato-vinagre foram agrupados em seus respectivos táxons e transformaram-se em Plano de Manejo para Pequenos Felinos e Grupo de Trabalho de Canídeos. Esses grupos foram oficializados e passaram a atuar nos zoológicos e criadouros amparados legalmente pelo Ibama até 2003, quando foram dissolvidos e foram criados os comitês de manejo.

Antes mesmo da dissolução do Grupo de Trabalho de Canídeos, em 2000, o cachorro-do-mato-vinagre passou a receber atenção especial voltando a ter um plano de manejo específico para a espécie.

Em 1998 foi realizado o Encontro sobre a Genealogia de Ariranhas (*Pteronura brasiliensis*) em Cativeiro no Brasil onde foram discutidas as primeiras ações para o manejo conservacionista da espécie em cativeiro, mas efetivamente não foi implantado um plano de manejo.

Como resultado dos trabalhos realizados pelos planos de manejo para espécies ameaçadas de extinção, diversas publicações que nortearam a manutenção das espécies em cativeiro foram produzidas, assim como reuniões científicas que auxiliaram na revisão e divulgação de protocolos de manejo, melhorando sensivelmente a qualidade de vida dos animais mantidos em cativeiro.

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Capítulo 17
Factors affecting the reproductive success of jaguars

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Introduction

The jaguar is the largest felid in the Americas, and the only living representative of the genus Panthera in the New World (NOWELL; JACKSON, 1996). The earliest pre-historic jaguar fossils in the Americas are ~850,000 years old, and have been recovered as far north as Washington state (SEYMOUR, 1989). The modern jaguar once ranged from mid-North America through Mesoamerica, and was widely distributed throughout South America. Most historic range has been lost in the last century due to rapid agricultural expansion and concomitant loss in native habitat (QUIGLEY; CRWSHAW, 1992). By 1989, Central and South American jaguar populations occupied only about one-third, and two-thirds of their historic range, respectively (SWANK, TEER, 1987). Continuous human persecution has reduced jaguar numbers within each of these regions. Perhaps 10,000 individuals remain (NOWELL; JACKSON, 1996) of this species that is an icon of strength and power throughout American cultures. The jaguar now is recognized as 'endangered' (USFWS) and is listed on Appendix I of Cites.

The North American captive population largely is of unknown origin or from genetically similar stock. Thus, there is a breeding moratorium for genetically undefined jaguars (the majority of the North American population) to avoid producing genotypically questionable offspring or cause inbreeding depression, known to compromise health and reproduction in felids (WILDT et al., 1986, 1987; ROELKE et al., 1993). Conversely, many captive jaguars in Latin America are wild-caught, but more than 90% of the Brazilian ex situ stock have untraceable lineages (MORATO; GASPARINI, 1994). The precarious nature of wild populations increases the importance of developing a self-sustaining captive population of jaguars to act as: 1) a reservoir for genetic diversity while avoiding more animal removals from nature; 2) a research resource for generating more species knowledge; 3) insurance against future catastrophes affecting wild populations; and 4) an educational resource for the public.

There is little information on reproductive status of jaguar females in Latin American facilities, but there is evidence that many captive males have poor ejaculate quality. Swanson et al. (1995) surveyed reproductive traits of indigenous felid species living in zoos in Mexico, Guatemala, Nicaragua, Costa Rica, Panama, Trinidad, Venezuela, Brazil, Paraguay, Bolivia, Uruguay and Argentina. During this team's extensive travels, they assessed 21 jaguars, the majority of which produced ejaculate traits (i.e., sperm concentration, motility and morphologies) inferior to counterparts living in North American zoos (SWANSON et al., 1995). Poor nutrition was suspected to be a contributing culprit, but no conclusive data were generated. More recently, Morato et al.(2001) found that captive jaguars in Brazil had reduced sperm quality compared to wild conspecifics. They speculated that the differences were unrelated to seasonal or weather factors (MORATO et al., 1999; MORATO, 2001), but likely associated with poor health and nutrition in the ex situ population (MORATO et al., 2001). Thus, the reason for this poor physiological reproduction remains unknown.

Many infectious, parasitic, and non-infectious diseases have been documented in jaguars, and are known to cause high morbidity and/or mortality. Additionally, many of these diseases have direct and/or indirect effects on the reproductive success of these felids. Prevention of disease should be the primary objective of veterinarians caring for these captive animals to ensure a healthy, reproductive population. It is also imperative that we learn the health and reproductive status, and how the two are correlated, of different populations of free-ranging jaguars. This will allow us to determine disease prevention strategies and the long-term conservation needs for these populations.

A priority should be to develop a scholarly database of knowledge on jaguar biology with an aim to identify factors limiting reproductive success ex situ and then, whenever possible, correcting through improved management. To achieve this ambitious goal, there is a need to identify key factors known to influence reproductive success. The reproductive health of captive jaguars, Panthera onca, is dependent on genetic make-up, sound husbandry practices, good veterinary care and proper nutrition. The underlying issues of genetic diversity, stress, health and nutrition that we will discuss here are universally germane to ex situ and in situ populations.

Genetic diversity

The effects of inbreeding depression and genetic management of small populations have been widely discussed when considering managing captive and wild populations and will be addressed elsewhere in this volume (cross-reference). It is known that inbreeding depression influences reproductive fitness in a variety of ways across a diverse range of species. For example, it has been established that increased inbreeding depression is closely correlated with decreased pupa's per generation in Drosphila flies, an increased genetic load in genes controlling oocyte sterility and zygote survival in butterflies (van OOSTERHOUT et al., 2000), decreased egg production in white leghorn chickens (FLOCK et al., 1991), decreased testicular sperm concentrations the oldfield mouse (MARGULIS; WALSH, 2002), decreased sperm quality in cuvier's gazelle (ROLDAN et al. 1998), reduced female fertility in Hereford cows (MacNEIL et al., 1989), increased juvenile mortality in ungulates (RALLS et al., 1979) and small mammals (RALLS; BALLOU 1982) and reduced male fertility in both clouded leopards and lions (WILDT et al., 1986; WILDT et al., 1987). Most of these experiments have been performed on captive populations, and there is some suggestion that the effects of inbreeding may be exacerbated in wild populations (JIMENEZ et al., 1994), leaving small populations of wild animals highly vulnerable. While there is significant gene flow within the jaguar as a species (EIZIRIK et al., 1998; JOHNSON et al., 1998; JOHNSON et al., 1999; EIZIRIK et al.,., 2001), the fragmented nature of present-day jaguar populations creates the potential for inbreeding depression and subsequent reduced fertility. The effect of inbreeding depression on reproductive traits highlights the need to immediately establish genetically management strategies for captive and wild populations to minimize the loss of valuable diversity (MEDELLIN et al., 1999). Assisted reproduction and the use of cryopreservation can play an important role in these strategies by extending generation length and allowing genetic variation to be preserved without the spatial and financial limitations facing most zoo facilities (BALLOU, 1992). Computer models predict that the transfer of preserved semen or embryos from unrelated individuals into a population would enhance genetic diversity and population survival of three species (HARNAL et al., 2002). Further understanding of reproductive physiology and assisted reproductive techniques are required increase efficiency of cell cryopreservation, artificial insemination and embryo transfer before these technologies become a routine facet of population management.

Stress

The effects of stress on temperament and reproductive behavior has been extensively discussed among the zoo community in recent years. Stress can be defined as an imbalance in homeostasis brought about by environmental stimuli (MOBERG, 1985A; MÖSTL; PALME 2002). It is well established that reproductive success is susceptible to severe stressors (RAMALEY 1981; MOBERG, 1985b; LASLEY; KIRKPATRICK, 1991; RIVIER; RIVEST, 1991; POTTINGER, 1999; MOBERG, 2000; DOBSON et al., 2001; DOBSON et al., 2003). These stressors result in the stimulation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Both beneficial (e.g., courtship, copulation, obtaining prey, giving birth) and detrimental (e.g., fighting, capture, transport) situations can elicit a stress response via an adrenal reaction (COLBORN, 1991; MOBERG, 2000). Subsequent acute (short-term) elevations in glucocorticoids can help an animal react appropriately to the challenge through rapid energy mobilization. In contrast, a chronic (long and sustained) glucocorticoid rise can reduce fitness through a variety of mechanisms ranging from immunosuppression to poor reproduction and offspring survival (MOBERG, 1985b; 2000).

Stress as a result of suboptimal housing and poor management also is known to elicit behavioral problems in captive individuals. This appears particularly prevalent in specialized carnivores (MELLEN, 1991; CARLSTEAD et al., 1993a; CARLSTEAD et al., 1993b; JURKE et al., 1997; CARLSTEAD, 2002; MOREIRA et al., 2002; WIELEBNOWSKI, 2002; WIELEBNOWSKI et al., 2002), perhaps because most of these species, like the jaguar, require comparatively large territories in nature that simply cannot be mimicked in zoos. Management factors significantly influence the number of litters produced by small-sized felid species in captivity (Mellen 1991), a phenomenon no doubt regulated hormonally. For example, changing a controlled environment for a laboratory cat so that it is more 'stressful' indeed decreases luteinizing hormone secretion from the pituitary while suppressing exploratory and play activity and increasing hiding behaviors (CARLSTEAD et al., 1993a). Similarly, placing the small sized leopard cat in a new environment causes persistently elevated glucocorticoid excretion while reducing exploratory behaviors (CARLSTEAD et al., 1993b). A negative relationship also has been measured between adrenal and ovarian activity in the cheetah (JURKE et al. 1997). Female cheetahs with high circulating cortisol concentrations experience less follicular activity and prolonged periods of anestrus. Perhaps most provocative have been recent studies by Wielebnowski and colleagues (WIELEBNOWSKI et al., 2002) who have studied the relationship among adrenal activity, husbandry and behavior in the clouded leopard, a species notoriously difficult to breed in captivity. Multiple regression analyses revealed that the highest corticoid concentrations were measured in clouded leopards living near the public (i.e., on exhibit), adjacent to potential predators (i.e., lions and tigers) or were served by multiple animal keepers. Clouded leopards with the lowest baseline excreted corticoid concentrations and the most appropriate response to acute stressors were housed out of public view (off-exhibit), away from larger-sized predators and received care from only one or two keepers. Confinement of any predator under intensive management conditions and the inevitable exposure to conspecifics (and often heterospecifics) is likely to impose a stress (MOBERG, 1985a). Thus, it should not be surprising that in the few solitary species studied (i.e., cheetah, margay, tigrina, clouded leopard), that sensitivity to a captive environment appears to contribute to low reproductive success (JURKE et al., 1997; WIELEBNOWSKI et al., 2002).

Monitoring corticosteroid concentrations in the peripheral circulation is a commonly accepted measure of stress in mammals and birds (POTTINGER, 1999; WASSER et al., 2001). However, regular blood sampling is impractical for most wildlife species, and this procedure itself can induce an adrenal response (MONFORT, 2003). An effective alternative to tracking corticosteroid hormones in blood is noninvasively monitoring corticoid metabolites in urine and feces, a process that has been accomplished in a host of carnivore species (CARLSTEAD et al., 1992; CARLSTEAD et al., 1993A; CARLSTEAD et al., 1993B; GRAHAM; BROWN, 1996; CREEL et al., 1997; JURKE et al., 1997; MONFORT et al., 1997; YOUNG et al., 2001; WIELEBNOWSKI et al., 2002), including the jaguar (PATERA et al., 2004). The relative ease and noninvasive nature of sample collection permits longitudinal studies, and the pooled measure of metabolites in the urine or feces allows examining long-term trends in adrenal function in captive or even free-living wildlife (see review (MONFORT, 2003). The results of such measures must be carefully analysed as activities as diverse as mating, fighting, hunting and being hunted all activate the secretion of corticoids (WIELEBNOWSKI, 2003). Thus increased corticoid levels may indicate positive stimuli and a beneficial environment, in fact short term elevated corticosteroids stimulates reproductive activity while the same condition over protracted periods of time suppresses reproductive activity (BRANN; MAHESH, 1991). Further, low levels of glucocorticoids may indicate a lack of negative stimuli, or a negative feedback mechanism, even in the presence of negative stressors (WIELEBNOWSKI, 2003), thus these measures alone cannot be used to assess the beneficial or detrimental effects of various environmental stimuli.

Now, a priority is to combine noninvasive monitoring of jaguar adrenal hormones with gonadal hormones to develop a comprehensive understanding of relatedness. Further using a combination of disciplines, we must now investigate the link between these endocrine relationships and parallel assessments of health, genetics, management conditions and behavior especially in the context of reproductive success.

Disease

Although seemingly obvious, it must be remembered that disease directly impacts reproductive success in all species. This is true for females (ie, ability to conceive, carry a fetus full term, have an uncomplicated parturition) and males (ie, sperm quality, the ability to copulate). In jaguars, non-infectious, infectious and parasitic diseases are known to cause morbidity and/or mortality and have direct affects on reproductive success. Additionally, nutrition and health are intimately linked and many health issues are either directly related to poor nutrition (ie, metabolic bone disease) or are compounded due to poor nutrition (ie, immunocompromise that results in higher susceptibility to infectious diseases). The prevention of disease should be the primary objective of veterinarians caring for captive jaguars to ensure a healthy, reproductive population in captivity. The health of captive jaguars is dependent on sound husbandry practices, proper nutrition, and good veterinary care (DEEM, 2003). It is also imperative that we learn the health and reproductive status, and how the two are correlated, of different populations of free-ranging jaguars. This will allow us to determine disease prevention strategies and the long-term conservation needs for these populations.

Disease can be defined as any impairment that interferes with or modifies the performance of normal bodily functions. The diseases of most concern for the reproductive success of jaguars can be classified into non-infectious, infectious, and parasitic diseases (Table 1). Although the list in Table 1 is not exhaustive of diseases known to occur in jaguars, we will focus on the diseases that occur most commonly in jaguars and are of greatest concern for their reproductive success.

Table 1 – Diseases of jaguars (*Panthera onca*) that may cause morbidity, mortality and/or directly affect reproductive success.

Non-infectious	Infectious	Parasitic
Metabolic Bone Disease*	Canine Distemper Virus*	*Toxoplasma gondii**
Starvation*	Feline infectious peritonitis*	*Dirofilaria immitis*
Dental disease*	Feline Immunodeficiency Virus*	Internal gastrointestinal parasites*
Obesity*	Rabies*	Ectoparasites*
Neoplasia*	Herpesvirus*	
Conspecific trauma*	Calicivirus*	
Kidney failure*	Feline parvovirus (panleukopenia)*	
	Feline Leukemia Virus	
Musculoskeletal abnormalities*	*Leptospira interrogans* spp.	
Reproductive tract abnormalities*	*Chlamydiophilia psittaci*	
	Salmonella spp.	

* Specifies those diseases that occur in jaguars and have been documented in the literature.

Noninfectious diseases include those related to nutritional imbalances. The three most significant nutritional diseases for reproductive success are metabolic bone disease, primarily in captive raised jaguar cubs, starvation that may occur in free-ranging jaguars associated with habitat fragmentation and prey base depletion or due to dental disease in captive or free-ranging jaguars, and all too frequently obesity in captive jaguars. Nutritional diseases are discussed in the following section. In captivity, common noninfectious diseases also include a high incidence of neoplasia, canine fractures, conspecific trauma, and kidney failure and musculoskeletal diseases in older jaguars. Jaguars appear to be particularly susceptible to malignant neoplasias of the female reproductive tract (MUNSON, 2003). The prevalence of these neoplasias in free-ranging jaguars is largely unknown. Fractured canines may lead to loss of body condition and have been caused by trapping of free-ranging jaguars due to inappropriate capture methods. Although kidney failure is hard to prevent in older captive jaguars, conspecific trauma and arthritis can be minimized with proper housing that ensures jaguars have enough space and the appropriate substrate.

There are a number of infectious agents (ie, viruses and bacteria) known to occur in jaguars. Each of these agents has a different morbidity and mortality rate that is influenced by the jaguar's nutritional status, stress level, and genetic make up. Some of these agents are known to have direct affects on the reproductive success either by causing decreased fertility, morbidity, or mortality of adult jaguars or by causing morbidity or mortality of the neonate. Transmission of these agents can be either horizontal, and may have a sexual component, or vertical through colostrum or in utero from the dam to her cub.

The five viruses, canine distemper virus (CDV) (APPEL et al., 1994), feline infectious peritonitis (FIP) (FRANSEN, 1973), feline immunodeficiency virus (FIV) (BARR et al., 1989), herpesvirus (HOFFMANN-LEHMANN et al., 1996), and rabies are all know to infect jaguars and are therefore of most concern for their health and reproductive success.

Canine distemper virus is an RNA virus that is highly contagious by aerosol spread, but short lived in the environment. Although named canine distemper, this virus has now been reported in all families of terrestrial carnivores (DEEM et al., 2000). Since 1991, CDV infections have been reported in five species of free-ranging and captive felids from at least eight dis-contiguous sites and epidemics in captive lions, tigers, leopards, and jaguars have been reported in the 1990s (APPEL et al., 1994). The most common clinical manifestations in non-domestic felids are gastrointestinal, respiratory, and neurological signs. Transmission from ocular, oral, or nasal secretions by direct contact and indirect via aerosol or fomites, although the virus is usually short lived in the environment, are the most likely routes. Transplacental transmission has been documented in domestic dogs. The epidemiologic role of vertical transmission in CD, and whether or not such transmission can occur in non-domestic species are unknown, although the isolate from lions in the Serengeti was transmitted vertically in hyenas (HASS et al., 1996). Additionally, involvement of the reproductive tract in many species has been document. Whether felids remain carriers or are immune following a primary infection is not known. There is a vaccine available for CDV however at this time the Felid Taxonomic Advisory Group (TAG) does NOT recommend the routine vaccination of non-domestic felid species.

Feline infectious peritonitis is a corona virus that causes severe disease in both domestic and non-domestic cats. It causes a chronic, progressive, immunologically-mediated, fatal disease. Peak incidence of disease occurs between 6 months and 5 years of age and transmission is by the fecal-oral route. Infection of a captive jaguar has been documented in the literature (FRANSEN, 1973). One problem in conducting serologic surveys of FIP in jaguars is that current serological tests cannot differentiate between exposure to FIP or to other less pathogenic coronaviruses. Additionally, serologic testing cannot differentiate between exposed and vaccinated jaguars. There is a vaccine available for FIP but it is not recommended for use in non-domestic felids.

Feline immunodeficiency virus was first described in 1987 and is a member of the genus Lentivirus of the family Retroviridae. Transmission in domestic cats has been shown to be via saliva from bite wounds, to kittens from queen's milk, and experimentally via artificial insemination with fresh semen. Although an often fatal and serious disease of domestic cats, there is no clear correlation between virus infection and disease in non-domestic felids. In domestic cats it produces an acquired immunodeficiency-like syndrome. Because insufficient data are available to eliminate the possibility of an association with clinical disease, it is prudent to test all captive jaguars for FIV-specific antibodies, and seropositive animals should be housed separately from uninfected ones. Serologic testing should also be considered when planning movement of captive jaguars and translocation of wild jaguars. An infected jaguar may shed FIV and is a potential source of infection for con-specifics. There is a new vaccine available for FIV but it interferes with serologic testing of cats post-vaccination. It is not routinely administered to non-domestic captive felids.

Feline herpesvirus (FHV) is a DNA virus that is short lived in the environment outside the host. Clinical disease associated with herpesvirus infection in domestic cats includes abortion, neonatal disease, classic rhinotracheitis in kittens, chronic conjunctivitis and keratitis, recurrent disease in older cats, chronic sinusitis, and miscellaneous other syndromes. One hundred percent of domestic cats develop latent infections. Pregnant cats infected with FHV may abort, resorb fetuses, or give birth to congenitally affected kittens. Clinical signs of rhinotracheitis due to FHV have been described in captive jaguars. There is a vaccine available for this virus and it is recommended that captive jaguars are vaccinated against this virus.

Exposure to feline panleukopenia (FPV) (parvovirus) and calicivirus was detected in wild caught jaguars kept in captivity in Costa Rica (DEEM, 2001). The significance of these two viruses for jaguar reproductive success is still largely unknown. However, the parvovirus responsible for FPV causes cerebellar disease in domestic kittens infected in utero so even if they survive fetal infection, the severe neurologic deficits in the neonatal period will lead to death in a free-ranging cat. Calicivirus causes respiratory disease in felids, with neonates often most severely infected. Therefore, both these viruses have the potential to impact reproductive success in jaguars.

Rabies is a highly fatal member of the rhabdovirus family which requires direct contact for transmission. All warm blooded animals are susceptible to clinical rabies disease. Infection is invariably fatal once clinical signs develop. However, some animals are exposed to the rabies virus, develop immunity based on the detection of antibody levels, and do not develop clinical disease. In regard to jaguar reproduction, the most important effect is central nervous system disease and death in adult jaguars. All captive jaguars should be vaccinated for rabies.

To the authors' knowledge, feline leukemia virus (FeLV) has not been documented in jaguars. However, this virus probably can infect jaguars and thus researchers working with jaguars should be familiar with the clinical signs associated with FeLV since it may cause significant morbidity and mortality of cats, especially in the neonatal period. In domestic cats, feline leukemia can be transmitted in utero from the dam to her kittens with high neonatal mortality.

Although less of an issue for reproductive success in jaguars, bacteria can also directly influence reproduction (ie, *Leptospira interrogans* spp.) or indirectly (ie, *Salmonella* spp. septicemia in neonates). In addition to the three bacteria discussed in this chapter, *Leptospira interrogans* spp., *Chlamydia (Chlamydiophilia) psittaci*, and Salmonella spp., there are many more bacteria species that cause infections in jaguars. Many of these infections occur in neonatal jaguars, often associated with immunocompromise with and without a viral component, and usually manifest as septicemias or pneumonias causing high morbidity and mortality.

Leptospira interrogans spp. are spirochetes that cause disease, usually associated with the reproductive and renal systems, in a number of animal species (BOLIN, 2003). However, cats appear highly immunoresponsive to leptospiral infection and leptospirosis does not seem to be a big disease problem of domestic cats. Most clinical cases in felids have been associated with the renal system. Leptospira are relatively resistant and persist for hours under moist, warm conditions. They are shed in the urine and transmitted by the fecal – oral route. The influence of leptospirosis on the reproductive success of jaguars is not known at this time. However, due to the high prevalence of this agent in the environment and the potential to cause significant disease, health evaluations of jaguars should include testing for L. interrogans spp.

*Chlamydia (Chlamydiophilia) psittac*i is frequently a cause of conjunctivitis, upper respiratory tract disease, pneumonia, urethritis, vaginitis, abortion, enteritis, encephalitis, and polyarthritis in domestic cats. Transmission is most commonly airborne or by fomites, but pregnant queens can transmit the infection to newborn kittens. There is often high morbidity but low mortality of this disease agent in domestic cats. Little is known about this disease in non-domestic cats and the influence of chlamydiosis on the reproductive success of jaguars is not known at this time.

Salmonella spp. are gram negative bacteria that can be highly pathogenic, especially in neonates or immunocompromised individuals or those cats exposed to large doses. This often occurs due to feeding of contaminated meat to captive felids. Three syndromes exist: 1. asymptomatic carrier state; 2. gastroenteritis, and 3. bacteremia. Salmonellosis can be fatal, particularly in neonates and immunocompromised animals, and septicemia may lead to fetal re-sorption or abortion. Salmonellosis is much more likely to occur in captive versus free-ranging jaguars due to poor hygienic standards.

Parasitic diseases are the third category of diseases, along with non-infectious and infectious diseases, of concern for the reproductive success of jaguars. By definition, parasites decrease either the survival or reproduction of host populations (ANDERSON; MAY, 1978). In jaguars, parasites of high concern include *Toxoplasma gondii, Dirafilaria immitis*, internal gastrointestinal parasites, and ectoparasites.

Toxoplasma gondii is an intracellular parasite that usually parasitizes domestic and non-domestic cats without producing clinical signs (WOLFE, 2003). However, occasionally severe clinical disease does occur in cats and is associated with the gastrointestinal, respiratory, ocular, and/or neurological systems. Why some cats become ill whereas others remain healthy is not fully understood. Age, sex, strain of *T. gondii* and number of parasites to which the animal is exposed may count for some of the reason. Toxoplasmosis is most severe during the neonatal period. Members of the Felidae family are the only known definitive host of *T. gondii*, and are the main reservoir of infection. The organism has been isolated from jaguars. Many non-felid species (including humans) can serve as the intermediate host and these species often have clinical disease, especially in offspring born to mothers infected during pregnancy. Clinical disease with *T. gondii* is rare in non-domestic felids but has been known to cause morbidity and mortality of non-domestic felids.

Dirofilaria immitis is a nematode parasite transmitted by mosquito vectors and responsible for the fatal condition called heartworm disease. Heartworm is a severe parasitic disease of many carnivore species. It has become an increasingly important health problem of domestic cats in the United States and has also been documented in a number of non-domestic cats, including jaguars (OTTO, 1975). In D. immitis endemic regions of the United States, ivermectin is recommended as a prophylaxis to minimize disease in captive non-domestic felid species and should be consider for use in all captive held jaguars where heartworm disease is known to occur. The impact of D. immitis on the health of free-ranging jaguars is not known at this time.

Jaguars, both free-ranging and captive, have a number of internal parasites that primarily cause clinical disease associated with the gastrointestinal tract (GIT) (PATTON et al., 1986; DEEM, 2003). Some may lead to high mortality, especially in neonatal cats. Reproduction may be affected due to general debility of an adult jaguar or severe disease, and death, in heavily infected neonates. Parasites of most concern for jaguars are 1) Toxocara cati which infects cubs when they ingest the larvae in dam's milk. Clinical signs include unthriftiness, potbellied appearance, and vomiting/diarrhea. These worms can also cause gastrointestinal impactions and obstructions; 2) coccidiosis which can cause severe and fatal, especially in neonatal cubs, disease; 3) giardiasis in which clinical disease in cubs may range from mild diarrhea to severe disease with profuse watery diarrhea, dehydration and obtundation (CIRILLO et al., 1990); and 4) Ancylostoma spp. which are transmitted from the dam to her cubs. Clinical signs are usually associated with gastrointestinal bleeding. In addition to internal parasites, a number of tick species and fleas have been identified from free-ranging and captive jaguars. These ectoparasites may transmit blood-borne protozoans (i e., *Hemobartellona* sp. and *Babesia* sp.) and may adversely affect the health of jaguars by modifying behaviour (ie, over grooming) and by causing anemia.

The importance of disease as a limiting factor in jaguar, and other non-domestic species', health and reproductive success in captivity has been appreciated for many years. Recently the role that disease plays in these same species in the wild has become increasingly appreciated (DEEM et al., 2001). This is most likely a result of anthropogenic changes that cause habitat fragmentation and an increased contact between wild animals, domestic animals and humans. For example, all the infectious and parasitic diseases discussed in this section do infect domestic cats, and some infect domestic dogs, both of which may act as reservoirs of infection for jaguars. It is imperative that health is evaluated, along with genetics, nutrition and stress, if we are to fully understand jaguar reproduction with the ultimate objective of ensuring reproductive success.

Nutrition

Nutrition plays a fundamental role in overall animal demeanor, sperm production, egg quality, hormone production and the ability to maintain pregnancy. For example, malnutrition alters luteinizing hormone and testosterone secretion patterns in rhesus monkeys (LADO-ABEAL et al., 2002) and delays pubertal onset, protracts post-parturition anestrus and reduces overall fecundity in the marmoset (TARDIF; JAQUISH, 1994) and water buffalo (OSWIN-PERERA, 1999). However, neither ex situ or in situ populations rarely suffer from insufficient supply of energy substrates, unless other factors contribute to an individuals inability to catch and eat prey, or absorb substrates. Indeed, obesity is more often of concern in the captive felid population due to lack of exercise and overeating. It is possible that overeating is exacerbated by the lack of specific nutrients, inducing a sensation of general hunger (BRAY, 2000). To avoid obesity and the potentially catastrophic effects of nutrient deficiency on health and reproductive capacity, the supply of micronutrients becomes vital.

One of the primary assaults on general health from malnutrition is an impaired immune response (GOGOS; KALFARENTZOS, 1995). Malnutrition impairs innate and adaptive defenses of the host, including phagocytic function, cell-mediated immunity, complement system, secretory antibody, and cytokine production and function. Deficient protein intake changes in the oral microbial ecology resulting in a preponderance of pathogenic anaerobic organisms, increased propensity of bacteria to bind to oral mucosal cells, attenuation of acute phase protein response, and dysfunction of the cytokine system (ENWONWU et al., 2002). Cats may be particularly susceptible to the effects of protein deficiency as they have an unusually high dietary requirement for protein (GREAVES; SCOTT, 1960). Cellular depletion of antioxidant nutrients promotes immunosuppression (GOGOS; KALFARENTZOS, 1995), accelerated replication rate of ribonucleic acid viruses, and increased disease progression (ENWONWU et al., 2002). Malnutrition also increases susceptibility to bacterial infection and parasite infestation (WATSON; PETRO, 1984). The transference of the ability to face immunochallenges passes from queen to kittens in the first hours after birth in the colostrum (CASAL et al., 1996). Without the benefit of these antibodies, kitten survival is severely impaired (YAMADA et al., 1991).

Livestock studies have consistently demonstrated that vitamins and minerals are essential in adult diets as micronutrients play key roles in steroid hormone synthesis, spermatogenesis, fertilization, embryo development, milk production and neonatal survival (SMITH; AKINBAMIJO, 2000). It is these very nutrients that are most often deficient in captive animal diets, due to the perception that supplementing base diets is an unnecessary expense (SMITH; AKINBAMIJO, 2000). Most carnivore diets in developing country captive facilities are comprised only of muscle meat without bones and viscera and, thus, are inadequate in calcium and other essential micronutrients (SOARES, 1995). The problems with sole muscle diets in captive felids have been known for decades. For example, such mineral/vitamin deficient diets were recognized early on to cause secondary hyperparathyroidism in captive lions (FIENNES; GRAHAM-JONES, 1960), a condition easily reversible by simple supplementation with bone meal or micronutrient mixes (KROOK et al., 1963; ULLREY; BERNARD, 1989).

Although the nutrient quality of whole prey varies with season, species, age and sex (DIERENFELD et al., 2002), whole prey is usually presumed to most closely approximate the natural diet of felids and is often used as the standard against which formulated or other captive diets are measured. The disparity in nutrient value between muscle meat only diets and that of whole prey is demonstrated in Table 2, based on information complied for the Jaguar Husbandry Manual (WARD; HUNT, 2003). While many nutrients that supply the need for energy and protein are supplied in muscle only diets, other micronutrient values are less than 10% that of average whole prey values (Calcium, Vitamin A, Vitamin D3, Manganese). Muscle meat only diets contain phosphorus and magnesium quantities less than 20% that of whole prey. These deficiencies can be devastating to the general health and reproductive fitness of felids.

Calcium is among the most important of the micronutrients for reproduction as it influences erectile function (MILLS et al., 2001), spermatogenesis (ANDONOV; CHALDAKOV, 1991), oocyte maturation (MACHACA; HAUN, 2002), fertilization (STRICKER, 1999) and embryo development (STRICKER, 1999). In short, calcium is required for reproduction, and active reproduction promotes calcium mobilization from bone reserves in the absence of an adequate diet (GAREL, 1987; KING, 2001). Clinical hypocalcaemia can also result in muscle spasms, secondary hyperparathyroidism, electrocardiographic changes and metabolic bone disease where the mineralization of the bones is altered in such diseases as osteoporosis (GRIER et al., 1996), as a result, bones are weakened and fracture easily and spinal and pelvic deformities are common (Lucke, 1993). Unfortunately, unlike other micronutrients or trace elements, calcium status cannot be accurately determined by assaying serum (WONG et al., 2001) or urine (PASTOOR, 1993). Therefore, calcium status via radiography (qualitative) (BIERY, 1985) or bone densitometry (quantitative) (CAPEN, 1985) is considered more reflective of a calcium deficiency than chemical analysis of bodily fluids (WEDEKIND et al., 1992). For this reason, calcium deficiency often goes undetected even after a medical examination.

Vitamin A and retinol play essential roles in sperm production and all processes of embryogenesis, consequently vitamin A deficiency causes male infertility, embryonic loss, decreased neonatal survival and fetal malformations (CLAGETT-DAME; DeLUCA, 2002). Cats have a unique requirement for vitamin A and retinol in the diet as they lack the ability to convert precursor compounds such as ß-carotene to vitamin A and retinol. Vitamin D deficiency reduces overall fertility (HALLORAN; DeLUCA, 1979) by controlling onset of puberty and fertility in males and females as well as pregnancy, lactation, and probably sexual behavior (STUMPF; DENNY, 1989). Vitamin D also facilitates calcium and phosphorus absorption in the gut, so Vitamin D deficiency can exacerbate hypocalcaemia (CHEW; MEUTEN, 1982). Manganese is considered one of the most important micronutrients for reproduction, and maternal supplementation of zinc, copper and manganese improves embryonic survival in dogs (KUHLMAN; ROMPALA, 1998). Magnesium is essential to sperm production (KISS; KISS, 1995), in fact measurement of magnesium levels is used as an index of reproductive health of males (SAARANEN et al., 1987). Animals fed muscle only diets are likely to be severely deficient in each of the micronutrients (Table 2). Further, micronutrients that have not yet been analyzed in muscle only diets are also likely to be lacking and significantly influence reproductive fitness. Zinc is essential for normal gametogenesis (APGAR, 1985), blastocyst formation and implantation in humans (VALLEE; FALCHUK, 1993), and Copper deficiency in felids prolongs anestrus and compromises conception (FASCETTI et al., 2000). In species with high rates of carotenoid accumulation (e.g. bovids, felids) deficiency can cause silent estrus, decreased conception, increased embryonic death and inferior composition of colostrums (SIMPSON; CHICHESTER, 1981). In a survey of 13 felid species, individuals accumulated less carotenoids when in poor health (SLIFKA et al., 1999), illustrating the need to examine general health and nutrition simultaneously.

Felids may be particularly susceptible to inadequate diets as they have specific and unique requirements for micronutrients such as arachidonic acid, which is essential for reproduction and spermatogenesis (MacDONALD et al., 1984). This is possibly due to a lack of desaturating enzymes that reduces their inability to produce this longer chain fatty acid (20 carbon) from the shorter chains (18 carbon) (RIVERS et al., 1975). Although cats may produce some longer chain fatty acids (PAWLOSKY et al., 1994) from 18-carbon fatty acids such as linoleic acid (making this a dietary requirement), it appears that their ability to synthesize arachidonic acid is highly deficient. A dietary source of niacin is also required, but this need is not due to a lack of enzymes. Cats are able to convert tryptophan to niacin as most mammals do, but this pathway is dominated by the synthesis of glutamate from tryptophan, so little niacin is formed (MORRIS; ROGERS, 1982). Amino acids such as methionine and cysteine serve as precursors for the amino acid taurine in most animals, and must be supplied in the diet. However, felids have a limited capacity to catabolize this amino acid, thus taurine must also be supplied to avoid retinal degeneration of young kittens (KNOPF et al., 1978), fetal resorptions and abnormalities, abortion, stillbirth and low birth weight of kittens (DA COSTA & HOSKINS, 1990; STURMAN; MESSING, 1991). Cats are also incapable of synthesizing arginine and are therefore more sensitive to arginine deficiency (MORRIS; ROGERS, 1983), which retards neonatal growth (ROGERS; MORRIS, 1979).

These peculiarities of feline metabolism leave them even more susceptible to suffer deficiencies and subsequent effects on general health and reproduction, given a nutrient poor diet, such as muscle meat only. To date, there have been no studies of the impact of micronutrient supplementation on felid semen quality with one exception. A recent thesis from São Paulo University suggested that supplementing vitamins and minerals to three jaguars (in Brazilian wildlife rehabilitation facilities) decreased some sperm malformations (PAZ, 2000). Further investigations into the influence of nutrition on reproduction and determination of optimal yet affordable diets for captive felids is an essential step to producing a viable, self-sustaining captive global population.

Table 2 – Nutrient content of prey items and muscle meat on a dry matter basis.

		Whole	Whole	Whole	Whole	Guinea	Skeletal muscle		
	Units	Chicken[1]	Rabbit[1]	Rat[1]	Deer[1]	Pig[1]	Horses[2]	Cattle[2]	Deer[2]
Moisture	%	32.5	26.2	33.9	41.1	31.3	27	28	30
Protein	%	42.3	65.2	61.8	47.4	51.4	76	63	65
Fat	%	37.8	15.8	32.6	41.4	46.1	18	29	29
Ash	%	9.4	3.4	9.8	11.4	9.2	4	3	3.4
Vitamin A*	IU/kg	35600	6200	151389	ND	16506	2593	1428	ND
Vitamin D3*	IU/kg	51.3	ND	139.2	ND	24.2	0	0	ND
Calcium*	%	2.22	5.93	2.62	3.09	3.02	0.05	0.03	0.03
Phosphorous**	%	1.4	3.43	1.48	2.26	ND	0.34	0.55	0.59
Potassium	%	ND	0.72	ND	0.95	ND	1.1	1.01	1.07
Sodium	%	ND	0.26	ND	0.39	ND	0.19	0.17	0.3
Magnesium**	%	0.5	0.18	0.08	0.19	0.07	0.05	0.06	0.06
Iron	ppm	122.2	100	148	164.5	56.4	232	78	165
Copper	ppm	3.6	4.6	6.3	26.1	5.6	3	2	5
Zinc	ppm	116.1	84	62.1	68.4	46.4	128	106	68
Manganese*	ppm	10.1	2.4	11	28.5	6.6	0.6	0.3	0.7

[1] (DIERENFELD et al., 2002).
[2] (ULLREY, BERNARD, 1989).
* Muscle only values are less that 10% of average whole prey values for same nutrient.
** Muscle only values are less that 20% of average whole prey values for same nutrient.

Conclusion

All of these data taken together clearly indicate that genetics, nutrition and disease affect the reproductive capacity of individuals. Less often discussed is the affect that reproduction has on the health of the individual. For example, it is well established in humans that estrogens help prevent the bone disease osteoporosis (NOTELOVITZ,1997; HALLWORTH, 1998). It is thought that estrogen prevents bone calcium resorption (TOBIAS; COMPSTON, 1999), but it may also act by stimulating osteoblast function in the elderly (WAHAB et al., 1997; TOBIAS; COMPSTON 1999; VEDI et al., 1999). This may also be true of pubertal females (SEEMAN, 1997; DELEMARRE-VAN DE WAAL et al., 2001) and males (WICKMAN et al., 2003), emphasizing the importance of normal reproductive hormones during puberty on the general health of the individual. Consider the case of some jaguars in captivity with high levels of stress (and corticosteroids), poor nutrition and medical care. It is likely that these individuals would have minimal levels of circulating reproductive hormones, resulting in a lack of reproductive capacity and poorly developed bone structure. This developmental weakness will likely never be overcome without intense therapy, and will therefore render the individual more likely to bone fractures, compounded health issues and poor reproductive performance throughout its life, even if optimal conditions are employed later in life.

This calls attention to the interrelated nature of all of these systems. Just as each system is dependent on one another in the body, so we must accept that data on each of these factors is most relevant when considered in unison. Given that reproduction is affected by all of these factors, we suggest that reproductive assessment in conjunction with medical examinations can be most helpful in determining the physiological health of an individual. Similarly, reproductive capacity of a population indicates its viability, and should therefore be a primary consideration in estimates of population health.

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Parte VI
Medicina da conservação

Capítulo 18
Macroparasites study in wild carnivores: a tool for species conservation

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Introdução

Os carnívoros figuram como espécies-chave para a conservação dos ecossistemas em que vivem, mantendo o equilíbrio de populações animais e vegetais. Segundo Redford (1997), a eliminação dos predadores topo de cadeia pode influenciar a dinâmica das comunidades animais e indiretamente as comunidades vegetais. Emmons (1987) sugere que a ausência de predadores resulta em densidades menos homogêneas de espécies de presas e que isto pode ser agravado, se ocorrerem, com aquelas que desempenham processos ecológicos importantes na predação e dispersão de sementes específicas. É notório que a ausência de espécies de topo de cadeia pode causar um efeito dominó, podendo haver tanto o aumento da quantidade de espécies de presas como a exclusão de outras espécies do mesmo sistema (ALHO, 1992). Adicionalmente, os carnívoros são freqüentemente citados como espécies bioindicadoras e sua presença pode servir para avaliar a qualidade de conservação do habitat (CIMARDI, 1996; SILVEIRA, 1999).

Em geral, os conceitos que remetem à importância dos carnívoros para a conservação dos ecossistemas ficaram claros e comprovados nas décadas passadas, bem como o conjunto de ameaças que influenciam diretamente na população desses animais. Na década de 1990, tornou-se mais evidente a importância de conhecer melhor o processo em que os parasitos e as doenças causadas por eles afetam a sobrevivência dos carnívoros. Primack e Rodrigues (2001) ressaltam que entre as principais ameaças à diversidade biológica estão a destruição, fragmentação e degradação do habitat, sobreexploração das espécies para uso humano, introdução de espécies exóticas, incluindo as domésticas e o aumento de ocorrência de doenças parasitárias. Funk et al. (2001) consideram que após a fragmentação ambiental, a perseguição e sobreexploração dos carnívoros e suas presas, as doenças emergem agora como ameaça central para a conservação dos carnívoros. Os autores ressaltam episódios de viroses como cinomose canina e raiva que afetam remanescentes importantes de populações ameaçadas de carnívoros selvagens como, por exemplo, *Lycaon pictus, Canis simeriensis, Panthera leo*, na África e *Mustela nigripes* na América do Norte.

Informações sobre doenças parasitárias que acometem populações de carnívoros selvagens neotropicais são desconhecidas ou pouco relatadas. As principais fontes de informação sobre parasitas que acometem espécies neotropicais provêm de poucos relatos de pesquisadores que capturam animais de vida livre. Essas informações estão disponíveis somente para poucas espécies, como *Puma concolor* ou *Panthera onca,* e pouco se sabe sobre doenças parasitárias que afetam outras espécies como *Lontra longicaudis* ou *Eira barbara*, que possuem sua distribuição natural semelhante à dos felinos mencionados.

Nesse campo, diante da ameaça eminente aos carnívoros selvagens, o maior obstáculo é compreender o real efeito das doenças na dinâmica das populações selvagens. Tarefa particularmente difícil quando os agentes etiológicos são capazes de infectar uma grande variedade de organismos. De maneira geral, o conhecimento sobre patogenia e dinâmica dos agentes etiológicos nas populações selvagens de carnívoros é deficiente (FUNK et al., 2001). O fator relevante nesse aspecto é que a presença de parasitos e outros agentes infecciosos no ecossistema precisa ser reconhecida como componente elementar, que não pode ser excluído, quando se planejam protocolos de manejo para as espécies de carnívoros, bem como para as unidades de conservação. Por se tratar de área onde ainda há uma enorme carência de informações, fica cada vez mais clara essa necessidade.

Com a perda de habitat, torna-se cada vez mais evidente a concentração e a maior proximidade entre indivíduos e espécies, confinados em áreas reduzidas, situação que facilita a transmissão e a mudança de parasitos entre diferentes hospedeiros. Adicionalmente, a fragmentação ambiental aumenta o contato entre áreas naturais, remanescentes alterados e campos de pecuária e agricultura, acrescendo o fluxo de espécies entre esses sistemas, o que inclui também os parasitos que sob tais condições podem desenvolver novas cepas mais virulentas e patogênicas (HOLMES, 1996). Também devido à fragmentação do habitat, a presença de animais domésticos no entorno das unidades de conservação e até mesmo dentro delas é bastante comum.

Combes (1996) revisa a importância dos parasitos em relação à estabilidade e biodiversidade do sistema e considera, principalmente, que os parasitos influenciam a fecundidade e a capacidade de sobrevivência dos hospedeiros, representando peças-chave na dinâmica dos ecossistemas e da diversidade global. Devemos ainda considerar que a presença de determinados parasitos em uma população de carnívoros não significa que os hospedeiros irão desenvolver quadros patológicos. Ao assumirmos que os parasitos fazem parte do sistema e co-evoluíram com seus hospedeiros naturais, devemos considerar sua presença principalmente como mais um indicador da biodiversidade local. Todavia, é fundamental entender que a presença desses parasitos, mesmo em seus hospedeiros naturais, pode representar uma ameaça à estabilidade populacional dos carnívoros, sobretudo quando restritos a áreas ou populações reduzidas.

Ainda que a amplitude total das interações entre parasitos e hospedeiros permaneça desconhecida, é fácil prever que a entrada de novos agentes parasitários no habitat representa uma situação crítica à sobrevivência dos novos hospedeiros. Tal situação, em geral, pode levar a considerável aumento nas taxas de mortalidade ou queda nos índices de natalidade dos hospedeiros, o que representa perda na diversidade biológica e genética original do sistema.

Para romper os obstáculos que hoje se interpõem ao entendimento mais amplo dessas inter-relações entre parasitos e hospedeiros é necessário que sejam elaborados e aplicados modelos de estudo que abordem a avaliação das características sanitárias das espécies-chave e outros fatores que caracterizem o ambiente biótico e abiótico. Apontando a mesma necessidade, Tabor et al. (2001) consideram que o uso de espécies-chave e sentinelas aumenta a eficiência de monitoramento, além de permitir o acesso rápido a informações sobre as condições ambientais da área.

Este capítulo propõe uma metodologia para a avaliação sanitária destinada à determinação dos principais macroparasitos que afetam os carnívoros neotropicais, ou que se mantêm em equilíbrio com esses e o ambiente. Com isso, contribui para o desenvolvimento de novos métodos aplicáveis à avaliação da saúde das populações de carnívoros e dos seus ecossistemas. O termo macroparasito aplica-se nesse caso aos vermes e protozoários de ciclo direto ou indireto, os quais podem ter sua presença determinada através da avaliação coproparasitológica. Esse uso concorda com o proposto por Dobson e May (1986), que apresentam as inter-relações epidemiológicas fundamentais à sobrevivência e coexistência entre parasitos e hospedeiros.

Metodologia proposta e considerações quanto aos métodos

A base para avaliação da saúde populacional está na integração entre diferentes áreas de estudo. Mais apropriadamente, Tabor et al. (2001) consideram o termo transdiciplinariedade a expressão correta quando consideramos a necessidade de agregar os diferentes temas que dizem respeito à saúde ambiental, a qual está relacionada intrinsecamente à saúde animal e humana. O modelo apresentado aqui foi elaborado para avaliar a presença e o perfil dos endoparasitos e protozoários entéricos de interesse sanitário, em diferentes populações de carnívoros, considerando aspectos relevantes da área de estudo e das espécies envolvidas nos ciclos parasitários.

O método permite avaliar se existe uma correlação entre o perfil parasitário dos carnívoros selvagens e as condições ambientais locais, incluindo as áreas adjacentes ao seu habitat. Adicionalmente, permite correlacionar o perfil parasitário dos carnívoros selvagens com as atividades humanas e as criações de animais domésticos. A integração entre essas características contribui para a determinação do status sanitário dos carnívoros selvagens, contribuindo também com a determinação dos riscos à sua conservação.

A coleta de dados passa por seis etapas distintas: a) Avaliação parasitológica de amostras fecais dos carnívoros selvagens; b) Avaliação da dieta dos carnívoros selvagens; c) Avaliação da presença de larvas viáveis no solo; d) Avaliação parasitológica dos animais domésticos na área e arredores; e) Avaliação das características ambientais básicas e f) Levantamento da história do uso e da ocupação humana e animal na região, as quais serão discutidas a seguir.

Cabe ressaltar que, preferencialmente, o levantamento parasitológico da população de animais domésticos e selvagens e a avaliação do solo sejam realizados concomitantemente, considerando-se que pode haver flutuações nas populações de parasitos, dependendo da estação do ano e as influências da sazonalidade sobre a dinâmica populacional dos hospedeiros.

a) Avaliação parasitológica dos carnívoros selvagens

As amostras fecais destinadas à avaliação parasitológica são colhidas durante os deslocamentos por transectos, em busca de vestígios deixados pelos carnívoros. Amostras frescas produzem resultados mais confiáveis, porém mesmo amostras ressecadas depositadas no ambiente há alguns dias podem ser avaliadas. Muitos ovos de helmintos e cistos de protozoários patogênicos resistem por vários dias no interior do bolo fecal. A avaliação parasitológica das amostras colhidas é feita pelos métodos de sedimentação e flutuação, consagrados pelo uso na parasitologia veterinária e humana (SANTOS, 1999). Os métodos podem apresentar sensibilidades diferentes, dependendo do grau de conservação da amostra e dos tipos de ovos contidos nela. Muitos tipos de ovos podem ser observados por ambos os métodos. Quando as amostras apresentam grande concentração de ovos ou a avaliação ambiental busca um perfil sazonal da incidência de ovos, pode-se realizar a técnica de contagem de ovos por grama de fezes (OPG). Essa técnica permite comparações mais precisas entre diferentes áreas e épocas do ano, uma vez que as técnicas de sedimentação e flutuação produzem resultados apenas semiquantitativos. Contudo, apesar das vantagens da técnica de OPG, realizar apenas ela pode limitar os resultados quanto à diversidade de ovos e cistos observados. Dessa forma, é recomendável associar as três técnicas, pois a presença de ovos em menores concentrações pode dificultar a avaliação quando se utiliza apenas OPG. Como principal limitação temos que os métodos de flutuação e sedimentação e OPG permitem, na maioria das ocasiões, apenas identificar o gênero do parasito; contudo, alguns ovos possibilitam apenas que se determine a classe ou família a que pertencem, não permitindo a diferenciação entre gêneros sem que seja feita a identificação da larva.

Como exemplo da possibilidade de utilizar o material escatológico proveniente dos carnívoros silvestres como instrumento para avaliação da sanidade populacional, usaremos resultados obtidos entre o período de janeiro de 2000 e dezembro de 2001, quando foi possível avaliar 26 amostras fecais de carnívoros da Reserva Natural Salto Morato (RNSM – FBPN), situada em Guaraqueçaba – PR. Entre todas as amostras avaliadas, apenas uma foi negativa, tanto para o teste de flutuação quanto para o de sedimentação. Apenas duas amostras apresentavam ovos de apenas uma espécie de parasito. As demais amostras continham de duas a cinco espécies diferentes de helmintos ou protozoários. Os ovos de parasitos encontrados nas amostras fecais na RNSM e sua freqüência absoluta e percentual dentro da amostragem estão relacionados na Tabela 1. A prevalência percentual e absoluta de parasitos por espécie amostrada está relacionada na Tabela 2.

Tabela 1 – Freqüência absoluta e percentual da ocorrência de endoparasitos nas amostras de fezes de carnívoros selvagens (Reserva Natural Salto Morato, entre janeiro de 2000 e dezembro de 2001).

Parasito	Freqüência absoluta	Percentual de ocorrência*
Helmintos típicos de carnívoros		
Diphylobothrium sp.	16/26	61,5%
Trichuris sp.	6/26	23,0%
Toxocara sp.	5/26	19,2%
Oxyuroidea	7/26	26,9%
Ancilostomatídeo	5/26	19,2%
Protozoários		
Balantidium sp.	2/26	7,7%
Oocisto desconhecido	1/26	3,8%
Helmintos típicos de Aves		
Ascaridia gali^{+}	1/26	3,8%
Helmintos comuns a vários grupos de vertebrados		
Capillaria sp.	3/26	11,5%
Estrongilídeo	2/26	7,7%
Exames Negativos	3/26	7,7%

* O percentual de ocorrência leva em consideração a presença de ovos e cistos do parasito em relação ao total de amostras (N=26).

Tabela 2 – Prevalência percentual e absoluta de parasitos nas amostras de fezes de carnívoros por espécie amostrada (Reserva Natural Salto Morato, entre janeiro de 2000 e dezembro de 2001).

Amostra	Parasitos	Número de positivos*	Percentual**
Cachorro-do-mato	*Diphylobothrium* sp.	06	100
Cerdocyon thous	*Trichuris* sp.	04	66,6
N=06	Oxyurídeo	03	50
	Balantidium sp.	02	33,3
	Capillaria sp.	01	16,6
	Ancylostoma sp.	01	16,6
	Toxocara sp.	01	16,6
	*Ascaridia gali****	01	16,6
Gato-do-mato	*Diphylobothrium* sp.	05	71,4
Leopardus tigrinus	Oxyurídeo	02	28,6
N=07	*Capillaria* sp	02	28,6
	Toxocara sp.	02	28,6
	Trichuris sp.	01	14,3
	Ancilostomatídeo	02	28,6
Jaguatirica	Oxyurídeo	02	66,6
Leopardus pardalis	*Diphylobothrium* sp.	01	33,3
N=03	Estrongilídeo	01	33,3
Puma	*Diphylobothrium* sp.	01	100
Puma concolor	*Toxocara* sp.	01	100
N=01			
Lontra	*Diphylobothrium* sp.	03	33,3
Lontra longicaudis	Ancilostomatídeo	02	22,2
N=09	*Trichuris* sp.	01	11,1
	Toxocara sp.	01	11,1
	Estrongilídeo	01	11,1
	Oocisto desconhecido	01	11,1
	Negativo	03	33,3

* Número de amostras positivas para o parasito ou grupo de parasitos observado nas amostras conforme as espécies avaliadas.

** Percentual com que determinado agente foi observado considerando o número total (N) de amostras avaliadas para cada espécie.

*** Parasito específico de aves.

Quando ponderamos a respeito dos grupos de parasitos observados nesse estudo, devemos inicialmente considerar que alguns dos gêneros possuem espécies responsáveis por ciclos zoonóticos, o que pode representar uma situação crítica para a conservação das espécies no local. Dessa forma, os dados obtidos devem ser cautelosamente utilizados, a fim de evitar maiores conflitos entre as comunidades humanas e os carnívoros silvestres, principalmente, por que as análises feitas não permitem que sejam determinadas exatamente as espécies de parasitos presentes no local.

Aiello e Mays (1998) citam como organismos envolvidos em ciclos zoonóticos *Balantidium coli*; diversas espécies de Trematódeos; *Diphylobothrium latum* e *D. pacificum*; *Capillaria hepatica* e *C. aerophila*; *Ancylostoma braziliense* e *A. caninum*; *Toxocara canis* e *T. cati*, todos os gêneros de parasitos observados nas amostras da RNSM. Contudo, naquele levantamento não foi possível determinar se as citadas espécies ocorrem nas populações locais de carnívoros. Entretanto, organismos como *Toxocara canis*; *T. cati*; *Ancylostoma braziliense* e *A. caninum* causadores das doenças humanas denominadas larva Migrans visceral e cutânea (bicho-geográfico) podem ser comuns tanto aos animais domésticos como aos animais silvestres.

Nas amostras colhidas na RNSM, foi notável a alta prevalência de ovos do gênero *Diphyllobothrium*, um parasito da ordem Pseudophyllidea, que possui considerável potencial zoonótico, além de grande capacidade de infectar diferentes espécies de mamíferos, aves, répteis, anfíbios e peixes. Tal agente possui um ciclo de vida que envolve hospedeiros intermediários (HI) principalmente peixes ou moluscos aquáticos, porém o parasito apresenta uma grande plasticidade na capacidade de contaminar outros grupos de vertebrados, tanto de forma intermediária quanto definitiva. A identificação do HI depende de isolamento dos cistos do parasito na musculatura dos animais afetados, o que significa uma grande variedade de peixes, anfíbios, aves e répteis (AIELLO; MAYS, 1998). Dessa forma, o modelo de estudo empregado não permitiu que os principais hospedeiros intermediários para *Diphyllobothrium* spp. fossem determinados naquela área.

Algumas amostras coletadas apresentavam aspecto diarréico e, em algumas dessas, foi possível observar a presença de cistos do protozoário entérico *Balantidium* sp. Em espécies domésticas, os episódios de diarréia representam a principal manifestação clínica desse agente patogênico. Foi possível observar algum grau de diarréia na maioria das amostras fecais de jaguatiricas coletadas, contudo nem sempre foram observados cistos do protozoário nas amostras.

Devemos ainda tecer duas considerações principais sobre o exame da ocorrência de parasitos nas fezes de predadores quando as amostras são coletadas nas trilhas. A primeira é que nas fezes dos predadores podem estar presentes também ovos ou larvas dos parasitos dos animais que serviram como presas. A segunda é que as fezes coletadas do solo podem conter ovos ou outros organismos, trazidos pelas águas da chuva. Dessa forma, os resultados obtidos devem ser observados com cautela. Nesse estudo pode-se considerar como achados incidentais a presença de parasitos como *Ascaridia gali*, parasito específico de aves, sobretudo galiformes. Esses achados eram esperados, pois os ovos de parasitos contidos no trato intestinal das presas podem passar intactos pelo trato digestivo dos predadores. Outro gênero de parasito observado com freqüência foi *Capillaria* o qual possui diversas espécies, em grande parte, específicas de aves. Porém uma grande variedade de mamíferos pequenos e médios também é afetada por parasitos desse gênero. Situação que deixa dúvida se os ovos observados pertenciam aos predadores ou às presas consumidas por eles.

Apesar de muitas dúvidas permanecerem, alguns dados obtidos na RNSM são indicativos de que a pressão humana e de animais domésticos em tempos passados gerou alta densidade de parasitos no ambiente estudado, pois toda a área, há pouco mais de uma década, era intensamente utilizada como rota de deslocamento de pessoas e campos para pecuária. De fato observou-se mais de 90% de amostras positivas para um ou mais parasitos, fato que, em uma criação de animais domésticos, seria motivo para intervenção com tratamento anti-helmíntico, contudo, por serem animais de vida livre, supõe-se que os parasitos e os hospedeiros tendam a manter um equilíbrio saudável. Entretanto, tal suposição vem acompanhada da necessidade de um acompanhamento da população de carnívoros no local, tanto com fins de censo populacional quanto de verificação da incidência e prevalência de parasitos.

Mesmo isoladamente a avaliação parasitária permite comparações superficiais entre áreas e biomas distintos. Para exemplificar essa possibilidade, podemos usar os dados coletados a partir de amostras fecais de *Chrysocyon brachyurus* e *Cerdocyon thous* obtidas no Parque Estadual do Cerrado (PEC), no município de Jaguariaíva – Paraná. Foram avaliadas oito amostras fecais de *Chrysocyon brachyurus*, e quatro de *Cerdocyon thous* entre 20/02/2001 e 20/10/2001. Apenas uma amostra foi negativa tanto para o teste de flutuação como o de sedimentação. Os parasitos encontrados e sua freqüência absoluta e percentual, dentro da amostragem, estão relacionados nas Tabelas 3 e 4.

Em linhas gerais, os resultados observados no Parque Estadual do Cerrado revelam que 83,4% das amostras analisadas foram positivas para um ou mais endoparasitos. Contudo, as amostras estavam pouco contaminadas, não excedendo o escore mínimo de infecção, ou seja, entre um e dois ovos de parasitos por campo de observação ao microscópio binocular. Adicionalmente, essa análise preliminar revela uma pequena variabilidade nas famílias de endoparasitos observadas, as quais se apresentaram em baixa ocorrência nas amostras avaliadas. O que representa uma diferença significativa dos resultados observados na Reserva Natural Salto Morato, em que os resultados apontam maior diversidade de espécies de parasitos

Tabela 3 – Freqüência absoluta e percentual da ocorrência de endoparasitos nas amostras de fezes de *Chrysocyon brachyurus* e *Cerdocyon thous* no Parque Estadual do Cerrado, Jaguariaíva – PR, entre 20/02/2001 e 20/10/2001.

Parasito	Freqüência absoluta	Percentual de ocorrência**
Helmintos típicos de carnívoros		
Família Oxyuridae	3/12	25
Família Spiruridae	4/12	33,3
Trematódeos	1/12	12,5
Ancilostomídeo	1/12	12,5
Estrongilídeo	3/12	25
Larva de nematódeo	2/12	16,6
Trichuris sp.	2/12	16,6
Protozoários		
Isospora sp.	2/12	16,6
Possíveis parasitos de roedores		
Oxyuroidea***	1/12	8,3
Exames negativos	2/12	16,6

* O percentual de ocorrência leva em consideração a presença de ovos e cistos do parasito em relação ao grupo de amostras.

** As presenças de larvas de nematódeos desenvolvidas nas amostras, podem ser referentes a nematódeos de vida livre, ou seja, não parasitos, os quais poderiam ser considerados "contaminantes" da amostra.

*** Uma das amostras apresentou ovos de Oxyuroidea semelhantes aos parasitos de roedores.

Tabela 4 – Prevalência percentual e absoluta de parasitos nas amostras de fezes de carnívoros por espécie amostrada no Parque Estadual do Cerrado, Jaguariaíva – PR, entre 20/02/2001 e 20/10/2001.

Amostra	Parasitos	Número de positivos*	Percentual**
Cachorro-do-mato	Oxyurídeo	01	25
Cerdocyon thous	Estrongilídeo	02	50
N=04	*Isospora* sp.	01	25
	Spiruroidea	02	50
	Larva de nematóide	01	25
Lobo Guará	Oxyurídeos***	03	37,5
Chrysocyon brachyurus	Estrongilídeo	01	12,5
N=08	*Isospora* sp.	01	12,5
	Larva de nematóide	01	12,5
	Trichuris sp.	02	25
	Ancilostomatídeo	01	12,5
	Trematódeo	01	12,5
	Spiruroidea	02	25

* O percentual de ocorrência leva em consideração a presença de ovos e cistos do parasito em relação ao grupo de amostras.

** As presenças de larvas de nematódeos desenvolvidas nas amostras, podem ser referentes a nematódeos de vida livre, ou seja, não parasitos, os quais poderiam ser considerados "contaminantes" da amostra.

*** Uma amostra possuía ovo de Oxyuroidea semelhante aos ovos de espécies parasitos de roedores.

e taxas de infecção maiores. Ainda que esses resultados sejam preliminares e pouco expressivos, não permitindo tratamento estatístico mais profundo, exemplificam satisfatoriamente a complexidade de fatores que podem estar envolvidos nesses ciclos parasitários.

b) Avaliação da dieta

As mesmas amostras coletadas para avaliação do perfil parasitário da população são utilizadas para determinação da dieta alimentar dos carnívoros selvagens. Para a avaliação da dieta as amostras são secas em estufa. A determinação da espécie que produziu as amostras deve ser feita com base no tamanho do bolo fecal, pegadas associadas e pêlos do predador. Após a secagem inicial, a amostra é lavada em água corrente sobre peneira granulométrica de 1,2 e 3 mm de malha, o seu conteúdo separado manualmente (EMMONS, 1987). Os itens encontrados serão separados em pêlos, penas, dentes, unhas, cascos e fragmentos de ossos, para identificação que deve ser criteriosa.

Os itens encontrados nas fezes podem ser quantificados quanto à freqüência de ocorrência e à porcentagem de ocorrência. A medida da amplitude do nicho alimentar pode ser calculada com base no proposto por Garla (1998). Esta estimativa serve para quantificar o grau de especialização da dieta de uma espécie, podendo ser identificada se é distribuída de uma forma uniforme, sem a predominância de qualquer presa, ou se presas específicas são consumidas com alta freqüência. Determinar o perfil alimentar dos carnívoros em estudo contribui também com a avaliação coproparasitológica, pois os ovos e larvas observados nas fezes podem ser provenientes de parasitos dos animais consumidos. Da mesma forma, a presença de predadores extremamente especializados no local de estudo favorece a determinação das cadeias epidemiológicas envolvidas.

c) Avaliação da presença de larvas infectantes no solo

Determinar a ocorrência de larvas infectantes no local de estudo contribui para avaliar as espécies de parasitos envolvidas no ciclo e o grau de contaminação ambiental. As amostras de solo, ou amostras ambientais, devem ser coletadas seguindo uma disposição aleatória dentro dos transectos estudados. Todavia, locais-chave, como a proximidade a cursos de água e carreiros, bastante utilizados, devem ser também contemplados. A quantidade de amostras de solo deve ser baseada no número e extensão dos transectos utilizados, sugere-se ser colhida uma amostra a cada 1.000 m de caminhada percorrida.

A determinação da presença de larvas, ovos e cistos viáveis no solo pode seguir o proposto por Santos (1999). Recomenda-se a coleta de no mínimo 100g de amostra, a qual deve ser obtida da camada mais superficial do solo. Para obter maior representatividade na amostra, deve-se delimitar uma área de aproximadamente 1m² de onde se coletam várias subamostras. O processamento da amostra deve iniciar com a remoção de detritos maiores. Cada 100g de amostra deve ser diluída em 500 ml de água, após isso, deve ser bem homogeneizada e filtrada com auxílio de uma gaze cirúrgica ou peneira fina. As amostras peneiradas devem ser divididas em cinco alíquotas de 100 ml e colocadas para sedimentar por 12 a 24 horas. Após a sedimentação espontânea, o material depositado na base do cálice deve ser centrifugado e observado ao microscópio óptico. A distinção entre larvas de vida livre pode ser feita pela técnica de Cort e Cols descrita por Santos (1999) onde se acrescenta uma solução de formol a 5% na lâmina avaliada. As larvas de vida livre morrem rapidamente enquanto que ancilostomídeos sobrevivem por períodos muito maiores. Outro ponto importante quanto à técnica empregada é que se recomenda fazer a leitura de todo o sedimento obtido a partir da amostra inicial, principalmente quando as amostras são fracamente positivas para larvas e/ou ovos de helmintos, ou cistos de protozoários.

Cabe ressaltar ainda que a avaliação do solo contribui para a identificação das espécies de helmintos e protozoários parasitos presentes no ambiente, possibilitando a avaliação de características morfológicas das larvas permitindo algumas vezes sua classificação taxonômica específica. Adicionalmente, o método permite quantificar o grau de infestação ambiental fornecendo resultados expressos em ovos, larvas ou cistos por 100g/solo.

d) Avaliação parasitológica dos animais domésticos na área e arredores

Avaliar o perfil parasitário dos animais domésticos nas áreas de estudo ou arredores é fundamental para determinar a presença de parasitos comuns para os animais selvagens e domésticos, sobretudo onde há sobreposição das áreas de uso desses dois grupos. A avaliação parasitológica dos animais domésticos também pode ter caráter pontual ou contínuo, o que permitirá a determinação das taxas de prevalência ou incidência, respectivamente, permitindo comparações diretas entre os dados obtidos a partir dos animais domésticos, carnívoros selvagens e amostras ambientais. O padrão de amostragem dos animais domésticos pode obedecer a diferentes métodos, variando com as espécies estudadas, número de animais, tamanho da área e até mesmo com a disponibilidade de recursos financeiros. Thrusfield (1995) ressalta que o tamanho da amostra pode ser definido ainda pelos objetivos e circunstâncias do estudo, propondo uma série de métodos de amostragem.

A amostragem dos animais domésticos deve abordar não apenas cães e gatos, mas também outros grupos taxonômicos como aves, lagomorfos e ungulados, sobretudo quando os carnívoros os utilizam como presas. Da mesma forma, recomenda-se avaliar os animais domésticos também nas áreas onde o contato com os carnívoros selvagens é pequeno e as distâncias com as comunidades humanas são consideráveis, pois a capacidade de dispersão dos agentes parasitários não está determinada para a maioria dos parasitos de importância médica.

e) Avaliação das características ambientais básicas

A caracterização ambiental das áreas deve levar em consideração principalmente aspectos fitossociológicos e grau de conservação ou uso da área. Todavia, características geomorfológicas, macroclimáticas e dos cursos hídricos podem ser incluídas na coleta de dados, reforçando as possibilidades de correlações com a presença e a incidência de macroparasitos no local.

A caracterização fitossociológica pode seguir o sistema fitogeográfico de Veloso et al. (1991), porém deve incluir de forma criteriosa a avaliação do estágio sucessional, pois a maior ou menor exposição do solo e dos rios pode alterar a sobrevivência ou dispersão de ovos e larvas. O grau de conservação e uso da área é talvez o ponto mais importante para ser determinado, porque os fatores antrópicos podem influenciar diretamente o perfil parasitário dos carnívoros selvagens no local. Nas áreas em estudos, devem ser coletados dados referentes à intensidade de uso e à presença ou ausência de fragmentação ambiental, atividade pecuária, extrativismo, caça, proximidade com núcleos habitacionais, rotas de deslocamento de pessoas e animais domésticos.

Entre os demais aspectos que podem ser avaliados, ressalta-se a caracterização dos recursos hídricos e as características do relevo. A disponibilidade de cursos d'água e a grandeza desses podem ser fator limitante na estabilidade das populações de parasitos, bem como a diferença entre áreas de serra e planícies alagadas, por exemplo, pode representar viabilidade ou não da sobrevivência de parasitos, principalmente daqueles de ciclo indireto que dependem de hospedeiros intermediários de hábitos aquáticos. Todavia, as características do solo local também podem ter influência direta sobre a viabilidade de larvas e ovos, principalmente quando se consideram características extremas como solos arenosos ou argilosos muito compactos.

Ainda que de forma subjetiva, é importante avaliar cada uma das características descritas anteriormente. Todavia atribuir graus às atividades antrópicas é fundamental para formar a matriz de dados que permitirá cruzar as informações ambientais com os achados coproparasitológicos. Outro ponto que pode ser considerado como fonte de informação sobre a qualidade da área de estudo é a disponibilidade de presas naturais e ocorrência de encontros dos carnívoros selvagens com pessoas, ou ataque a animais domésticos.

f) Levantamento da história de uso e ocupação humana e animal na região

Levando em conta que a presença de parasitos e hospedeiros em uma determinada área é sujeita a variações dinâmicas, em decorrência de diversos fatores ambientais, é difícil considerar o perfil parasitário de uma determinada população de forma pontual. Certamente o tipo de uso da área no presente é o fator de maior influência sobre essa dinâmica, contudo o histórico de ocupação pode esclarecer a presença de determinados grupos de parasitos, ou a freqüência desses dentro das amostras. Dessa forma, a avaliação da história de ocupação da área estudada deve levar em consideração as principais atividades atuais e da década passada, a história de colonização e, quando disponíveis, índices populacionais, presentes e passados, tanto dos animais domésticos quanto do homem.

Como exemplos da importância dessa análise, podemos tomar novamente o do Parque Estadual do Cerrado (PEC) e da Reserva Natural Salto Morato (RNSM). No PEC observamos menor diversidade de espécies parasitas e menores taxas de prevalência entre os carnívoros estudados, quando comparamos com a RNSM. Em parte tal diferença pode estar relacionada com a história de ocupação dessas unidades; o PEC está isolado por áreas de lavoura e plantio de *Pinus* sp., sua utilização para pecuária foi pouco expressiva e ocorreu há mais de duas décadas. Como contraste temos as altas prevalências e a maior diversidade de parasitos observados na RNSM, que era utilizada intensamente como rota de deslocamento humano e para criação de búfalos domésticos (*Bubalus bubalis*). Todavia não devemos considerar apenas os aspectos da ocupação humana como determinante dessas taxas parasitárias, evidentemente fatores como a susceptibilidade dos hospedeiros envolvidos, as densidades populacionais dos carnívoros e características macroclimáticas locais possuem forte influência sobre a dinâmica atual entre parasito e hospedeiro.

Ainda que esses dados sobre a ocupação da área muitas vezes não contribuam com valores numéricos, o entendimento do histórico local colabora para o entendimento da dinâmica parasitária atual, auxiliando na extrapolação do risco a que outras áreas podem estar submetidas.

Considerações finais sobre o método

A característica transdisciplinar do método permite avaliar em conjunto aspectos importantes da dinâmica populacional de carnívoros selvagens. As variações entre a carga parasitária do ambiente, das espécies selvagens e domésticas, a especificidade da dieta dos carnívoros e as formas de uso do habitat compõem uma matriz de dados que caracteriza, em parte, o estado atual de ameaças sobre a saúde dos carnívoros selvagens. Como o método permite uma caracterização integrada, fornecendo uma imagem ampla da interferência humana sobre os carnívoros selvagens, é possível utilizar as informações de uma determinada população para fazer inferências precisas sobre outras populações, mesmo em ambientes diversos.

Entretanto, o modelo de estudo não permite entendimento completo da dinâmica populacional, pois carece de instrumentos que revelem taxas de natalidade e mortalidade, ou informações mais profundas sobre área de uso dos indivíduos no local de estudo. Contudo, a associação desse método a modelos de estudo de dinâmica populacional e *home range* é facilmente conseguida. Outra forma de estudo clássica que contribui sobremaneira com os resultados desse método é o uso de armadilhas fotográficas, as quais contribuem com a caracterização individual dos animais e, sob certos aspectos, oferece alguma informação sobre o estado geral e sanidade aparente de cada indivíduo fotografado na área de estudo (Figuras 1 e 2). Outro ponto positivo sobre a possibilidade de associar o método a outras formas de estudos populacionais é que, conforme os interesses do estudo o modelo proposto, pode-se adaptar tanto a avaliações rápidas ou longas, fornecendo dados sobre prevalência ou incidência dos parasitos, todavia fornecendo um perfil parasitário da área, sem que sejam necessárias alterações de metodologia.

Figura 1 – Dois exemplares de *Puma concolor* flagrados por meio de adaptadores fotográficos, Reserva Natural Salto Morato, Guaraqueçaba – PR. À esquerda, um exemplar apresentando uma cicatriz cutânea na região torácica. À direita, um exemplar adulto em deslocamento. Ambos apresentando boa condição corporal. (Imagens gentilmente cedidas por G. Paula Vidolin, captadas com adaptadores fotográficos artesanais.

Figura 2 – À esquerda, um exemplar de *Cerdocyon thous*; à direita, um exemplar de *Eira bárbara*, ambos aparentemente saudáveis, com boas condições corporais, evidenciadas principalmente pelas condições de pelagem e musculatura. Reserva Natural Salto Morato, Guaraqueçaba – PR (Imagens gentilmente cedidas por G. Paula Vidolin, captadas com adaptadores fotográficos artesanais).

Quanto à confiabilidade dos resultados obtidos a partir das amostras fecais, podemos verificar que mesmo amostras depositadas no ambiente há alguns dias demonstraram-se úteis, sendo possível determinar a presença de ovos viáveis de parasitos e cistos de protozoários. Ainda devemos considerar que as possíveis contaminações que as amostras poderiam sofrer, recebendo ovos e larvas provenientes do solo ou água, são de menor relevância. Principalmente pelo fato de que o método busca determinar não a saúde individual, mas sim o grau de contaminação ambiental e comparar a carga parasitária na população de carnívoros com outros fatores ambientais. Dessa forma, mesmo que ocorra contaminação da amostra por ovos, cistos ou larvas vindas do solo onde a amostra foi depositada, esses agentes serão também representantes da população de parasitos do local.

Quanto ao valor dos achados parasitológicos, devemos ter clareza de compreender que o método apenas contribui para o entendimento das cadeias parasitárias, porém não permite determinar com exatidão as intrincadas relações entre parasitos e hospedeiros. A principal limitação desse método é o fato de ele estar baseado, sobretudo, nos exames coprológicos, que na maioria das vezes só possibilitam a determinação do gênero do parasito, ou grupo taxonômico superior a este. Como complementação deve-se empregar outras metodologias de análise parasitológica. Incluir a cultura de larvas no método requer mais tempo de uso de laboratórios e maiores custos. Porém essa possibilidade deve ser seriamente considerada, em uma segunda etapa, mediante a presença de gêneros e grupos importantes de parasitos, devendo-se considerar relevantes as espécies de parasitos que poderiam causar altas infestações e maiores taxas de mortalidade, bem como aquelas causadoras de zoonoses ou economicamente importantes para as populações de animais domésticos.

Ainda devemos considerar que a avaliação do solo é fundamental para correlacionar os achados coproparasitológicos com a situação ambiental no mesmo momento, tendo em vista que a transmissão do agente parasitário e a viabilidade dos seus ovos e larvas dependem, em grande parte, de condições ambientais.

Sobre a saúde populacional, devemos considerar que o método não permite determinar o impacto ou a pressão que os parasitos causam diretamente sobre os hospedeiros. A fim de esclarecer tais efeitos, seriam necessários estudos específicos. Contudo, dados obtidos a partir de achados incidentais e da avaliação de animais capturados ou mortos, podem contribuir de forma importante no entendimento sobre a saúde das populações selvagens dos carnívoros neotropicais. Em outras palavras, a presença de exames positivos para diversos parasitos não significa que os carnívoros selvagens estão doentes, quer dizer apenas que os parasitos estão presentes no ambiente. As conseqüências dessa presença dependem de inúmeros fatores, porém sabemos que, em determinadas condições, as interações entre parasitos e hospedeiros podem resultar na morte individual, ou na instabilidade populacional (COMBES, 1996; DOBSON; MAY, 1986).

Em linhas gerais, o modelo de estudo proposto nesse artigo usa inferências quanto ao possível risco que determinados grupos de parasitos podem representar, mediante uma determinada situação socioambiental atual. Tais inferências são baseadas principalmente em achados parasitológicos, na forma e intensidade de uso da área de estudo e arredores, considerando ainda ações humanas tomadas no passado. Nesse aspecto, o principal valor do entendimento histórico sobre a ocupação da área de estudo é caracterizar a evolução local e ajudar a prever possíveis problemas que aconteceriam em outras áreas, mediante ocupações semelhantes. Ainda que os dados apresentados nesse artigo sejam preliminares, necessitando principalmente de amostragem mais abrangente, os resultados são indicativos de que a influência das comunidades humanas sobre as populações de carnívoros pode estar relacionada também com a transmissão de agentes entre animais domésticos e selvagens.

Finalmente, devemos considerar que, a despeito do modelo empregado, os estudos parasitológicos em animais selvagens de vida livre podem ser considerados como uma ferramenta importantíssima para o melhor conhecimento das espécies e sua conseqüente conservação. Adicionalmente, a determinação das prevalências ou incidências parasitárias nas espécies selvagens é uma fonte de informação importante sobre as condições do ambiente e as conseqüências da ocupação humana frente aos diferentes tipos de habitat onde se encontram os carnívoros selvagens.

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Capítulo 19
Impact of viral infections in wild carnivore populations

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Introduction

Microbial pathogens are integral components of natural ecosystems and play an important role in the evolution and ecology of host communities. However, the infection dynamics of many pathogens are changing rapidly in the face of expanding human and domestic animal populations, with important consequences for wildlife health. Although habitat loss, persecution and over-exploitation are thought to have been the most important endangering process for wild carnivores in the past, disease has now emerged as a central issue in carnivore conservation, with several dramatic epidemics and population declines documented in the past 20 years. In all recent surveys of infectious disease in wild carnivores, two key characteristics emerge in relation to those diseases that pose the greatest threat to wild carnivore populations. First, most recent disease outbreaks in wild carnivores have been caused by viral pathogens and second, it is the generalist pathogens that can co-infect and be maintained in more abundant carnivore species (particularly domestic dogs) that pose the greatest threat to small, endangered wild carnivore populations.

In this presentation, we will discuss the impact and implications of infectious diseases in wild carnivores, with particular reference to viral diseases.

Viral diseases

Recent studies indicate that viral diseases are a particular concern in human and veterinary medicine, with viral pathogens disproportionately represented among those classified as emerging human and domestic animal diseases and those of major transboundary importance (TAYLOR et al., 2001; CLEAVELAND et al., 2001). Comparable studies of risk factors for disease emergence in wildlife have not yet been carried out because of a lack of quantitative data on the overall range of diseases affecting wildlife. Nonetheless, viral diseases dominate in many of the recent wildlife disease outbreaks. For example, most of the pathogens causing wildlife epidemics between 1998 and 2000 were viral in origin (DOBSON; FOUFOPOULOS, 2001). A survey of infections of free-ranging large carnivores indicated that the majority (44%) were viral in origin (MURRAY et al., 1999) and a study listing disease outbreaks that caused declines in wild carnivore populations identified viral pathogens as the cause of 12 out of 14 outbreaks (FUNK et al., 2001).

Several explanations have been proposed for the disproportionately high number of viral pathogens associated with emerging diseases in human and domestic animals, and these factors may also play a role in viral infections of wildlife. First, as mutation rates are higher in viruses than in other pathogens, viruses are able to evolve more quickly and are there likely to be successful at exploiting new niches. Second, short generation times and relatively simple life cycles may facilitate sudden increases in abundance or host range (making epidemics appear dramatic). Third, the relative difficulty of treating viral diseases (in humans and domestic animals) may favour the establishment and spread of viral pathogens that can be transmitted to wildlife. Fourth, advances in diagnostic technology allow viruses to be detected more readily now than in the past.

Impact of viral diseases infecting wild carnivore populations

Infectious diseases have a range of direct and indirect impacts on wild carnivore populations. Diseases may affect wildlife populations through impacts on host survival (through morbidity, mortality and an increased likelihood of predation or injury) and host reproduction (GULLAND, 1995). The outcome of infection in individual hosts will depend upon a number of interacting factors, including the virulence of the virus, infective dose and host immunity. These parameters can be modified by other interacting factors, such as malnutrition, stress, and pollutants that affect host immune responses as well as have direct effects on host survival. Several characteristics of carnivore ecology may increase susceptibility to infection and disease (WOODROFFE et al., in press). First, carnivores occupy high trophic levels, which exposes them to infections carried both by prey as well as conspecifics. A high trophic position also increases the likelihood of contamination with immunotoxic chemicals, which may increase susceptibility to disease and the severity of disease. Second, many wild carnivores are susceptible to many diseases derived from domestic dogs (*Canis familiaris*). Domestic dogs are the most abundant and widespread large carnivore, with more than 500 million animals estimated worldwide (WANDELER et al., 1993; Macpherson et al., 2000) and their large populations are capable of sustaining multiple infections and acting as a persistent source of infection for other species. Where dogs are permitted to move around freely, contact with wild carnivores and the risk of disease transmission is likely to increase. Such contact may be exacerbated by the large home ranges of many carnivores, which often extend beyond the boundaries of protected areas. Hence, disease transmission from domestic dogs may constitute an anthropogenic 'edge effect' influencing nominally protected populations (WOODROFFE; GINSBERG, 1998).

Over the past 20 years, considerable advances have been made in our understanding of the way in which pathogens can influence host population dynamics. The dynamics of viral pathogens depend on several key factors relating both to the virus (e.g. transmission characteristics), individual host responses (e.g. immunity, host survival) and host population parameters, such as population size, density, contact patterns, demographic characteristics and spatial distribution (ANDERSON; MAY, 1991). In general, viruses that can be easily transmitted have a short infectious period and cause high host mortality (or immunity) are likely to generate epidemics, with wide fluctuations in disease incidence. As epidemics progress, animals either die or become immune, with the result that the virus may disappear from the host population during the troughs between epidemics (known as epidemic 'fade-out'). (ANDERSON; MAY, 1991). Many questions remain about the mechanisms by which these types of viruses persist in host populations. In many cases, persistence is only possible in host populations that are large enough to generate sufficient new susceptibles (e.g. through birth or immigration) to sustain infection. However, increasing attention is focussed on the importance of spatial, genetic and behavioural heterogeneities in host populations for viral persistence (MAY; ANDERSON, 1984; SWINTON et al., 1998; SWINTON et al., 2002). In contrast to viruses that generate epidemics, viruses that show low pathogenicity and prolonged infectiousness tend to exhibit a relatively high and constant prevalence, with the possibility of persistence in relatively small populations.

The complexity of viral, host and population factors presents a considerable challenge for assessing impacts of viral pathogens on host fitness and the dynamics of wild carnivore populations. Not only is it necessary to detect and identify the causes of disease in individual hosts, but also to relate patterns of infection or disease to temporal changes in host population dynamics. Detection of pathogens is notoriously difficult in free-living wildlife (GULLAND, 1995) and this is particularly true for viruses, which often require specialist storage or collection of field specimens. For viruses, it is usually simpler to detect prior exposure of the host through the presence of antibodies in blood (serology) and the overwhelming body of published data relating to viral infections of wildlife has been derived from seroprevalence studies. These data have limitations in that they demonstrate only that an animal has been exposed at some time in the past. Animals that die from infection, by definition, will not contribute to serological surveys. Thus, a high seroprevalence does not necessarily indicate that the infection has a large impact on host population dynamics; it may simply reflect a high degree of exposure to a virus that is relatively non-pathogenic in that population. Conversely, low seroprevalences may arise when case mortality rates are high and most infected animals die. Without knowing the patterns of mortality caused by the disease in a given population, it can often be difficult to interpret seroprevalence data in isolation. Interpretation is further complicated by the growing recognition that the pattern and impact of viral infections can vary widely, both for the same population at different times, and between different populations of the same species. Despite these caveats, serological data can have great value, particularly if the age of animals is known. For an infection that induces long-lasting antibodies, age-seroprevalence patterns differ in endemic and epidemic situations. With stable endemic infections, seroprevalence increases with age, reflecting the increasing probability of exposure to the pathogen with time. In epidemic infections, a more step-like age-seroprevalence pattern is seen, with most animals alive at the time of the epidemic seropositive, and those born since the epidemic all seronegative. Longitudinal studies have particular value in determining when the exposure occurred and the stability of infection patterns.

In terms of conservation threats, viruses have the potential to cause population extinction directly (in small populations) or indirectly, by reducing the size and resilience of populations, thereby increasing the probability of extinction due to other factors. As many viral pathogens have short infectious periods and cause high mortality, they are predicted to persist only in large, spatially-structured populations with high turn-over (SWINTON et al., 2002). It is not surprising that such pathogens cannot persist in the small, isolated populations typical of many threatened species. However, many carnivore viral pathogens are capable of infecting multiple hosts (CLEAVELAND et al., 2001) and when these pathogens are maintained in relatively common sympatric species, they have the potential to cause extinction through 'spill over' into threatened populations.

Several major reviews have recently focussed on diseases of wild carnivore and wild canid populations (YOUNG, 1994; WOODROFFE et al., 1997; MURRAY et al., 1999; FUNK et al., 2001; WOODROFFE et al., in press). In this review, we aim to present examples that address specific issues relating to the impact of viral infections in wild carnivore populations, focussing on selected major pathogens.

A selected review of carnivore viral infections

Morbilliviruses

Morbilliviruses are a group of RNA viruses that include several pathogens of major medical and veterinary importance, such as measles, rinderpest and peste de petits ruminants. The morbillivirus group is characterized by several emerging pathogens that have caused devastating epidemics in a range of marine and terrestrial carnivores: canine distemper virus (CDV), phocid distemper virus (PDV) and dolphin morbillivirus (DMV).

CDV induces a multisystemic, often fatal disease in a wide and expanding range of carnivore species (HARDER; OSTERHAUS, 1997) and has resulted in population declines in many wild carnivore populations, including species of canids, procyonids, felids, mustelids, and pinnipeds (Table 1). The well-documented example of CDV as a cause of decline and near-extinction of the black-footed ferret (*Mustela nigripes*) was one of the first to highlight the potential threat of infectious diseases to the survival of endangered populations (THORNE; WILLIAMS, 1988). CDV has since been documented as a cause of high mortality in the highly endangered African wild dog, both in Botswana (ALEXANDER et al., 1996) and Tanzania (van de BILDT et al., 2002) and indirect evidence has implicated CDV in the disappearance of African wild dogs (*Lycaon pictus*) from the Serengeti-Mara ecosystem in the early 1990s (ALEXANDER; APPEL, 1994; CLEAVELAND et al., 2000).

Although sporadic cases of CDV have been reported in large cats in captivity (BLYTHE et al., 1983; FIX et al., 1989), CDV had generally not, until recently, been considered pathogenic in cats. However, in the 1990s, outbreaks among large cats in captivity (APPEL et al, 1994) drew attention to CDV as a potential new disease threat for large felids. Subsequently, in 1994, a CDV epidemic killed 30% of lions (approximately 1,000 individuals) in the Serengeti National Park and Masai Mara National Reserve (ROELKE-PARKER et al., 1996; KOCK et al., 1998), the first documented case in free-ranging large cats, and CDV has since been reported as a cause of death in bobcats (WOODFORD, 1995). In hindsight, it appears that CDV may not have been new to felids; a retrospective study of lion and tiger deaths in Swiss zoos between 1972 and 1992 revealed that 19 out of 42 (45%) were antigen positive, with many showing neurological, respiratory or gastrointestinal signs (MYERS et al., 1997). The question remains as to why CDV epidemics in large cats have not occurred earlier (HARDER; OSTERHAUS, 1997). For example, age-seroprevalence data from the Serengeti lion population indicate that lions were exposed to CDV in the early 1980s, but no disease signs or unusual mortality were observed at the time (PACKER et al, 1999). Possible explanations for increased pathogenicity of CDV in large cats include aggravating co-pathogens, predisposing genetic factors or strain variation of the virus. In the Serengeti lion outbreak, co-infection with other viral pathogens appears not to have been a factor (ROELKE-PARKER et al., 1996) and evolution of a new more pathogenic strain considered a more likely explanation (Carpenter et al., 1998). However, since the end of the epidemic, CDV has continued to circulate in Serengeti lions but has caused no major pathogenic effects (CLEAVELAND; PACKER, unpubl.). Many questions still remain about the epidemiology and pathogenesis of CDV in Serengeti lions, but recent data from a lion die-off in the Ngorongoro crater, adjacent to the Serengeti National Park, supports the hypothesis that factors associated with drought, such as poor nutritional status and high parasite burdens, may play a role in increasing host susceptibility to disease (Packer, unpubl. data).

Although CDV was implicated in a die-off of crab-eater seals (*Lobodon carcinophagus*) in the Antarctica as far back as the 1950s (BARRETT, 1999; BENGSTON et al., 1991), concerns have been growing about its recent impact on pinnipeds. CDV caused massive mortality of Lake Baikal seals, *Phoca sibirica*, in 981-1988 (GRACHEV et al., 1989; MAMAEV, 1995) and of

Caspian seals (*P. caspica*) in 1997 (FORSYTH et al., 1998; KENNEDY et al., 2000). The disease is considered a serious risk to the long-term survival of these species (BARRETT, 1999).

A common theme in recent CDV outbreaks in wild carnivore populations is the close similarity between viruses isolated from wild carnivores and sympatric domestic dogs (MAMAEV et al., 1995; CARPENTER et al., 1998; KENNEDY et al., 2000). Although virus strains were not isolated from crab-eater seals in the 1951 epidemic, dogs were implicated as the outbreak occurred in close proximity to unvaccinated dogs stationed at a dog-sled base. Serological and demographic evidence has also implicated domestic dogs as a likely source of infection in the Serengeti CDV outbreak (CLEAVELAND et al., 2000). Domestic dogs are emerging as a crucial factor in wild carnivore diseases and their role will be discussed in more detail in a later section, in relation to CDV as well as other viral diseases.

In addition to CDV, several newly-recognised morbilliviruses have emerged over the past 15 years as a major cause of die-offs in marine mammal species. Phocine distemper virus (PDV) is closely related to, and may have originated from CDV (BARRETT, 1999). In 1988 a PDV epidemic decimated populations of harbour seals (*Phoca vitulina*) in the North Sea, with some mortality in grey seals, *Halichoerus grypus* (DIETZ et al 1989; HEIDE-JØRGENSON et al 1992; SWINTON et al., 1998). It has been suggested that PDV was introduced into the harbour seal population through atypical contact with harp seals, *P. groenlandica*, usually found at more northern latitudes, which are believed to maintain endemic PDV due to their large population size (Goodhart 1988). The outbreak affected all major European harbour seal colonies, with mortality varying between 15% and 58% in different areas. This variation has been attributed to a range of factors, including differing levels of exposure to immunosuppressive PCBs (de KOEIJER et al., 1998), genetic heterogeneity (STANLEY et al., 1996; GOODMAN, 1998) and seasonal variation in contact rates (for example, at haul-out sites) (HÄRKÖNEN et al., 1999).

In the 14 years since 1988, no mortality due to PDV was recorded in the harbour seals, but a new outbreak in 2002 spread rapidly throughout most of the North Sea colonies (JENSEN et al., 2002). With few immune survivors of the 1988 epidemic remaining in the population, immunity probably played a negligible role in the most recent outbreak. However, mortality levels in English and Scottish populations have been lower than in 1988, probably as a result of the timing of the epidemic, which occurred later in the year when most animals were at sea and transmission rates relatively low.

Soon after the 1988 PDV epidemic, another newly-recognised morbilliviruses, dolphin morbillivirus was identified as the cause of mass mortality among striped dolphins (*Stenella coeruleolaba*) in the Mediterranean from 1990 to 1992 (DESWARt et al., 1995) and detected in common dolphins (*Delphinus delphis ponticus*) stranded on the northern shores of the Black Sea in 1994 (BIRKUN et al., 1999). In 1997, approximately 70% of the local population of Mediterranean monk seals, *Monachus monachus*, inhabiting the western Saharan coast of Africa washed ashore in a mass die-off. A morbillivirus, closely resembling a previously identified dolphin morbillivirus, (DMV), was detected in the tissues of these animals and attributed as the cause of mortality (OSTERHAUS et al., 1997). However, the primary cause of mortality and the exact role of morbilliviruses have been debated. The die-off also coincided with high concentrations of dinoflagellates in coastal waters, suggesting that algal toxins may be involved (HERNANDEZ et al., 1998). Algal toxins may have interacted with morbilliviruses in earlier epidemics of marine mammals, possibly contributing to the deaths of half a million Cape fur seals (*Arctocephyalus pusillus*) in the 1820s and mass mortality of bottlenose dolphins (*Tursiops truncates*) off the eastern seaboard of the US in 1987 (HARWOOD, 1998). Other toxins, including pollutants such as PCB and DDT, also increase susceptibility or severity of viral diseases in marine mammals and may contribute to the emergence of viral diseases in some populations (ROSS, 2002). Indeed, higher concentrations of PCB have been detected in harbour porpoises dying from infectious diseases than those dying from other causes (mainly traumatic) (JEPSON et al., 1999) and toxic environmental contaminants have been shown to have adverse effects on virus-specific immune responses in seals (ROSS et al., 1996).

Serological studies have shown that morbilliviruses are widespread among cetaceans and pinnipeds and are probably transmitted periodically between species (DUIGNAN et al., 1995; MULLER et al., 2002). In the case of monk seals, transmission of cetacean morbilliviruses from other species is probably not an infrequent event, with distinct viruses isolated from seals in Mauritania and Greece (OSTERHAUS et al., 1998). The potential for inter-species transmission of marine morbilliviruses coupled with their widespread distribution and circulation in more abundant species, undoubtedly poses a conservation threat for small, endangered populations. Particularly worrying is the apparent ease of morbillivirus transmission from species of different taxonomic orders, not just close relatives (HARWOOD, 1998).

Viral infections may also predispose animals to other causes of mortality and morbilliviruses have been implicated as a factor in strandings of both cetacean (DUIGNAN et al., 1995; BIRKUN et al., 1999) and pinniped species (JAUNIAUX et al., 2001). However, the relative impact and importance of viral infections in marine mammal strandings remains unknown.

Rabies

Rabies is a RNA virus that causes a fatal neurological disease in a wide range of mammalian species including humans. Rabies occurs throughout the world, but extensive disease control initiatives have largely contained the public health threat in Europe and North America and it is the developing world that carries the major burden of the disease. The World Health Organization (WHO) reports approximately 35,000 human deaths each year (WHO, 1998) with the majority occurring in the developing world, but this figure almost certainly underestimates the true magnitude of the problem. Rabies is widely considered to be a growing problem throughout Africa and Asia (PERRY, 1995; CLEAVELAND, 1998; WHO, 2002). In many African countries, the incidence of disease has increased dramatically over the past three decades, with evidence that infection is expanding, both in terms of geographic range and the number of species affected (PERRY, 1995).

Although all mammals may be infected by rabies, not all species are capable of acting as a reservoir. Whether a population can act as a reservoir host depends on several interacting factors involving the virus strain, host demographics, social behaviour, and the environment (WANDELER, 1991). Throughout the world, carnivores and bats are the principal reservoirs of the virus (Table 2). Carnivore reservoir hosts are characterized by opportunistic species that are capable of attaining high population densities (facilitating virus transmission) and with high birth rates (allowing rapid generation of new susceptible hosts following epidemics). The paradigm of rabies epidemiology is that, within a given geographic area, distinct antigenic and genetic strains are maintained in a single host population, with other species infected by 'spill-over' transmission (SMITH, 1989; RUPPRECHT et al., 1991; KING et al., 1994). Although other species may sporadically acquire infection from the principal host, they appear unable to maintain infection independently.

Rabies has the potential to cause disease in all mammals but species vary in their susceptibility. Canids, such as foxes and jackals, appear to be highly susceptible, whereas other carnivores, such as felids and hyaenids less susceptible. A recent study in the Serengeti has identified a novel pattern of infection, with spotted hyaenas appearing to maintain a distinct strain of rabies virus within the population, without any apparent signs of disease or death and no evidence of transmission to other host species (EAST et al., 2001).

Rabies illustrates two key issues with respect to disease impacts in wild carnivore populations: (a) extinction threats to endangered populations and (b) human-wildlife conflicts arising from the public health threat of the disease.

Conservation threats

Over the past 15 years, rabies has been identified as a major threat to several rare and endangered wild canid species (reviewed by MACDONALD, 1993). Here we present two case studies illustrating some of the conservation implications of rabies in (a) the Ethiopian wolf (*Canis simensis*) and (b) the African wild dog (*Lycaon pictus*).

The Ethiopian wolf is the world's most endangered canid, with about 500 individuals surviving in small, isolated and fragmented populations in the Ethiopian highlands (SILLERO-ZUBIRI; MACDONALD, 1997). Outbreaks of rabies in the early 1990s resulted in the loss of almost 70% of wolves in the largest and most critical population in the Bale Mountain National Park (SILLERO-ZUBIRI et al., 1996). Only now, more than ten years later, are pack numbers and sizes approaching pre-epidemic levels (HAYDON et al., 2002). Domestic dogs living in or near wolf habitat are the most likely reservoir of rabies and source of infection for wolves. With the continued expansion of these dog populations, the potential for rabies transmission remains a high risk and poses the most immediate threat to the survival of the Ethiopian wolf (LAURENSON et al., 1998).

Rabies has posed a similar threat to the African wild dogs in the Serengeti-Mara ecosystem of east Africa. Over the past four decades, the African wild dog has been declining throughout its range in sub-Saharan Africa, with fragmentation and isolation of populations. Declines have been attributed to a range of factors, including habitat loss, persecution and competition. However, in the late 1980s and early 1990s, rabies was suspected or confirmed in several packs that disappeared in the Serengeti-Mara ecosystem (GASCOYNE et al., 1993; KAT et al., 1995; WOODROFFE et al., 1997). Subsequently, in 1991, the population became locally extinct. Although disease was thought to be a factor in the final extinction event, the exact cause is unknown and remains the subject of debate (MACDONALD et al., 1992; BURROWS et al., 1994; KAT et al., 1995; WOODROFFE, 1997). Nonetheless, rabies was clearly a major factor in the demise of the population.

As with the Ethiopian wolf, the close similarity of viral strains isolated from wild dogs and domestic dogs, coupled with epidemiological evidence for persistence of disease in domestic dogs, suggests that domestic dogs are the likely reservoir and source of infection for wild dogs in the Serengeti (KAT et al., 1995; CLEAVELAND; DYE, 1995).

A feature of viral disease outbreaks in African wild dogs has been the loss of complete packs associated with outbreaks of both rabies (WOODROFFE, 1997) and CDV (ALEXANDER et al., 1996). Once a pathogen is introduced into a group, transmission between individuals can occur rapidly, resulting in high infection rates. For social species, a single transmission event (i.e. introduction into the group) can therefore have a severe impact on populations. However, even in close social groups, it is unlikely that pathogens infect or kill 100% of individuals. The failure of surviving individuals to re-establish packs in the wake of epidemics may be a manifestation of the Allee effect. African wild dogs are obligate co-operators requiring helpers and, below a threshold pack size, inverse density dependence may occur, increasing the probability of pack extinction (COURCHAMP et al., 2000). In cooperative species, any disease agent that reduces a social group to below this threshold has the potential for disproportionately large impacts on the population.

Population viability analyses

The growing recognition of disease threats for endangered carnivores has prompted several studies using population viability analyses (PVAs) to evaluate the impact of different pathogens on extinction risks. Most studies include disease simply as an additional mortality factor in models, such as the VORTEX-based models (MACE; SILLERO-ZUBIRI, 1997; GINSBERG; WOODROFFE, 1997) and individual-based models (VUCETICH; CREEL, 1999), while more sophisticated approaches have explicitly incorporated epidemiological dynamics (HAYDONet al., 2002). In all cases, diseases that cause high adult mortality (such as rabies) appear to be a significant threat to small populations of Ethiopian wolves and African wild dogs and a critical factor determining their persistence. In contrast, diseases that cause moderate mortality (such as CDV), or where mortality occurs mainly in pups and juveniles (e.g. canine parvovirus) have relatively little effect on population persistence (HAYDON et al., 2002; GINSBERG; WOODROFFE, 1997).

Viruses as a source of human-wildlife conflicts – indirect impacts on wild carnivores

Rabies illustrates a growing area of concern in wildlife disease management, which relates to the potential for conflict in situations where wildlife act as reservoirs of human diseases. Recent studies have identified pathogens that co-infect wildlife as a particular risk for emerging human diseases (CLEAVELAND et al., 2001). This may reflect changing patterns of contact between humans and wildlife, arising for example through increased size and mobility of human populations, expansion of human-related activities and encroachment into wildlife areas. Whatever the underlying cause, identification of wild carnivores as a source of infection for humans generates the potential for conflict. For example, in the past, rabies control measures have largely relied upon culling of wildlife reservoirs (such as foxes) to control disease. Culling is one of the few tools available to control infectious diseases in wildlife, reducing population sizes below the threshold density required for persistence. However, in practice, culling has largely been ineffective for rabies control (MACDONALD, 1980; AUBERT, 1994; ARTOIS et al., 2001). Culling is costly, difficult to implement and requires high levels of sustained effort to be effective. Furthermore, culling may disrupt social behaviour and alter contact and dispersal patterns, which may enhance, rather than reduce, transmission and dissemination of infection. The recent development of oral rabies vaccines for mass immunization of wild and domestic carnivores provides a potential solution for disease control in wildlife. However, oral vaccines are currently only available for rabies and trials still needed before their implementation in any new setting (WHO, 1994).

Parvoviruses

Various parvoviruses infect and cause disease in a range of wild carnivore species. These include several closely-related viruses, such as feline panleukopenia virus (FPV), canine parvovirus (CPV-2), mink enteritis virus (MEV), Aleutian mink disease (ADV) and racoon parvovirus (RPV). Parvovirus infections in wildlife have recently been reviewed in detail (Steinel et al., 2001) and host ranges of the different viruses are summarized in Table 3.

The epidemiology of parvoviruses is characterized by acute infection, transient faecal excretion of virus, long-lasting immunity and persistence of virus in the environment for prolonged periods. In comparison to rabies and morbilliviruses, the period of infectiousness is relatively long and once infection is introduced into an area, it is likely to persist. Viral infection dynamics might therefore be expected to follow an endemic pattern. However, evidence for this is contradictory. In California coyote populations, CPV seroprevalence increased with age, suggestive of an endemic pattern of infection (with increasing probability of exposure with age) (CYPHER et al., 1998). CPV seroprevalence was remarkably constant in coyotes in Colorado over a four-year period (1985-1988) (GESE et al., 1991), also consistent with a stable endemic pattern of infection. In contrast, the pattern of feline parvovirus exposure in Serengeti lions appears more episodic, with significant variation in seroprevalence of juveniles between years (PACKER et al., 1999). Consistent with this, CPV age seroprevalence data in Serengeti domestic dogs is more suggestive of epidemic, rather than endemic, infection, and temporal and spatial patterns difficult to explain if CPV persists for prolonged periods in the environment (CLEAVELAND, unpub.).

The age of an infected animal is an important determinant of infection, as fetal and newborn tissues are rich in mitotically active cells, which are required for parvovirus replication (STEINEL et al., 2001). In younger animals, brain tissues and myocardium are often affected, causing neurological signs and myocarditis. In adult animals, dividing cells of the gut epithelium and lymphatic tissues are targets for viral infection, leading to different disease manifestations characterized by gastroenteritis, lymphopenia and leukopenia.

For CPV, the major concern in wild carnivore populations relates to pup mortality, with substantial mortality reported in young wolves (Peterson & Krumenaker, 1989; Johnson et al., 1994) and coyotes (*Canis latrans*) (THOMAS et al., 1984). For wolves, CPV has not been shown to cause population declines, but may hinder recovery after a decline (MECH; GOYAL, 1993). Although there is no direct evidence that CPV has caused mortality in free-living African wild dogs, some populations have been exposed to infection and it is reasonable to hypothesise that CPV is a factor in some pup deaths (CREEL;CREEL, 2002). A comparison of litter sizes in different populations suggested that exposure to CPV might reduce litters before pups emerge from the den, but does not cause significant mortality after the denning period or low recruitment of yearlings (CREEL et al., 1997).

Feline immunodeficiency virus

Feline Immunodeficiency virus (FIV) is a novel lentivirus within the Retroviridae family, first isolated from a domestic cat in 1986 (PEDERSEN; BARLOUGH, 1991). Serological surveys have subsequently shown that a wide range of non-domestic felids have FIV antibodies (OLMSTED et al., 1992; BROWN et al., 1993). Seroprevalence varies greatly between different species and different wild populations, and FIV is endemic in certain natural populations, but absent in others. Seroprevalence is highest for both free-ranging and captive lions. However, patterns vary widely in wild populations. For example, infection appears to be endemic in lions in Kruger National Park (South Africa) and Serengeti National Park (Tanzania). In the Serengeti, 91% lions are seropositive and seroprevalence reaches 100% in animals over four years of age (PACKER et al., 1999). In contrast, lions in Namibia (Etosha National Park) and India (Gir Forest) are seronegative (Kennedy-STOSKOPF, 1999). The patterns of infection suggest that natural geographic barriers probably restrict the distribution of the virus in wild populations (BROWN et al., 1993). However, 8 out of 31 lions tested in Botswana were FIV seropositive and concerns exist about the spread of the virus into Namibia (OSOFSKY et al., 1996).

Although infection rates are highest in the lion, exposure to FIV has also been detected in other species, including nearly all natural populations of the North America puma (*Felis concolor*) and in one cheetah population (BROWN et al., 1993).

FIV causes T-cell deficiency in the domestic cat, but clinical manifestations of the disease vary and are often non-specific, with signs such as generalized lymphadenopathy, fever, depression and neutropaenia leading to progressive secondary infections. Although FIV is pathogenic in domestic cats, the impact in non-domestic felids, both at the individual and population level is uncertain. FIV-associated clinical signs have been reported in zoo lions (KENNEDY-STOSKOPF, 1999), but there is currently little evidence for morbidity or mortality among free-ranging lions. One difficulty is that where FIV is endemic, infection rates are high with few uninfected individuals in the population to allow comparison of fitness traits. Limited data from the Serengeti lion population demonstrate no detectable effects of FIV infection on mortality and no clinical signs of immune deficiency have been seen in the population except during the CDV outbreak (PACKER et al., 1999). However, FIV is not thought to have played a major role in the epidemic, as there was no association between clinical pathology and FIV status of CDV victims (ROELKE-PARKER et al., 1996).

Human impacts

Anthropogenic factors are likely to affect the infection dynamics of viral pathogens of carnivores in many different ways. Here we highlight three key areas in relation to (a) domestic carnivore populations, (b) direct transmission from humans to wildlife and (c) changing land-use patterns.

The role of domestic carnivores

In many of the examples illustrated in this review, disease transmission from domestic carnivores poses a direct threat to wild carnivore populations. In developing countries, several factors may interact to increase the threats of spill-over infection from domestic animals to wildlife, including the rapid growth and expansion of human (and domestic animal) populations, increasing mobilisation of rural populations and a deterioration in the infrastructure and resources for veterinary services and animal disease control (CLEAVELAND, 1998). Throughout much of the developing world, domestic dog populations are growing faster than human populations (between 5-10% per annum) and are characterized by high birth and death rates (CLEAVELAND, 1996; BUTLER, 1998; KITALA et al., 2001). Rapid population growth, coupled with high birth rates, generates a rapidly increasing supply of susceptible animals to maintain cycles of infection. Thus, populations which previous could sustain only sporadic, short-lived epidemics may now be large enough to maintain infection and act as reservoirs of diseases for wild carnivores. Increased risks of transmission are most evidence where human population expansion occurs close to protected areas, as in the Serengeti, Tanzania. During the past two decades, the annual rate of human population growth in villages close to the protected area boundary has been substantially higher than the national and regional average (CAMPBELL; HOFER, 1995). Similarly, in Ethiopia, human settlements have increased substantially over the past last ten years in areas close to the Bale Mountains National Park (STEPHENS et al., 2001), bringing dogs into increasing contact with Ethiopian wolves.

Human-wildlife disease transmission

For primates, disease risks associated with contact with local people and tourists have long been recognised, with outbreaks of measles in mountain gorillas (HASTINGS, 1991), and polio and pneumonia in chimpanzees (VAN LAWICK-GOODALL, 1971; TANAPA, 2001) attributed to transmission from people. Although fewer examples exist of human-to-carnivore disease transmission, 24% (n=344) of all known human pathogens can also co-infect carnivores (CLEAVELAND et al., 2001), raising the potential for transmission between humans and carnivores. The recent emergence of the human bacterial pathogen, *Mycobacterium tuberculosis*, as a cause of severe epidemics in meerkats (*Suricata suricatta*) and banded mongooses (*Mungos mungo*) in southern Africa highlights this potential threat (ALEXANDER et al., 2002). In Caspian seals, detection of antibodies to strains of influenza A and influenza B prevalent in the human population has been taken as evidence of transmission from humans to seals (OHISHI et al., 2002). A particular concern relates to the human HIV/AIDS epidemic, as co-infection with HIV has the potential to enhance transmission of other pathogens. High levels of pathogen excretion coupled with increasing contact between humans and wildlife will almost inevitably result in the emergence of new wildlife epidemics involving human pathogens.

Changing land-use patterns

In addition to the growth of domestic dog populations, human activities may also affect carnivore viral infection dynamics through land-use changes and effects on social behaviour. For example, changes of habitats through cultivation or urbanization generally result in reduced biodiversity, which may favour opportunistic species, such as foxes, jackals and raccoons that tolerate and can exploit these changes. These species are thus able to attain densities far higher than those in species-rich conservation areas, and their population may then exceed the threshold for maintenance of infectious diseases, such as rabies. A second factor relates to persecution of wildlife species on agricultural land, which may disrupt social systems and behaviour patterns resulting in increased contact rates and aggressive encounters between animals.

Although natural ecosystems have always responded to change, it is likely that the rapid growth and expansion of human activities has accelerated the pace of ecological perturbation. It remains to be seen how rapidly wildlife populations can respond to changes in infectious disease dynamics. As diseases become more persistent and outbreaks more frequent, selection for disease resistance is likely to become more intense. However, evolutionary time scales required for the evolution of resistance are unlikely to be sufficiently rapid for many endangered populations currently threatened by disease.

Indirect effects

Viral impacts on community structure

Although the focus of this review is the direct impact of viral infections on wild carnivore populations, some viruses affecting herbivores have major repercussions on populations, communities and ecosystems, with profound indirect impacts on wild carnivores. Rinderpest, a highly virulent fatal disease of cattle and wild ungulates caused by a morbillivirus, provides a classic example. Over the past 100 years, rinderpest has been a crucial factor shaping the Serengeti ecosystem and the consequences of the great pandemic that ravaged eastern and southern Africa at the turn of the last century resulted in the establishment of the Serengeti as a wildlife-rich area (SINCLAIR, 1979). Following the implementation of mass cattle vaccination in the 1950s, rinderpest was eliminated from the reservoir host (cattle) and subsequently disappeared from wildlife. As a result, the wildebeest population, now released from the limiting mortality of rinderpest, increased from about 200,000 animals in the 1960s to a carrying capacity of about 1.3 million in the late 1970s (SINCLAIR, 1995). The increase in resident and migratory prey led to a rise in the number of lions and hyaenas (HANBY; BYGOTT, 1979), which in turn have probably limited the number and distribution of smaller endangered carnivores, such as the cheetah (Kelly et al., 1998) and African wild dogs (CREEL; CREEL, 1996). The introduction and subsequent elimination of rinderpest have thus triggered two major perturbations in the Serengeti, with consequences for both herbivore and carnivore populations.

Table 1 – Examples of morbillivirus diseases that have resulted in population declines in wild carnivore populations.

Species	Locality	Cause	Reference
Black-footed ferret	USA	CDV	Williams et al., 1988
African wild dog (*Lycaon pictus*)	Botswana Tanzania (Mkomazi) Tanzania (Serengeti)	CDV CDV CDV	Alexander et al., 1996 Van de Bildt et al., 2002 Schaller, 1972; Cleaveland et al., 2000
African lions	Tanzania (Serengeti) Kenya (Masai Mara)	CDV CDV	Roelke-Parker et al., 1996; Kock et al., 1998
Lake Baikal seals	Lake Baikal	CDV	Grachev et al., 1989; Mamev et al., 1995
Caspian seals	Caspian sea	CDV	Kennedy et al., 2000
Crab-eater seals	Antarctica	CDV	Bengston et al., 1991; Barrett, 1999
Channel Island foxes	Santa Catalina Island, California, USA	CDV	Timm et al., 2000; 2001
Harbor seals	North Sea (1988, 2002)	PDV	Heide-Jorgensen and Härkönen, 1992; Dietz et al., 1989; Jensen et al., 2002
Mediterranean monk seals	Mauritania	DMV/toxin?	Osterhaus et al., 1998; Hernandez et al., 1998
Striped dolphins	Mediterannean	DMV	Domingo et al., 1990
Bottlenosed dolphins	NW Atlantic Gulf of Mexico	DMV DMV	Lipscomb et al., 1994 Lipbscomb et al., 1996
Common dolphins	Black sea	DMV	Birkun et al., 1999

Table 2 – Characteristics of Carnivora species that can act as reservoirs of rabies. It is important to note that a particular species may act as a reservoir for rabies in only in a limited part of its geographic range. All species are small to medium-size (0.4-20 kg) species, usually generalist foragers and have the potential to attain high host densities and high birth rates. Table adapted from WANDELER, 1991.

Species	Distribution	Food sources	Density/km²
Canis familiaris (domestic dog)	Worldwide – free-roaming populations common throughout Africa, Asia and latin America	Generalist – household refuse	5-10 (rural Africa)[1-3] >200 (urban)[4] >300 (Korea)[5]
Canis aureus *Canis mesomelas* *Canis adustus* (jackals)	Asia, Africa, Middle East Africa Africa	Generalist – refuse, carrion, invertebrates, small mammals	0.4-7.1 (Zimbabwe)[6] 4-7 (Botswana)[7] 0.4 (Natal)[8]
Vulpes vulpes (red fox)	Europe, northern Asia, NE North America	Generalist – refuse, carrion, invertebrates, small mammals	0.5 – 6 (Europe)[9]
Mephitis mephitis (skunk)	American Midwest, California	Generalist – refuse, carrion, invertebrates, small mammals	0.7-20 (USA)[10]
Procyon lotor (raccoon)	SE and mid-Atlantic USA	Generalist – refuse, carrion, invertebrates, small mammals	3-35 (Niagara)[11]
Herpestes sp. (mongooses)	Carribean islands SE Asia (role as reservoirs unclear)	Generalist – refuse, carrion, invertebrates, small mammals	250-1260 (Grenada)[12]
Otocyon megalotis (bat-eared foxes)	Eastern and southern Africa	Specialist – termites, beetles	1.1-3 (up to 28)[13]

References: 1. Cleaveland (1996) 2. Kitala et al. (2001) 3. Butler (1998) 4. Eng et al. (1993) 5. Lee et al (2001) 6. Foggin (1988) 7. McKenzie (1993) 8. Rowe-Rowe (1976) 9. Wandeler (1991) 10. Olson and Lewis (1999) 11. Flack (1986) 12. Everard and Everard (1985) 13. Malcom (1986).

Table 3 – Parvovirus infections in wild carnivores (data from Steinel et al., 2001; Ikeda et al., 2001).

Virus	Host species	Characteristics of disease
Feline Parvovirus Group	Felidae, canidae, mustelidae, procyonidae	Acute disease can result in fatality, but subclinical infections with or without mild clinical signs are common. Infection induces long-lasting immunity with elimination of virus
Feline panleucopenia (FPV)	Domestic cats, felids, raccoons, arctic foxes*, (hedgehog?, beaver?, porcupine?)**	Has caused disease in wide range of non-domestic felids (lions may be more resistant), raccoons, arctic foxes; impact in wild carnivore populations uncertain
Canine parvovirus-2	Canidae, domestic cats, cheetahs Siberian tiger (large cats may be more susceptible than domestic cats), stone marten	Clinical signs observed in coyotes, dingo pups, wolves, maned wolves; mainly affects pups; most data from serological surveys and impact not certain
Mink enteritis virus	Mink; can replicate in cats	High case fatality in mink, occurs worldwide in farmed mink
Canine Parvovirus Group		
Aleutian mink disease virus	Mink, ferrets	Chronic, persistent and progressive disease; common in farmed mink and ferrets; impact in free-ranging carnivores unknown
Canine minute virus (CPV-1)	Domestic dogs only	Mild disease, mainly affects young pups

* Arctic fox isolate may represent a virus intermediate between FPV and CPV-2 (STEINEL et al., 2001)
** Parvovirus infection suspected on histopathology and immunohistochemisty

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Capítulo 20
O impacto potencial de doenças bacterianas e protozoários patogênicos sobre populações selvagens de carnívoros brasileiros

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Introdução

Doenças infecciosas possuem grande influência sobre a conservação da biodiversidade e podem contribuir significativamente para a inclusão de diversas espécies da fauna selvagem na lista de animais em extinção (AGUIRRE et al., 2002). Quando comparada com a destruição dos ecossistemas, a poluição e a ação antrópica causada, em geral, pela construção de hidrelétricas, condomínios, rodovias, entre outras, as doenças infecciosas parecem ter um menor papel na diminuição da biodiversidade. Essas doenças normalmente existem como eventos constantes na natureza, cujos animais selvagens são hospedeiros de vasta gama de parasitas, sejam eles bactérias, fungos, vírus, riquétsias, protozoários, helmintos ou ectoparasitas (SCOTT, 1988). O parasitismo é ecologicamente tão importante quanto a predação, a competição ou qualquer outra relação ecológica (LYLES; DOBSON, 1993).

As doenças infecciosas sempre representaram uma importante força na geração e manutenção da biodiversidade e, hoje, paradoxalmente, representam uma séria ameaça de extinção para muitas espécies (GOMES, 2002). Da mesma forma, doenças que seriam consideradas normais com ausência de impacto significativo nas populações animais em diversas circunstâncias podem ter conseqüências graves em populações reduzidas ou fragmentadas (AGUIRRE et al., 2002).

Dessa maneira, o risco das doenças emergentes da vida selvagem pode aumentar à medida que crescem o contato com humanos e seus animais domésticos, e as alterações ecológicas locais (DASZAK et al., 2000; Cleaveland et al., 2002). Isso particularmente torna-se muito importante para os predadores naturais, pois o aumento dos ataques aos animais domésticos, como gado bovino e galinhas, nas fazendas próximas às áreas naturais, também podem representar um risco de infecção e fatores de risco para esses animais (CLEAVELAND et al., 2001; TAYLOR et al., 2001).

Diversas espécies de carnívoros são suscetíveis a doenças bacterianas e causadas por protozoários tais como: leptospirose, salmonelose, clostridiose, toxoplasmose, leishmaniose, babesiose. Além disso, os carnívoros selvagens e ferais podem servir como fontes de infecção, reservatórios e portadores de agentes etiológicos (bactérias e protozoários).

No Brasil, existem poucos estudos sobre o papel dos carnívoros neotropicais na epidemiologia das doenças bacterianas e daquelas causadas por protozoários e o impacto destes agentes nas populações selvagens. Da mesma forma, existem poucos relatos sobre a morbidade e a mortalidade dos carnívoros selvagens devido a doenças tropicais. O maior número de estudos realizados foi em populações de lobos-guarás (*Chrysocyon brachyurus*), cachorros-do-mato (*Cerdocyon thous*), jaguatiricas (*Leopardus pardalis*), suçuaranas (*Puma concolor*) e onças-pintadas (*Panthera onca*), abordando ecologia, distribuição, hábitos alimentares e avaliação da área de vida buscando a oferecer mais conhecimento para a preservação das espécies (PESSUTTI et al., 2001).

Os agentes patogênicos – bactérias e protozoários – são geralmente transmitidos para os carnívoros pela via oral, pela ingestão de água e por alimentos contaminados; por vetores biológicos e mecânicos (moscas, pulgas, piolhos, carrapatos, etc.); pelo contato direto com a pele, por meio de feridas e mordeduras; pelo contato com urina, fezes, aerossóis, fômites e solos contaminados; ou pela via transplacentária e venérea.

Com objetivo de melhor analisar o impacto das doenças nas populações selvagens de carnívoros é importante caracterizar a diferença entre infecção e doença. A infecção pode ser definida como a presença de um agente patógeno microparasita (bactéria, vírus, protozoário) ou macroparasita (helminto, artrópode parasita) sem produzir sinais clínicos no hospedeiro ou na sua população. Já a doença usualmente refere-se a uma condição clínica que deve ser observada ou mensurada. A distinção entre a sua presença ou ausência dependerá da realização de um exame clínico e/ou de um diagnóstico laboratorial (SCOTT, 1988).

A presença de estágios nos ciclos biológicos dos parasitas e na cadeia epidemiológica das doenças na vida selvagem pode indicar o potencial da infecção numa população de hospedeiros. Embora a doença seja geralmente caracterizada como uma condição associada a um indivíduo animal, ela também pode ser relacionada como uma condição populacional. A avaliação da presença de doenças numa população depende de análises das características demográficas das populações de hospedeiros relacionadas aos estudos epidemiológicos (SCOTT, 1988). O mesmo autor afirmou que existem poucos exemplos para mostrar o impacto de alguns patógenos como causadores de doenças e sua influência na sobrevivência desses hospedeiros. Entretanto, agentes bacterianos e protozoários, mesmo que em menor escala que os agentes virais podem indiretamente afetar a sobrevivência dos carnívoros selvagens devido ao aumento da suscetibilidade à infecção.

Em várias situações, carnívoros capturados em vida selvagem, por alguma fatalidade, foram encaminhados a parques zoológicos, centros de triagem e reabilitação, e nestes estabelecimentos, os animais também poderiam sofrer danos irreparáveis. Em condições de cativeiro, diversos elos do equilíbrio parasita-hospedeiro podem ser rompidos. A subnutrição decorrente do desconhecimento das necessidades básicas das diferentes espécies, a limitação espacial que vai predispor a reinfecção ou reinfestação, assim como o estresse crônico, decorrente de ambientes inadequados, totalmente diversos dos nichos ecológicos ocupados pelos animais, pode produzir alterações orgânicas e comportamentais, que favorecem a instalação de doenças e o desenvolvimento de quadros clínicos mais severos (MUNSON; COOK, 1993).

Dessa forma, o cativeiro oferece diversas oportunidades para o estudo de animais selvagens em situações controladas e proporciona a aplicação de novas tecnologias no diagnóstico das enfermidades transmissíveis nesses animais (MUNSON; COOK, 1993) e constitui importante fonte de informação para estudos epidemiológicos (THRUSFIELD, 1995).

Não é incomum o diagnóstico de agentes patógenos e doenças em zoológicos. Esses resultados positivos levam a duas hipóteses. A primeira é a de que os carnívoros se infectaram no próprio zoológico por meio de contaminação ambiental ou outra via de transmissão pela inerente suscetibilidade do animal ao agente infeccioso. A segunda hipótese reside no fato dos carnívoros já chegarem infectados da natureza e atuarem como fontes de infecção, reservatórios ou portadores, e por algum estímulo de ordem imunossupressiva ou de outra natureza, o agente se multiplica e provoca a infecção ou doença no hospedeiro.

A disseminação de agentes patógenos em áreas naturais pode estar relacionada também com a presença de cães (*Canis familiaris*) e animais domésticos residentes nas propriedades ao entorno de Unidades de Conservação e na participação de cães errantes ou ferais na transmissão de doenças para os carnívoros selvagens. Há relatos na literatura de estudos realizados buscando o melhor entendimento desta questão (MARVULO et al., 2002; CANON-FRANCO et al., 2003; SILVEIRA; JÁCOMO, 2003 – Comunicação pessoal). Importante considerar também que populações de animais selvagens podem servir como fontes de infecção para a população de domésticos. Esta análise de ambas vias de transmissão constitui de um imenso objeto de estudo para a medicina da conservação.

Neste artigo apresentamos o impacto das doenças bacterianas e causadas por protozoários nas populações de carnívoros selvagens. A escassez de literatura sobre este tema em populações de vida livre conduziu-nos a referenciar trabalhos com carnívoros selvagens em cativeiro, já que esse indivíduo poderá atuar como fonte de infecção, reservatório ou portador do agente, tanto em cativeiro como em vida livre.

Doenças bacterianas

Clostridioses

O *Clostridium perfringens* compreende um grupo de tipos de microorganismos formadores de toxinas. As enterotoxemias podem ser produzidas por todos os tipos de *C. perfringens*, A, B, C, D e E. A enterotoxemia pelo tipo A já foi referenciada em carnívoros como marta e raposa-prateada (KOHLER; BEER, 1999). Em cativeiro, no Brasil, os lobos-guarás apresentaram diarréia hemorrágica, anorexia e desidratação quando infectados por esta bactéria. Na necropsia, os linfonodos mesentéricos estavam enfartados e os intestinos estavam hemorrágicos. Cultura anaeróbia de fígado, rins e conteúdo intestinal pôde isolar o microorganismo. Alimentos estocados inapropriadamente poderiam ser a via de transmissão (SANTIAGO et al., 1997).

O botulismo é uma intoxicação sofrida nos animais por meio do consumo de alimentos ou rações com a toxina de *Clostridium botulinum* ou de seus esporos (KOHLER; BEER, 1999). Muitos mustelídeos são suscetíveis ao *C. botulinum* tipo C, e em menor extensão nos tipos A, B e E (PIMENTEL et al., 2001). Essa doença é geralmente fatal e pode também ser causada pelo consumo de carne crua contaminada (REED-SMITH, 1994-1995). O diagnóstico é realizado pela observação dos sinais clínicos e o histórico de doença hiperaguda (PIMENTEL et al., 2001). Em populações de carnívoros de vida livre, o possível risco de infecção deste agente é a ingestão de carcaças de bovinos ou outros animais suscetíveis.

Leptospirose

A leptospirose é uma zoonose cosmopolita causada por bactéria do gênero *Leptospira* pertencente à família Leptospiraceae e à ordem Spirochaetales. Recentemente, ocorreu nova classificação dividindo o gênero *Leptospira* em sete espécies patogênicas: *L. borgpeterseni*, *L. inadai*, *L. noguchii*, *L. santarosai*, *L. weillii*, *L. kirschneri* e *L. interrogans* (sensu stricto). A leptospira não-patogênica compreende apenas uma espécie: *L. biflexa.* Existem mais de 200 variantes sorológicas patogênicas que se relacionam com diferentes hospedeiros vertebrados, num processo dinâmico, originando uma epidemiologia bastante complexa (TORTEN; MARSHALL, 1994).

Do ponto de vista epidemiológico, é possível identificar três formas de ocorrência da leptospirose: silvestre, rural e urbana. De acordo com as interações estabelecidas entre os grupos de animais e as variáveis ambientais, a doença manifesta-se sob a forma de surtos epidêmicos ou permanece dentro do limiar de endemicidade (VASCONCELLOS, 1987).

Muitos animais selvagens estão perfeitamente adaptados a esse agente e não manifestam sinais clínicos (ACHA; SZYFRES, 1986). Os sinais clínicos que podem ser encontrados nos animais acometidos são espasmos musculares, falta de coordenação motora, febre, descarga catarral, hemoglobinúria, icterícia, estomatites, vômitos, perda progressiva de peso e morte (HORSCH, 1999).

Carnívoros selvagens e ferais podem ser reservatórios de leptospiras e levantamentos sorológicos podem indicar infecção ou doença em populações selvagens (Faine, 1994). Em cativeiro, o Grupo de Trabalho de Canídeos-GTC (Sociedade de Zoológicos do Brasil-SZB e o Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – Ibama) recomenda a vacinação para leptospirose dose única cada seis meses, para animais nos zoológicos que já ficaram expostos ou foram comunicantes de um surto desta doença em áreas próximas do zoológico (SANTIAGO; OLIVEIRA, 2001). Farias et al., (1999) descreveram um surto de leptospirose em ariranhas mantidas em zoológico, com a presença dos sinais clínicos da doença.

Maia et al.(2000) pesquisaram a presença de aglutininas anti-*Leptospira* spp. em 20 lobos-guarás (adultos e filhotes) de zôos brasileiros (Fundação Zoobotânica de Belo Horizonte; Fundação Riozoo; Jardim Zoológico de Brasília; Zoológico de Curitiba; Parque Natural da CBMM) e em espécimes capturados da natureza. Entre os animais estudados, foram encontrados cinco animais positivos, examinados pela prova de soroaglutinação. Os autores sugeriram que o grande número de roedores encontrados nos zôos pôde ser responsável por tal achado.

Salmonelose

A salmonelose é provocada por bactérias pertencentes à família das enterobactérias do gênero *Salmonella*. As salmonelas distribuem-se por todo o mundo, sendo os portadores crônicos, freqüentemente não diagnosticados, os indivíduos mais importantes para manutenção do agente no ambiente devido à sua excreção pelas fezes. A *S. typhimurium* tem maior importância em carnívoros e a infecção pode ocorrer a partir da ingestão de alimento ou água contaminada (STELLMACHER, 1999).

Essa doença é caracterizada clinicamente por um ou mais dos três principais sinais clínicos: septicemia, enterite aguda ou crônica. Espécies de *Salmonella* spp. têm sido isoladas em fezes de animais selvagens clinicamemte saudáveis sem a presença da doença (ACHA; SZYFRES, 1986). Giorgi (1973) encontrou em 114 amostras de fezes de animais da Fundação Parque Zoológico de São Paulo, 8,7% mamíferos positivos, sendo a maioria, carnívoros. O autor sugeriu como fatores predisponentes da ocorrência dessa doença, o ambiente de zoológico diferindo do natural, podendo desencadear uma quebra de resistência orgânica favorecendo a instalação da doença e, em segundo lugar, a ingestão de alimentos contaminados, pois esta é a principal via de transmissão.

Micobacterioses

A tuberculose é uma doença infecciosa causada por bactéria do gênero *Mycobacterium*. Para os carnívoros podemos considerar três espécies: *M. bovis, M. avium* e *M. tuberculosis*, sendo que a ocorrência da infecção está associada à via alimentar ou aerógena. Nos felinos, a infecção pode ocorrer pelos ferimentos causados pela arranhadura quando se coçam (MATTHIAS, 1999). Os principais sinais clínicos da tuberculose são perda progressiva de peso, dificuldade respiratória, linfadenites e distúrbios gastrintestinais. Existem poucos relatos na literatura sobre a importância dos carnívoros selvagens na epidemiologia dessa doença, contudo devido à aproximação humana em áreas naturais o risco potencial da disseminação do *M. tuberculosis* aumentou (ALEXANDER et al., 2002) e a presença de bovinos próximos às unidades de conservação expõe a população de animais selvagens ao *M. bovis*.

Brucelose

Essa doença possui maior importância nos ungulados selvagens (ACHA; SZYFRES, 1986). Considerando os carnívoros, a brucelose canina é causada por *Brucella canis*, um cocobacilo aeróbico Gram-negativo. *B. canis* é encontrado no sêmen (venéreo), em corrimentos vaginais (no estro, acasalamento e pós-aborto), em tecidos fetais abortados e na urina. A infecção ocorre por meio da penetração das mucosas oronasal, conjuntival e genital pelos organismos (SHERDING, 2003).

Contudo, relacionando as outras espécies de *Brucella* spp. poucas são as descrições em carnívoros. Entretanto, estes animais podem se infectar pela ingestão de fetos abortados de bovinos, ovinos, caprinos e suínos contaminados com outras espécies de brucelas como *B. abortus*, *B. ovis, B. suis* ou conforme mencionado, também, pela via venérea através da cópula com a *B. canis* (CARMICHAEL, 1976). Este objeto de pesquisa torna-se de suma importância nas populações de carnívoros selvagens de vida livre, assim como, nos cães domésticos provenientes de fazendas no entorno de unidades de conservação.

Em ambiente de zôo, existe o relato de Maia et al. (2000) que descreveram a presença de aglutininas anti-*Brucella* sp. em 58 lobos-guarás, sendo 12 espécimes capturados na natureza e 46 provenientes de cativeiro. Para a detecção de aglutininas anti-*Brucella* lisa foi empregado o teste do Antígeno Acidificado e Tamponado (TAAT). Os animais positivos no TAAT foram submetidos às provas de Soroaglutinação Lenta (SAL) e 2-mercaptoetanol (2ME). Para detecção de aglutininas anti-*Brucella* rugosa foi empregado o teste de imunodifusão em gel de ágar (IDGA). Ao TAAT, 15 espécimes apresentaram resultado positivo, sendo três destes resultados confirmados e um considerado suspeito pelas provas de SAL e 2ME. No teste de IDGA, três animais apresentaram resultados positivos. Segundo os autores, estes resultados sugeriram que estes carnívoros tiveram contato com bactérias do gênero *Brucella* e outros estudos devem ser realizados para avaliar a importância clínica e adoção de medidas preventivas.

Outras doenças bacterianas

Outras doenças bacterianas descritas em mustelídeos incluem tuberculose, tularemia e pasteurelose (PIMENTEL et al., 2001).

Doenças causadas por protozoários

Hepatozoonose

Hepatozoon canis é um protozoário parasita pertencente à família Haemogregarinidae e à ordem Eucoccidia, sendo transmitido pelo carrapato-marrom do cão *Rhipicephalus sanguineu*s. Este parasita já foi descrito em roedores e em alguns carnívoros tais como o cão, o gato, o chacal e a hiena (SOULSBY, 1982). O hospedeiro infecta-se com este parasita pela ingestão de carrapatos com esporozoítos do agente (GEORGI; GEORGI, 1992). A presença de gametócitos de *Hepatozoon* spp. no interior de leucócitos também já foi descrita em hiena, leão, leopardo, guepardo, chacal (MCCULLY et al., 1975) e raposa (MAEDE et al., 1982), em diversos países. A patogenicidade do *H. canis* varia de uma inaparente e branda infecção, a uma séria doença, que pode levar o animal ao óbito (CRAIG, 1984).

Com relação aos carnívoros selvagens brasileiros, existe apenas um relato (ALENCAR et al., 2000) sobre a ocorrência de *H. canis* em um cachorro-do-mato, que morreu atropelado por um carro numa rodovia próxima de Botucatu, São Paulo. Dessa forma, torna-se de suma importância a realização de necropsias nos animais atropelados para colheita de informações, realização de diagnósticos, pesquisa e isolamento de agentes patogênicos, buscando á estruturação de um banco de material biológico dos animais selvagens da fauna brasileira.

No Brasil, há necessidade de estudos mais profundos para determinar os vetores desse protozoário, já que nas áreas rurais, onde a ocorrência do *H. canis* é maior, a espécie de carrapato que mais ocorreu foi a *Amblyomma cajennense* (O´DWYER et al., 2001).

Leishmaniose visceral

A leishmaniose visceral é uma zoonose de grande expressão na saúde pública em muitos países da América do Sul e Central (ACHA; SZYFRES, 1986). Da mesma forma que em outras áreas endêmicas da leishmaniose visceral, os cães domésticos são relacionados como os principais reservatórios do agente causador dessa doença - *Leishmania chagasi* (DEANE; DEANE, 1962; SILVA et al., 1997). O vetor biológico deste protozoário é o mosquito-palha do gênero *Lutzomyia*.

Em relação aos carnívoros selvagens, dois canídeos brasileiros também foram considerados reservatórios desse parasita, entre eles, o cachorro-do-mato *Dusicyon thous* proveniente do Nordeste do Brasil (DEANE; DEANE, 1954) e o cachorro-do-mato *C. thous* e a raposa-do-campo *Lycalopex vetulus* (REY, 2001) da região amazônica (LAINSON et al., 1969; SILVEIRA et al., 1982), e do Estado do Mato Grosso do Sul, Brasil Central (Mello et al., 1988). Mais recentemente, Courtenay et al. (1996) questionaram a original identificação do *D. vetulus* e sugeriram que deve ser a mesma espécie que o *C. thous*.

Da mesma forma, recentemente Silva et al. (2000) descreveram a infecção natural de *L. chagasi* em um cachorro-do-mato que foi atropelado em uma rodovia no Sudeste do Brasil no Vale do Jequitinhonha, norte do Estado de Minas Gerais. Esses estudos confirmaram o papel de alguns canídeos selvagens na epidemiologia da leishmaniose visceral do Brasil. Contudo, esses relatos tiveram enfoque apenas na saúde pública, não se dispondo de dados sobre o real impacto desse protozoário na saúde dos animais infectados ou doentes, assim como, para as suas populações.

Toxoplasmose

A toxoplasmose é causada por um protozoário intracelular obrigatório, o *Toxoplasma gondii*. Os felídeos são importantes na disseminação da infecção pelo *T. gondii* em animais e humanos no mundo inteiro, pois são os únicos animais que excretam oocistos no meio ambiente (FRENKEL et al., 1970; MILLER et al., 1972).

Embora o *T. gondii* seja também transmitido transplacentariamente por taquizoítos e pela ingestão de cistos teciduais com bradizoítos na carne de animais infectados, existem evidências de que a infecção desse parasita não seja possível sem a presença de felídeos (MUNDAY, 1972; WALLACE, 1972; DUBEY et al., 1997). Não só os gatos domésticos, mas possivelmente todas as espécies de felídeos selvagens são capazes de excretar oocistos de *T. gondii* (DUBEY; BEATTIE, 1988). Esse fenômeno já foi comprovado em várias espécies de felinos. Infecções experimentais com esse agente já foram descritas em felídeos neotropicais como a suçuarana (MILLER et al., 1972), o gato-mourisco *Herpailurus yagouroundi* (JEWELL et al., 1972) e a jaguatirica (JEWELL et al., 1972), que excretaram oocistos após serem alimentados com cistos teciduais com bradizoítos. Em condições naturais, oocistos de *T. gondii* foram descritos em gato-mourisco (PIZZI et al., 1978), gato-do-mato-grande *Oncifelis geoffroyi* (PIZZI et al., 1978) e no gato-palheiro *O. colocolo* (PIZZI et al., 1978). Dessa maneira, os estudos de *T. gondii* em felídeos selvagens assumiram uma grande importância para o melhor entendimento da relação hospedeiro-parasita, auxiliando no conhecimento da história natural da toxoplasmose.

Entretanto, pouco se conhece sobre o papel dos felídeos selvagens na epidemiologia da toxoplasmose e a importância do *T. gondii* como causa de mortalidade e morbidade nesses animais. Mesmo assim, poucos são os relatos no Brasil da ocorrência de anticorpos anti-*Toxoplasma gondii* em felídeos neotropicais em cativeiro (SOGORB et al., 1977; SILVA et al., 2001) e em vida selvagem (FERRARONI; MARZOCHI, 1980; FERRARONI et al., 1980).

A toxoplasmose clínica é raramente vista em felídeos domésticos ou selvagens, embora a infecção toxoplásmica seja comum (DUBEY; BEATTIE, 1988; DREESEN, 1990). Contudo, a doença pode ocorrer em animais jovens, imunologicamente imaturos, ou em animais velhos com resposta imune debilitada (DUBEY et al., 1977). Desta maneira, podemos inferir que os felídeos são importantes na contaminação ambiental por este agente, de suma importância na medicina da conservação porque diversas espécies de primatas neotropicais são bastante suscetíveis e geralmente morrem de toxoplasmose (DUBEY; BEATTIE, 1988; EPIPHANIO et al., 2000).

Outros Coccídios

Pereira e Lopes (1983 e 1988) relataram o cachorro-do-mato como hospedeiro definitivo de *Sarcocystis capracanis* (Apicomplexa: Sarcocystidae) e a infecção pela *Hammondia heydorni* (Apicomplexa: Sarcocystidae) a partir de um caprino infectado.

Até o momento, não há evidência da excreção de oocistos de *Neospora caninum* pelos canídeos selvagens.

Outras doenças causadas por protozoários

Outras doenças causadas por protozoários descritas em carnívoros selvagens incluem babesiose em lobos-guarás (NUNES, 1981; NUNES; PUGLIA, 1983; SERRA FREIRE et al., 1995) e tripanosomose (FOWLER; CUBAS, 2001).

A citauxzoonose felina foi recém-descrita em nosso país (Soares, 2001) em felídeos selvagens (leão, onça-pintada e gato-do-mato-pequeno) de um zôo do Estado do Rio de Janeiro.

Como é fácil encontrar carrapatos, piolhos e pulgas em carnívoros selvagens, a ocorrência de *Erlichia* sp. e *Haemobartonella* sp. nesses animais é verificada.

Conclusões

A avaliação do impacto de doenças em populações de carnívoros selvagens está diretamente ligada à medicina da conservação que tem por definição estudar a saúde humana, animal e do ecossistema cuja intersecção dessas três áreas caracteriza o que se denomina de saúde ecológica. A medicina da conservação é uma importante ferramenta para o desenvolvimento de políticas públicas e ambientais e de saúde pública e animal. Dessa forma, levantamentos epidemiológicos devem ser realizados para melhor entendimento dos fatores de risco associados às doenças emergentes e ao impacto dos patógenos, como bactérias e protozoários, nas populações de carnívoros selvagens.

Além disso, o desenvolvimento dessas pesquisas permitirá avaliar quais os impactos que as doenças podem causar sobre uma população de carnívoros selvagens e estabelecer as medidas de controle para evitar essa disseminação. Dessa forma, a identificação das fontes de infecção, dos fatores de risco, e o conhecimento da ocorrência das doenças numa população se torna a base para o desenvolvimento de um programa de monitoramento e de vigilância epidemiológica, para a preservação da saúde humana, animal e para a conservação das populações naturais.

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Capítulo 21
A review of ecological aspects of disease spillover in carnivores, and possibilities for intervention in the Bolivian Chaco

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Introduction

Interest in diseases of wildlife has been growing for a variety of reasons, including concern for animal welfare, conservation of threatened species, and the increasing recognition of emerging zoonotic diseases (DOBSON; MILLER 1989; LYLES; DOBSON 1993; HESS 1996; LAURENSON et al., 1998; HARVELL et al., 1999; WOODROFFE 1999; BRIONES et al., 2000; DASZAK et al., 2000; DEEM et al., 2001). As humans continue to modify the environment, new diseases are discovered (KRAUSE, 1992; BINDER et al., 1999), new host ranges are identified (KRAUSE, 1992; ROELKE-PARKER et al., 1996; FORREST; GUSHULAK, 1997; HAHN et al., 2000), and the risks to wildlife populations are appreciated (WOODROFFE, 1999; MUNSON; KARESH, 2002). The human population is increasing and continually encroaching on wildlife habitat, and wherever humans go, they bring domestic animals. As humans and domestic animals continue to spread out and impact our environment, additional infectious diseases affecting domestic and wild carnivores will likely be recognized. This review explores some of the ecological issues surrounding the transmission of disease between populations of domestic and wild carnivores, and discusses the potential impacts of domestic animal diseases on wild populations. Preliminary results from an ongoing study in Bolivia are presented, and selected intervention strategies that may protect wild carnivores are discussed.

Many carnivore species are susceptible to domestic carnivore diseases both in captivity (FIX et al., 1989; van HEERDEN et al., 1989; APPEL et al., 1994; MAINKA et al., 1994; HARDER; OSTERHAUS 1997; CHANDA et al., 2000; STEINEL et al., 2000), and in the wild (JESSUP et al., 1993; ALEXANDER et al., 1995; APPEL; SUMMERS, 1995; ROELKE-PARKER et al., 1996; EVERMANN et al., 1997; LAURENSON et al., 1998; PETERSON et al., 1998; ARTOIS; REMOND, 1999; DANIELS et al., 1999; LEUTENEGGER et al., 1999; CROOKS et al., 2001) (Table 1). In some cases, when wild carnivores succumbed to these diseases, domestic (often feral) carnivores have been suspected – but never proven – as the source (FIX et al., 1989; GOLTSMAN et al., 1996; CROOKS et al., 2001). Proving such links is especially challenging when dealing with free-ranging carnivore populations. Unfortunately, we often don't know enough about ecological interactions among wild and domestic carnivores to determine whether cross-species transmission is occurring, or in which direction it is going (TRUYEN et al., 1998; SHAMIR et al., 2001). For example, a recent serological survey of domestic dogs and giant pandas (*Ailuropoda melanoleuca*) found that pandas, dogs, and cats had antibody titers to canine parvovirus, and both dogs and pandas had antibody titers to canine distemper virus, canine coronavirus, and canine adenovirus (MAINKA et al., 1994). Although the authors speculate that transmission of viruses occurs between the giant pandas and the domestic pets, no evidence is available to support or refute this supposition. In order to properly manage and protect endangered carnivores, an understanding of the ecology and epidemiology of disease is essential.

Spillover

The concept of spillover has been used to describe various situations in which a disease has spread from one host species to another (NEL et al., 1997; WOODROFFE, 1999). Daszak et al. (2000) define spillover as the transmission of infectious agents from reservoir animal populations (often domesticated species) to sympatric wildlife. The reservoir host may be asymptomatically infected, mildly affected, or severely affected. In some cases, the nonreservoir host may be a dead-end host, and be unable to transmit the pathogen to other hosts (WOOLHOUSE et al., 2001). Unless the population of the host species is very small, diseases for which endangered species serve as a dead-end host are probably not a serious threat to the species because only one or a few individuals are affected. Spillover is more problematic when a pathogen is contacted from the reservoir host and subsequently transmitted such that an epidemic in the nonreservoir host results. This can be disastrous when the nonreservoir host population is small and threatened (THORNE;WILLIAMS 1988; GOLTSMAN et al., 1996; MURRAY et al., 1999; WOODROFFE, 1999). Diseases that disproportionately kill (or render sterile) young animals may also cause major population declines (WOODROFFE, 1999).

The most common scenario for the diseases of conservation concern occurs when the reservoir host is equally susceptible to a given disease agent as the host of interest. The species is called a reservoir not because of a difference in susceptibility, but because its population density is high enough to maintain the disease. Infectious diseases require a minimum population in order to be sustained; if the population is smaller than this minimum, the disease will die out (ANDERSON; MAY 1979). Virulent diseases that require a large population will go extinct if the population of their host is too small; the disease kills off its hosts before the disease agent can be transmitted to a new host, and the pathogen dies with the host (WOOLHOUSE et al., 2001). Problems arise when more than one species can host a highly virulent pathogen

(DOBSON; FOUFOPOULOS, 2001). When a nonendangered species shares its range with an endangered one, the conservation implications become clear. The large population of the common species may be large enough to sustain a disease endemically, preventing the pathogen from going extinct (WOODROFFE, 1999). If the disease spills over into the endangered species, a serious population decline or extinction could occur. Such spillover events may occur repeatedly; even though the disease dies out in the threatened population, it is maintained in the more common species. If the two species have regular contact, periodic outbreaks should be expected.

Morbilliviruses

Canine distemper virus (CDV) is probably the morbillivirus with the broadest host range. The most well-known and best studied case of a domestic carnivore disease affecting a wild carnivore population is undoubtedly the 1994 canine distemper epidemic among the Serengeti lions (*Panthera leo*) (HARDER et al., 1995; ROELKE-PARKER et al., 1996; KOCK et al., 1998; CLEAVELAND et al., 2000). This outbreak may have decreased the population by as much as 30 percent (Roelke-Parker et al., 1996). Molecular (HARDER et al., 1995) and epidemiological (CLEAVELAND et al., 2000) data indicate that the ultimate source of the virus in this epidemic was the domestic dog population living near Serengeti National Park. The exact ecological mechanism by which the virus was transmitted is unknown, but the most likely route was probably via spotted hyenas (*Crocuta crocuta*) and silver-backed jackals (*Canis mesomelas*), both of which are frequently found in and around villages where opportunities for contact with domestic dogs abound (Kruuk 1972). Once infected, these carnivores could pass the virus on to lions at kills, where the three species commonly interact (KRUUK, 1972; CLEAVELAND et al., 2000). Spotted hyenas are susceptible to CDV, and an outbreak of canine distemper was observed in Serengeti hyenas during late 1993 and early 1994 (APPEL; SUMMERS, 1995; HAAS et al., 1996).

The ecological factors influencing disease transmission may be obvious, or subtle and complex. In the Serengeti, lions occasionally ventrure into villages; it is possible that a lion was exposed to CDV simply through direct contact with a dog, and then transmitted the infection to other members of the pride. Although lions are not frequently found near villages, a severe drought in 1993 led to large numbers of wildebeest being hunted by humans, who killed them to protect their grazing land (CLEAVELAND et al., 2000). As a result, many fresh carcasses were available for scavenging, and it is likely that wild carnivores and dogs both took advantage of this food source (CLEAVELAND et al., 2000). In contrast to lions, even in the absence of abundant carcasses to scavenge, hyenas and jackals routinely enter villages to forage (KRUUK, 1972). Alexander et al. (1995) found that low-ranking hyenas were more likely than high-ranking animals to have antibodies to CDV. These lower ranking animals are more likely to scavenge in villages and thus encounter infected dogs, or scavenge on dog carcasses (ALEXANDER et al., 1995). The lower ranking hyenas may have passed the virus along to others in their pack, or directly to lions at kills. Lions also sometimes attack or prey upon hyenas, and so the virus could have been transmitted via direct contact (KRUUK, 1972).

African wild dogs (*Lycaon pictus*) are highly endangered top carnivores that are susceptible to infection with CDV (MCCORMICK, 1983; vAN HEERDEN et al., 1989; van de BILDT et al., 2002). Ecologically, they have been observed to have considerable contact with domestic dogs in the Masai Mara region of Kenya (GASCOYNE et al., 1993; KAT et al., 1995). The Maasai keep a large number of dogs for livestock protection (ALEXANDER; APPEL, 1994). During an epidemic of canine distemper among the domestic dogs of the Maasai, two wild dog packs entirely disappeared after some animals were observed dead or ill (ALEXANDER; APPEL, 1994). It is strongly suspected that these two packs were depopulated by the same disease that affected the sympatric domestic dog population; unfortunately, tissue samples were not available to confirm these suspicions.

Canine distemper virus, phocine distemper virus, and monk seal morbillivirus can all affect marine carnivores. Morbilliviruses are transmitted by direct contact between individuals, and since most marine mammals are highly social, these viruses can move rapidly through the population. In some cases, these viruses have spread from terrestrial to aquatic species. For example, a mass mortality event occurred in 1987-88 among Lake Baikal seals (*Phoca siberica*), killing several thousand animals (GRACHEV et al., 1989). This outbreak was caused by CDV (Osterhaus et al., 1989a), and domestic dogs were almost certainly the source of the virus (Mamaev et al., 1996). Dogs have been implicated as the possible source of the virus in several other epidemics as well (GRACHEV et al., 1989; OSTERHAUS et al., 1989a; BENGSTON et al., 1991; KENNEDY et al., 2000). Although there has been a great deal of controversy regarding the roles of algal toxins and polychlorinated biphenyl-induced immunosuppression in some of these outbreaks (ROSS et al., 1996; HARWOOD, 1998; HERNANDEZ et al., 1998), it is clear that in most cases the immediate cause of morbidity and mortality was viral infection (OSTERHAUS et al., 1998; O'SHEA, 2000).

Parvoviruses

Canine parvovirus (CPV), mink enteritis virus (MEV), and feline panleukopenia virus (FPV) are closely related viruses of carnivores; although FPV has been known for at least a century, MEV was recognized in the 1940's, and CPV and its variants (types 2a and 2b) emerged in the last 30 years (TRUYEN, 1999). Canine parvovirus-2a and 2b both have an expanded host range, and can infect dogs and cats (Truyen 1999), making these viruses a greater risk to wildlife species. In fact, they have recently been detected in a variety of wild felids (STEINEL et al., 2000). Currently, it is thought that the ancestor of CPV was a variant of FPV in a wild carnivore (IKEDA et al., 2000), and that although transfer between species is probably not common, it may allow for recombination and the emergence of new variants with differing host ranges and pathogenicity (HORIUCHI et al., 1998).

Canine parvovirus occasionally spills over into grey wolf (*Canis lupus*) populations from domestic dogs (MECH; GOYAL; 1993; PETERSON et al., 1998), causing periodic outbreaks in the wild canids. Wolves are permissive hosts for the virus and can continue to spread it within their population, although adult animals are rarely affected. Because the virus generally affects pups under four months of age (PETERSON et al., 1998), it may cause population declines or, at least, prevent small populations from growing (MECH; GOYAL 1993; PETERSON et al., 1998).

Antibodies to parvoviruses have been found in many other captive and free-ranging wild carnivores, including European wildcats (LEUTENEGGER et al., 1999), leopard cats (IKEDA et al., 1999), Giant pandas (MAINKA et al., 1994), civets (Ikeda et al., 1999), jaguars (Fiorello, unpublished data), a bat-eared fox, cheetahs, African wildcats, African hunting dogs, and honey badgers (STEINEL et al., 2000). The significance of this virus in these populations is still unknown, although the bat-eared fox, African wildcats, cheetahs, and honey badgers all had clinical signs typical of parvovirus infection and several animals did not survive (STEINEL et al., 2000). In one study, most leopard cats (*Felis bengalensis*) sampled did have antibodies against FPV, and the authors speculate that this is due to indirect contact with domestic cats (IKEDA et al., 1999). These authors found that exposure to viruses spread by direct contact, such as calicivirus and herpesvirus, were uncommon in leopard cats, but exposure to FPV, a virus capable of surviving for extended periods in the environment, was common. This highlights the fact that wild carnivores need not come into direct contact with domestic carnivores to catch their diseases.

A recent study compared red foxes (*Vulpes vulpes*) from rural and urban counties in Germany with respect to seroprevalence of viral diseases (TRUYEN et al., 1998). These authors found that although there were no statistically significant differences in seroprevalence between regions, overall the seroprevalence of CPV-2 was 13%. This was far greater than the seroprevalence of the other viruses tested (CDV, canine herpesvirus (CHV), canine adenovirus (CAV)). These authors also analyzed DNA sequences from a parvovirus isolated from two foxes. The sequences represent a true intermediate between CPV and FPV, and therefore indicate that CPV may have evolved from the fox parvovirus (TRUYEN et al., 1998). Domestic carnivores could therefore be exposed when walking through or investigating fox feces, and vice versa. Cross species transmission certainly occurs at least occasionally, and parvoviruses may be similar to influenza viruses in their ability to shift host range and virulence with minimal mutations (HORIUCHI et al., 1998). Even host-adapted strains of parvoviruses can cause mortality in wild carnivores (PETERSON et al., 1998), so this group of viruses should be of major conservation concern.

Retroviruses

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are major pathogens of domestic cats, causing severe immunosuppression and often resulting in death. Both viruses are also known to cause disease in captive and free-ranging nondomestic felids (BARR et al., 1989; JESSUP et al., 1993; LEUTENEGGER et al., 1999; WORLEY, 2001), although such reports are rare. In one case, a wild cougar (*Felis concolor*) in California was found to be infected with FeLV, and this animal died despite treatment (JESSUP et al., 1993). The virus was isolated from the cougar, verifying active viral replication, and the clinical signs were consistent with those reported for domestic cats with this infection (JESSUP et al., 1993). This animal was found on a university campus in an urban area, thus illustrating that domestic and wild felids do sometimes share the same habitat.

Eight leopard cats, representing free-ranging animals, zoo animals, and pets from Vietnam and Taiwan, were all negative for FeLV antigens (IKEDA et al., 1999). However, in France, three of eight European wildcats (*Felis silvestris*) were positive for FeLV. The authors consider domestic cats a possible source of infection, although they emphasize the importance of additional surveys (ARTOIS; REMOND, 1994). The sample size in this study was very small, but the authors point out that caution is warranted, as this species is considered threatened (ARTOI; REMOND, 1994). It is not known with certainty if the above data reflect differences in susceptibility or differences in exposure, but this virus has been isolated from a leopard cat cell line (WORLEY, 2001), so it is likely that this species (and probably all members of the Felidae) are susceptible to infection with FeLV.

Feline immunodeficiency virus has been intensively studied, thanks to its similarity to human immunodeficiency virus (OLMSTED et al., 1992; CARPENTER; O'BRIEN, 1995; PLOTNICK; LARSEN 1995; CARPENTER et al., 1996). It is rarely associated with disease in nondomestic felids, and there appear to be many different strains of the virus—possibly each species has its "own" strain (OLMSTED et al., 1992; CARPENTER; O'BRIEN, 1995; CARPENTER et al., 1996). Carpenter and O'Brien (1995) speculate that, in some nondomestic felids, long associations between virus and host have resulted in nonpathogenic viral strains. However, cross species infection is considered a possibility, and in at least one case, a puma was found to be infected with a strain characteristic of domestic cats (CARPENTER et al., 1996). It is possible that nondomestic felids are adapted to "their" species viral strain, but susceptible to the strains of other species. There have been a few cases in which infected nondomestic felids have had reproductive problems and chronic infections, both of which are commonly documented in infected domestic cats (BARR et al., 1989). More work needs to be done to determine the relationships between viral strain, host species, susceptibility to infection, and susceptibility to disease.

Thus far, FIV has not been found in free-ranging European wildcats from France, Germany, Switzerland, or Scotland (DANIELS et al., 1999; LEUTENEGGER et al., 1999), or in leopard cats from Vietnam or Taiwan (IKEDA et al., 1999), although a captive leopard cat was found to have antibodies to FIV (BROWN et al., 1993). Populations of both free-ranging pumas and lions have been found to have high percentages of individuals with FIV antibodies (OLMSTED et al., 1992; EVERMANN et al., 1997), and a population of free-ranging cheetahs (*Acinonyx jubatus*) had a seroprevalence of over 20% (OLMSTED et al., 1992). Several authors have expressed concern that domestic cats can serve as a reservoir for this virus, but at this point we don't know enough about the epidemiology of this virus to know whether nondomestic felids are at risk (PLOTNICK; LARSEN, 1995).

Rabies

Much has been written about the rabies virus, but its importance in wildlife populations nonetheless warrants its mention here. Rabies outbreaks have occurred and been well-documented in numerous carnivore species, including raccoons (SCHUBERT et al., 1998), foxes (ANDERSON et al., 1981), jackals (BINGHAM et al., 1999), wolves (CHAPMAN, 1978; WELLER et al., 1995), African wild dogs (GASCOYNE et al., 1993; KAT et al., 1995), bat-eared foxes (MAAS, 1993) and various viverrids (ENURAH et al., 1988; Nel et al., 1997) and mustelids (RUPPRECHT et al., 2001). In addition to its public health implications (MURPHY, 1998; RUPPRECHT et al., 2001), rabies can devastate wildlife populations, making it an important concern for carnivore conservation (Sillero-ZUBIRI et al., 1996; WOODROFFE, 1999; DASZAK et al., 2000). Recent evidence shows that canid biotypes of rabies can spill over into viverrids, and vice versa (NEL et al., 1997), illustrating that the pathogenicity of a given biotype cannot be predicted.

The Ethiopian wolf (*Canis simensis*) is a highly endangered carnivore that has suffered major population declines due to rabies outbreaks (SILLERO-ZUBIRI et al., 1996). Fewer than 500 individuals are left; therefore even a relatively small epidemic is a great risk to the species. In one long-term study of Ethiopian wolves, 77 of 111 animals died or disappeared during a rabies epidemic (SILLERO-ZUBIRI et al., 1996). Rabies virus was isolated from two animals, and the serotype of the virus was similar to those isolated from domestic dogs in Ethiopia, indicating that dogs were the likely source of the virus. Although in general wolves seem to avoid direct interactions with domestic dogs, one major threat to this species is hybridization with dogs, which proves that direct interactions do occur (JOHNSON et al., 1996; SILLERO-ZUBIRI et al., 1996; WAYNE; KOEPFLI, 1996). Dogs often travel with their owners from villages to mountainous areas in the wolf's range, where they can effectively spread diseases picked up from other dogs in the village

(SILLERO-ZUBIRI et al., 1996). Wolves may also forage at the periphery of villages, where they may compete with domestic dogs for carrion, garbage, or other food items.

Local extinctions of African wild dog populations due to rabies epidemics have also been documented (GASCOYNE et al., 1993; GINSBERG et al., 1995b; KAT et al., 1995). In Kenya, researchers involved in a long-term study of wild dogs documented the death of 20 out of 30 pack members due to rabies (KAT et al., 1995). The virus was isolated from tissue samples, and clinical signs of the ill animals were consistent with rabies (RUPPRECHT et al., 2001). The isolates from wild dogs were essentially identical to those taken from rabid domestic dogs in the same region (KAT et al., 1995). In Tanzania, one pack of wild dogs disappeared; the one animal that could be found was thin and showed neurologic signs consistent with rabies (GASCOYNE et al., 1993). A dead wild dog was sampled and the cause of death was determined to be rabies. In addition, three unvaccinated wild dogs from other packs in the area had rabies antibody levels consistent with (although not proof of) previous infection with rabies (GASCOYNE et al., 1993). Once again, the serotype of the virus isolated was similar to that of viruses isolated from domestic dogs in eastern Africa (GASCOYNE et al., 1993).

Mange

Mange is a layperson's term for a variety of dermatological diseases caused by microscopic mites. Sarcoptic mange, caused by *Sarcoptes scabiei*, is a notorious zoonosis that is highly contagious among both individuals and species (BORNSTEIN et al., 2001). Epidemics of sarcoptic mange have caused high mortality and population declines in European red foxes (LINDSTROM ET AL., 1994; BAKER et al., 2000; SIMPSON, 2000) and raccoon dogs (SHIBATA; KAWAMICHI, 1999). Otodectic mange, although not commonly associated with mortality, may be the major threat to the Mednyi Arctic fox (*Alopex lagopus semenovi*), a highly endangered subspecies endemic to one island in the Bering Sea. The current population of these small canids is estimated to be only about 100 individuals (IUCN, 1998).

Otodectic mange is caused by *Otodectes cynotis*, a common cause of ear infections in domestic cats and dogs (GREENE, 1998b). In the mid-1970's, when there were about 600 Mednyi foxes, a sharp population decline was noted and attributed to high pup mortality (GOLTSMAN et al., 1996). Otodectic mange was diagnosed in pups, and the disease appeared to be much more severe in this species than it is in domestic dogs. The fox pups had skin lesions not only around the ears but on the abdomen and inguinal region, and affected animals lost weight and became markedly lethargic (GOLTSMAN et al., 1996). It seems that few or no pups survive the infection. Adult foxes were not affected. At the time when the epizootic began, dogs visited the island frequently with hunters, and the authors strongly suspect that the mite was introduced by a domestic dog (GOLTSMAN et al., 1996).

Although the mite infestation has not yet been proven to be the cause of the population decline (for example, the timing of pup death is around two to four months of age, just when maternal antibodies are waning and pups are most susceptible to, for example, canine parvovirus), it is certainly a contributing factor, and obvious signs of other diseases have not been observed (GOLTSMAN et al., 1996). Further study will hopefully determine the ultimate cause of the mortality, but if this subspecies is to be saved, the exclusion of domestic dogs from the island must be strictly enforced.

Investigating the problem

To positively document spillover, one would have to be present at the start of the domestic dog epidemic, isolate the disease agent from the dogs, document appropriate contact between the dogs and wild species, recognize the start of the epidemic in the wild species (which must follow the domestic epidemic in time), and isolate the same agent from the wild species. This is extremely difficult in any situation, and nearly impossible where a long-term study of the target wild population is not in place.

An alternative approach, however, to documenting the risk of spillover is possible and may give enhanced predictive power. Serologic studies, which rely on pathogen exposure and not isolation, can be done at any time, not just during a disease outbreak, and can help uncover the pathogen exposure "history" of a population. Most serologic tests detect antibodies to disease agents; these antibodies are formed when an individual is infected with the pathogen. The presence of antibodies indicates that the animal was infected, but it does not provide any information about the effect that this infection had on the organism. Antibodies can be present after severe disease or inapparent (subclinical) infection. Depending on the pathogen and the host immune system, antibodies may persist for months, years, or even the lifetime of the animal. There is individual variation in the length of persistence of antibody titers, but there are data on how long we can generally expect them to be detectable, at least in domestic species. For example, CDV antibodies tend to last the lifetime of an animal (GREENE; APPEL, 1998), whereas sarcoptic mange antibodies last only several weeks to months (LOWER et al., 2001). Disadvantages of serology include possible cross-reaction with antibodies to other pathogens, lack of information on disease severity, inability to distinguish between different strains of a pathogen (and therefore its host species of origin), and the presence of maternal antibodies in very young animals, which make it impossible to determine if the individual or its mother was exposed. Despite these disadvantages, in many cases serology is an extremely useful tool for describing the disease exposure of a population.

Previous serologic studies have assessed disease exposure in wild carnivore populations, but unfortunately these have not simultaneously examined domestic carnivores or have not assessed contact between the domestic and wild carnivores. For example, three studies sampled European wildcats (*Felis silvestris silvestris*) in an effort to explore disease risks to the population (ARTOIS; REMOND, 1994; DANIELS et al., 1999; LEUTENEGGER et al., 1999). Although some wildcats did have antibodies to feline diseases, a corresponding survey of domestic cats was not done, nor was any assessment of contact between the domestic and wild populations performed. Domestic dogs and cats from towns on the border of a national park in Bolivia were tested, but wild carnivores were not surveyed (FIORELLO et al.,, in review). A serologic survey of giant pandas (*Ailuropoda melanoleuca*) and domestic dogs in China showed that pandas did have antibodies to common canine diseases (MAINKA et al., 1994). However, no data were available regarding the amount or type of contact between the pandas and dogs. Similarly, sympatric African wild dogs and domestic dogs were subjected to serologic testing, but contact data were not collected (ALEXANDER et al., 1993).

One of the few studies that included contact data between wild and domestic carnivore populations was done in South America (COURTENAY et al., 2001). Radiotelemetry of crab-eating foxes (*Cerdocyon thous*) was carried out to assess the amount of time that individual animals spent near houses; foxes and domestic dogs were also sampled and tested for antibodies to CDV and CPV. These workers found that foxes spent a considerable amount of time near houses; on average, 38 minutes were spent in villages per night (COURTENAY et al., 2001). Although they did not find antibodies in the foxes they sampled (N=37), these authors nonetheless concluded that spillover from domestics is a potential threat to these canids and should be further investigated (COURTENAY et al., 2001).

In an effort to clarify some of these issues, and assess the risk of disease transmission from domestic carnivores to free-ranging carnivores, I have begun a project in Bolivia that involves sampling domestic dogs and cats, sampling wild canids and felids, and interviewing villagers about dog demographics. The remainder of this paper will briefly cover the preliminary results of the serosurvey, and discuss in more depth the demographics of domestic dogs and possibilities for intervention.

A relatively new national park in Bolivia, Kaa-Iya del Gran Chaco, is a large protected area of tropical dry forest located in the southeastern part of the country. The park’s western border is contiguous with the Izoceño-Guaraní indigenous territory (TCO), and is approximately 40km from a group of Izoceño villages (TABER et al., 1997). The Izoceños use the region between the park and the villages for subsistence and commercial hunting. Because hunting almost always involves dogs, there is potential for contact between domestic dogs and wild carnivores. This area has a high diversity of carnivores, despite the dry climate (TABER et al., 1997). Camera trap studies have documented healthy populations of carnivores in this buffer zone between the park and the communities (MAFFEI et al., in press; PROYECTO KAA-IYA, unpublished data). Geoffroy’s cats (*Oncifelis geoffroyi*), pampas foxes (*Pseudalopex gymnocercus*), and crab-eating foxes (*Cerdocyon thous*) are seen frequently near the villages. Hunters commonly report seeing signs of pumas (*Puma concolor*), ocelots (*Leopardus pardalis*), jaguarundis (*Herpailurus yaguarondi*), and jaguars (*Panthera onca*), and sightings of the actual animals occur regularly (PROYECTO KAA-IYa, unpublished data). Most of these species are protected in part or all of their range (NOWELL; JACKSON, 1996).

Methods

This research was conducted with assistance from Proyecto Kaa-Iya, a collaborative effort of the Izoceño indigenous organization, Capitanía del Alto y Bajo Izozog (CABI) and the Wildlife Conservation Society (WCS). Proyecto Kaa-Iya staff has worked in the Izozog and the park since 1996, training local parabiologists in wildlife monitoring and resource management. The project also maintains the research camps where wildlife trapping and sampling was carried out.

Domestic animals were sampled in March 2001, December 2001, and March–April 2002. Three villages were targeted for domestic animal sampling on the basis of ease of access and previous involvement with researchers. Animals were identified for sampling by walking from house to house and asking residents if they had dogs or cats and were willing to participate. Local residents and/or parabiologists accompanied me during sampling. Sampling was basically opportunistic; no effort was made to randomize subjects. Only animals five months of age and older were included, to avoid interference and false positive results due to the presence of maternal antibodies (GREENE, 1998a). Blood samples were taken from dogs with the aid of manual restraint and muzzles. Cats required chemical restraint; 10 mg of tiletamine–zolazepam (Telazol; Fort Dodge Laboratories, Iowa, 50501 USA) given intramuscularly (IM) was used. Blood was placed into red top clot tubes was spun down within two hours of collection so that the serum could be separated and frozen in liquid nitrogen.

Wild carnivores were trapped in March 2001, October–December 2001, March–April 2002, December 2002, and February–March 2003. Wild carnivores were captured using cage-style box traps (Tomahawk Livetrap Comp., Tomahawk, WI, 54487 USA and Havahart Traps, Woodstream Corp., Lititz, PA, 17543 USA) modified to house a live chick. Baited traps were placed along dirt roads and trails about 200 meters apart in three general sites: (1) within a village; (2) outside of the protected area, but inside the Izoceño TCO; (3) within the National Park, about 200 km from the village. Parabiologists and local hunters assisted me in choosing sites for the traps to maximize captures. Traps and chicks were checked once or twice daily, and chicks were provided with food, water, and shade.

Trapped canids and the jaguarundi were immobilized with Telazol at 5mg/kg given IM. Felids were immobilized with an IM combination of ketamine (5 mg/kg) and medetomidine (Pfizer Corp., New York, NY, 10017 USA)

(0.05mg/kg); the latter was reversed with an equal volume of atipamezole given IM (Pfizer Corp., New York, NY, 10017 USA) when all procedures were complete. Heart rate, pulse, respiratory rate, temperature, and oxygen saturation were monitored throughout anesthesia. Animals were weighed, measured, given a complete physical examination, photographed, ear tagged, and sampled for blood, feces, and ectoparasites. Once recovered, animals were released at the site of capture. Blood was processed similarly to the domestic carnivore samples.

Serologic analysis and parasite identification were performed at commercial laboratories in the United States and Europe (Table 2). Domestic felids were tested for exposure to FeLV, FIV, feline calicivirus (FCV), FPV, feline herpesvirus (FHV), feline coronavirus (FCoV), *Toxoplasma gondii*, *Leptospira interrogans*, and *Dirofilaria immitis*, the causative agent of heartworm disease (HWD). Wild felids were tested for the above as well as CDV. Domestic and wild canids were tested for exposure to CDV, CPV, CHV, CAV, canine coronavirus (CCV), *T gondii*, *L. interrogans*, the mange mite *Sarcoptes scabiei,* and HWD.

In addition to wild carnivore sampling, data on domestic dog demographics and hunting ecology were collected. Using a standardized questionnaire, villagers were interviewed and questioned about dog ownership, dog mortality, birth rates, and vaccination rates. Interviews were conducted by a parabiologist well-known to the members of the community. Most interviews were conducted in the village of Iyobi, one of the largest settlements in the Izoceño TCO (Proyecto Kaa-Iya 2001). This should result in a conservative estimate of the number and hunting activity of dogs, as Iyobi's economy is slightly more diversified than that of most of the communities, thus hunting is less important there (A. Noss, personal communication). Projections based on these data were created to explore how the population dynamics may change if vaccination for common diseases was performed.

An equation was constructed to represent the dog population (see Appendix A). The total population was calculated to be 522 dogs (see below). Although dog owners stated that their female dogs had one litter per year, to be conservative, I assumed that only 75% of adult (>1 year) female dogs bred each year. I then set the death rate to be equal to the birth rate, so that the population was defined at equilibrium (ANDERSON; MAY, 1982). Leaving the birth rate unchanged, the death rate of puppies and the number of breeding females were altered to reflect various scenarios involving vaccination and sterilization.

Results

Sufficient serum for at least one diagnostic test was obtained from 97 dogs and six cats (Table 3). The prevalence of antibodies to CDV and CPV in the dogs was greater than 95%. Antibodies against sarcoptic mange were found in 62.5% of dogs tested. Antibodies were also found to CHV, CAV, CCV, *L. interrogans,* and *T. gondii*. In addition, several dogs were positive for *D. immitis* antigen. All of the cats had antibodies to FPV and FCV; over 80% had positive titers to *T. gondii*, and one cat of five tested had *L. interrogans* antibodies. None of the six cats tested had antibodies to FHV, FeLV, FIV, FCoV, or *D. immitis*.

Analyses of wild carnivore samples is still in progress, but some preliminary results are available (Table 4). Sera from three *C. thous*, five *P. gymnocercus*, one *H. yaguarondi*, eight *O. geoffroyi*, and ten *L. pardalis* have been collected from the four study sites. Canine distemper virus antibodies were found in *L. pardalis* and *P. gymnocercus*. Antibodies to CPV were common in both species of canids. Feline calicivirus antibodies were found in *O. geoffroyi* and *L. pardalis*, but FPV antibodies were found only in *O. geoffroyi*. Positive titers to *T. gondii* were found in all species except *H. yaguarondi*. One *P. gymnocercus* individual had antibodies to *L. interrogans*, and two of three *C. thous* were positive for HWD. Antibodies to CAV, CCV, CHV, FHV, FCoV, and FeLV were not found in any wild carnivores. More detailed results of the wild carnivore serosurvey will be published elsewhere when analyses are complete.

The results of the questionnaires revealed that essentially all households have dogs. Dogs in the Izozog are all owned, and considered necessary for hunting all species except brocket deer (*Mazama* spp.). The average number of dogs per household is 3.6, and the average number of people per household is 4.9 (Proyecto Kaa-Iya, Official Census 1996). This means that there is roughly one dog for every one and a half persons. Using these estimates and the population census, a community the size of Iyobi has approximately 522 dogs (Table 5). We also asked if residents thought there was a shortage or excess of dogs in the community; all respondents said that there were “many dogs” in their community. Seventeen of 121 dogs, or 14%, were vaccinated against rabies; none were vaccinated against other pathogens. Eighty-two percent of dogs hunt, which in a village like Iyobi, equals 428 dogs. Sixty-four percent of hunting dogs were reported to hunt weekly or more often. This results in over 270 dogs entering the forest each week.

The age structure of the dog population indicates that turnover is quite high. The sex ratio of dogs is roughly 1.3:1, although the average age of male and female adult dogs is similar (Table 5). The average annual mortality of adult dogs is 38%; dog owners reported an average of 1.8 dogs out of 5.1 dying during the past year. Average litter size is 3.7, and an astonishing 70% of puppies die as neonates. The age structure of the dog population did not differ among communities, or between the east and west banks of the river. When questioned about the cause of death of puppies, dog owners mentioned mange (47.8%), diarrhea (43.5%), worms (30.4%), vomiting (21.7%), anorexia (4.3%), and infanticide (4.3%); 34. 7% said they did not know.

Discussion

The large number of positive titers to a variety of pathogens indicates a significant level of disease exposure among domestic carnivores. Although in most cases the titers indicate infection and do not document disease, it is reasonable to assume that many of the animals with antibodies have indeed been clinically ill and have been capable of transmitting the pathogen to other hosts. For example, 25% to 75% of dogs infected with CDV are estimated to become clinically affected with the disease (GREENE; APPEL, 1998). During informal discussions with residents, dog owners frequently commented on dog illnesses and previous epidemics. Mange and "moquillo"—a term that technically refers to canine distemper but in the Izozog is used for any serious diarrheal disease — were most commonly cited by dog owners as major problems for their dogs.

Exposure to CDV and CPV was extremely common. All dogs one year of age and older had antibodies to parvo, and all but one dog of that age class had antibodies to distemper. Although dogs of any age can suffer clinical signs from these viral infections, they are generally considered diseases of puppyhood (GREENE; APPEL, 1998; HOSKINS, 1998). In the Izozog, it appears that virtually all dogs are infected with these viruses, and the comments of local residents confirms that diarrhea—a prominent feature of both CDV and CPV infection—is a common clinical sign. In order for a highly virulent pathogen to be maintained in the population, a constant supply of susceptible hosts must be entering the population (ANDERSON; MAY, 1979). This is likely the case in the Izozog. If there are about 390 female dogs in the community of Iyobi, and 75% of them had one litter of puppies each year, given an average litter size of 3.7 puppies/litter, there would be over 1000 pups born in Iyobi in a year. For highly contagious viruses like distemper and parvo, there is no doubt that this would be sufficient to sustain the infections.

Evidence of exposure to other common canine pathogens was found in many dogs, although none were as common as CDV and CPV (Table 3). However, these may be significant sources of morbidity, as several studies have shown that dual infections with some of these pathogens can be much more severe than single infections (KOYABASHI et al., 1993; PRATELLI et al., 1999; DUBEY; ODENING, 2001; PRATELLI et al., 2001). Most dogs had antibodies to more than one disease agent, but without longitudinal data, it is impossible to determine if infections were simultaneous or sequential.

Current, or very recent, infection with the sarcoptic mange mite was noted in two-thirds of dogs (Table 3). Based on personal observation, many of these dogs were suffering moderate to severe clinical signs. This pathogen is of major conservation concern, as it has been documented that free-ranging *P. gymnocercus* living in the Izoceño TCO have been infected with, and exhibit clinical signs resulting from, this parasite (DEEM et al., 2002; FIORELLO, unpublished data). Other canid species have suffered population-level declines from this pathogen (LINDSTROM et al., 1994), and cross-species transmission is known to occur (DAVIDSON; NETTLES, 1997). While we don't know if the foxes are being infected from the domestic dogs, we do know that literally hundreds of dogs are entering the forest each week, and foxes are very common in and around communities, so opportunities for transmission abound.

Domestic cats are uncommon in the Izozog and are not favored as pets. The few cats I was able to locate and sample all had similar exposure profiles (Table 3). All had FCV and FPV antibodies, and most had been exposed to *T. gondii*. The feline panleukopenia virus is a contagious pathogen that is an important cause of clinical disease in captive nondomestic felids, although its importance in free-ranging populations is unknown (BARKER; PARRISH, 2001). The low number of cats in the area makes it tempting to discount the importance of their role in disease transmission to wild felids; however, three of eight *O. geoffroyi* had antibodies to this virus, and all three were captured within a village. While the virus may be circulating in the wild population independently of the domestic cats, the lack of exposure in *O. geoffroyi* captured outside the village argues against this. A larger sample size is required to further elucidate this issue.

Evidence of exposure to common pathogens of domestic carnivores was found in all species of wild carnivores sampled except the single *H. yaguarondi* (Table 4). Canine distemper virus, perhaps the most worrying of the disease agents investigated, appears to have infected both canids and felids in this area. Both canid species had CPV antibodies. *Toxoplasma gondii* antibodies were found in all species but the jaguarundi. This last infection is probably of little concern in felids, as they are the definitive host for this parasite, and it is not often highly pathogenic to them (TENTER et al., 2000; DUBEY; ODENING, 2001). However, it may become a clinical problem in the canids, or in felids with concurrent infections (DUBEY; ODENING, 2001). Sixty-five percent of wild felids had antibodies for FCV. Calicivirus is extremely common in domestic cats; for example, one study in the United States found a prevalence of 53% in pet cats (SYKES, 2001). While it generally causes a milder disease than FHV, which was not found in any felid (wild or domestic) tested in Bolivia (FIORELLO et al., unpublished), some strains of FCV are responsible for severe and highly fatal illness (PEDERSEN et al., 2000).

The discovery of antibodies to common domestic canine and feline pathogens in wild carnivores is not evidence that the wild carnivores were exposed by the domestics. However, it does raise the question of whether these pathogens are circulating in the wild populations independently from the domestic animals, or being repeatedly introduced to the wild carnivores by the domestics. Large numbers of dogs are brought into the forest on a daily basis to hunt, and even without direct contact, transmission can occur. For example, parvoviruses can remain viable and infectious in the environment for weeks to months (HOSKINS, 1998; BARKER; PARRISH, 2001). Although cats are not common and are not brought into the forest by humans, wild carnivores do enter the villages and may interact with domestic felids or be exposed to their territory markings. As further results from this and other studies become available, we may be able to better elucidate the ecology of disease transmission in the carnivores of this region. In the meantime, however, we should consider disease spillover from domestic to wild carnivores a real possibility.

Given this potential risk to these threatened carnivores, what can be done to protect the wildlife? Although vaccination of susceptible wild carnivores may sometimes be effective (KIRKWOOD, 1993; HAYDON et al., 2002), it is difficult, costly, and controversial, and may be surprisingly dangerous to the species targeted (van HEERDEN et al., 1989; BURROWS et al., 1994; GINSBERG et al., 1995a; CURLEE, 1999; WOODROFFE, 2001). On the other hand, vaccination of domestic carnivores is safe, effective, and much more practical than vaccination of wild animals. While this may seem like an ideal solution, upon closer study, the drawbacks become clear.

The mortality rate of puppies in the Izozog is quite high, and based on the observations of owners, much of this mortality may be due to disease. Five out of the six clinical signs mentioned by dog owners could be caused by pathogens, and the extremely high prevalence of antibodies to canine disease agents supports the conclusion that these diseases are very common in the dog population. Although the data are not sufficient to prove it, it is likely that the dog population is limited by disease. If this is the case, eliminating even a portion of mortality due to disease may allow the population to increase dramatically. In order to explore this possibility, I considered three scenarios of intervention using vaccination against CDV, CPV or both in a very simple mathematical model (Appendix A). All scenarios assume that half of the reported 70% puppy mortality is due to disease, leaving nondisease causes such as infanticide, malnutrition, trauma, and congenital disorders to make up for the remaining half, or 35%. I further assumed that the vaccine used is 90% effective and that 50% of the puppies are successfully vaccinated.

In scenario 1, I assume that all puppy mortality due to disease is due to CPV. This results in a reduction of mortality, to 54%. If 50% of puppies are vaccinated every year for 10 years, the dog population at the end of this time is 2,031 — an increase of almost 300%. This is obviously unrealistic, because CPV is only one of several pathogens that are likely contributing to mortality, and vaccinating half of all puppies for 10 years would require a tremendous investment of time and money. On the other hand, given the common reports of diarrhea and vomiting as causes of death in pups, parvo is probably a major source of mortality among puppies, so this example illustrates what might happen if it were drastically reduced.

In scenario 2, a more conservative approach is taken. If 10% of disease mortality is due to parvo, and 10% is due to distemper, and 50% of puppies are vaccinated for both diseases each year for 10 years, the total mortality drops from the reported 70% to 67%. This small drop, however, results in an increase to 709 dogs after 10 years, an increase of 36%. Scenario 3 is intermediate between the previous two scenarios; I assume that 20% of disease mortality is due to CPV and 20% to CDV, but dogs are only vaccinated one year. In ten years, this results in a population increase of 12%, to 586 dogs.

These simple models illustrate that even a one-time intervention can substantially increase the dog population, in a community where there are already many dogs. By eliminating the effect of disease in limiting the population, these communities may literally become overrun with domestic dogs. This reduction in the disease burden may not be beneficial for wildlife if in preventing two diseases, the dog population burgeons such that there are many more hosts for other diseases. Does this mean that there are no steps we can take to protect wild carnivores from domestic animal diseases? Certainly not—but it does mean that simple intervention may not have simple results. An alternative scenario that may protect wildlife while keeping the dog population in check involves both vaccination and sterilization. If in addition to the conditions of Scenario 2, 10% of the female dogs are sterilized in the first year, the result is 519 dogs in ten years. Ten percent of female dogs is roughly 25 dogs, which represents a reasonable number of dogs that could be sterilized in a two week period. Further work will incorporate the effect of vaccination on disease transmission and exposure level within the domestic dog population. But it may not be possible to eliminate disease as a population regulator without intense, ongoing efforts to limit the dog population in other ways.

With proper consideration and planning, intervention with vaccination and sterilization may be a beneficial and feasible strategy to reduce the risk of disease spillover from domestic to wild carnivores. However, simply reducing the disease burden on a domestic animal population may have negative consequences if ecological and demographic factors are not considered and investigated. The mathematical method of predicting the change in the dog population in response to various interventions is purely theoretical; I hope that in the near future it will be possible for me to evaluate its utility. This is just one possible way of estimating the effect of a proposed conservation intervention—certainly more, and better, methods will be devised as more research is devoted to this area.

Conclusion

Contact among humans, wild animals, and domestic animals is increasing as humans venture further into wildlife habitats, and wild animals are forced to make their living in close proximity to human settlements. Even for species whose ecology is relatively well understood, we must consider the fact that carnivores are intelligent, highly adaptable mammals whose ecology and behavior will change as they are pressured by human activities and anthropogenic environmental impacts. Both new studies and ongoing surveillance are required for an adequate understanding of the ecology and disease dynamics of wild carnivores, without which we risk additional disease outbreaks in wild carnivore populations, our domestic carnivores, and ourselves. Preventing such outbreaks will be a continuing challenge as our planet continues to change, and intervention will become more and more necessary. This study is a preliminary attempt to explore the options of wildlife managers, biologists, and veterinarians when trying to design practical and effective strategies to protect wildlife from domestic animal diseases.

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Table 1 – Disease agents reported in selected nondomestic carnivores

Disease Agent	Captive animals	Free-ranging animals	References
Canine distemper virus	crab-eating fox,* maned wolf,* coati,* others	lion,* African wild dog,* others	Mann et al. 1980; Cubas 1996; Roelke-Parker et al. 1996; Murray et al. 1999
Canine parvovirus	crab-eating fox,* maned wolf,* bush dog,* coyote*	coyote*	Ervermann et al. 1982; Young 1994; Cubas 1996
Canine coronavirus	coyote*	African wild dog, coyote	Evermann et al. 1980; Murray et al. 1999
Canine herpesvirus		coyote	Davidson et al. 1992
Canine adenovirus-1		wolf, African wild dog, coyote	Murray et al. 1999
Rabies virus		leopard,* puma,* cheetah,* others	Murray et al. 1999
Feline leukemia virus		puma*	Jessup et al. 1993; Murray et al. 1999
Feline immunodeficiency virus	puma, jaguar, ocelot, margay, Geoffroy's cat, jaguarundi, others	jaguar, lion, puma	Carpenter and O'Brien 1995; Murray et al. 1999
Feline panleukopenia virus	jaguar,* margay,* ocelot,* leopard cat	puma, leopard cat	Paul-Murphy et al. 1994; Cubas 1996; Ikeda et al. 1999
Feline herpesvirus	leopard cat	puma, lynx	Paul-Murphy et al. 1994; Ikeda et al. 1999; Murray et al. 1999
Feline coronavirus		puma	Paul-Murphy et al. 1994
Feline calicivirus	leopard cat	puma, leopard cat	Ikeda et al. 1999; Murray et al. 1999
Toxoplasma gondii		puma, coyote, jaguar, puma, lynx*	Paul-Murphy et al. 1994; Murray et al. 1999
Dirofilaria immitis		wolf, coyote, jaguar, jaguarundi	Otto 1974
Sarcoptes scabei		coyote,* raccoon dog,* pampas fox,* red fox*	Pence and Windberg 1994; Young 1994; Shibata and Kawamichi 1999; Deem et al. 2002; Lindstrom et al. 1994
Leptospira interrogans		coyote,* wolf, lynx	Murray et al. 1999; Labelle et al. 2000
Brucella canis		wolf, coyote	Murray et al. 1999

* indicates that clinical signs were observed

Table 2 – Methodologies used by commercial laboratories to detect disease agents or disease exposure. Tests are performed on serum except where indicated otherwise.

Pathogen	Methodology	Lab
Canine adenovirus	antibody SN	Cornell-V
Canine coronavirus	antibody SN	Cornell-V
Canine distemper virus	antibody SN	Cornell-V
Canine herpesvirus	antibody SN	Cornell-V
Canine parvovirus	antibody HAI	Cornell-V
Dirofilaria immitis	antigen ELISA	Cornell-V
Sarcoptes scabiei	antibody ELISA	Laupeneck
Feline calicivirus	antibody SN	Cornell-V
Feline coronavirus	antibody KELA (IFA-equivalent)	Cornell-V
Feline herpesvirus	antibody SN	Cornell-V
Feline immunodeficiency virus	antibody ELISA	Cornell-V
confirmatory	Western blot	Cornell-V
Feline leukemia virus	antigen ELISA	Cornell-V
Feline panleukopenia virus	antibody HAI	Cornell-V
Leptospira interrogans	antibody MA	Cornell-V
Brucella canis	slide agglutination/AGID	Cornell-V
Toxoplasma gondii–canine	antibody IHA	Cornell-V
Toxoplasma gondii–feline	antibody KELA	Cornell-V
Enteric parasites	zinc sulfate flotation (feces)	Cornell-P
Insect ectoparasites (fleas)	microscopy (whole parasites)	Cornell-P
Non-insect ectoparasites (ticks)	microscopy (whole parasites)	WR

AGID = agar gel immunodiffusion, ELISA = enzyme-linked immunosorbent assay, HAI = hemagglutination-inhibition, IFA = immunofluorescent antibody, IHA = indirect hemagglutination, KELA = kinetic ELISA, MA = micro-agglutination,; SN = serum neutralization

Cornell-V = Cornell University Veterinary Diagnostic Lab, Section of Virology; Cornell-P = Cornell Diagnostic Lab, Section of Parasitology; Laupeneck = Labor Laupeneck, Switzerland; WR = Dr. R. Robbins, Walter Reed Army Hospital

Table 3 – Number of positive titers over number of individual domestic carnivores tested for various pathogens.

	CAV	CCV	CDV	CHV	CPV	FCV	FPV	HWD	Toxo	Lepto	Sarcoptes
cats	N/A	N/A	N/A	N/A	N/A	6/6	6/6	0/6	5/6	1/5	N/A
						100%	100%	0%	83%	20%	
dogs	32/96	48/96	92/96	65/95	92/96	N/A	N/A	8/95	36/96	10/35	52/82
	33%	50%	96%	68%	96%			8%	38%	29%	63%

CAV = canine adenovirus; CCV = canine coronavirus; CDV = canine distemper virus; CHV = canine herpesvirus; CPV = canine parvovirus; FCV = feline calicivirus; FPV = feline panleukopenia virus; HWD = heartworm disease

Table 4 – Number of individuals captured (N) and number with positive results over number tested for exposure to various pathogens.

Species	N	CDV	CPV	FCV	FIV	FPV	HWD	Lepto	Toxo
Cerdocyon thous	3	0/2	2/3	N/A	N/A	N/A	2/3	0/2	2/3
Oncifelis geoffroyi	8	0/8	N/A	4/8	0/8	3/8	0/8	0/8	2/8
Herpailurus yaguarondi	1	0/1	N/A	0/1	0/1	0/1	0/1	0/1	0/1
Leopardus pardalis	10	6/8	N/A	7/8	1/8*	0/8	0/8	0/8	8/8
Pseudalopex gymnocercus	5	4/5	4/5	N/A	N/A	N/A	0/5	1/5	1/5

CDV = canine distemper virus; CPV = canine parvovirus; FCV = feline calicivirus; FIV = feline immunodeficiency virus; FPV = feline panleukopenia virus; HWD = heartworm disease
HWD = heartworm disease; Lepto = *L. interrogans*; Toxo = *T. gondii*;
* One ocelot had equivocal results for FIV

Table 5 – Average responses to resident questionnaires (N = 41).

Number of dogs owned	3,6
Number of female dogs	1,5
Number of male dogs	2,2
Age of female dogs (years)	3,3
Age of male dogs (years)	2,7
Litter size	3,7
Number pups that die per litter	2,6
Pup mortality	0,7
Adult mortality	0,38
Percent of dogs that hunt	82,0%

Appendix A

$$A = P-(D*P)-S/2*P+L*Y$$

Where A = projected dog population
D = death rate of adult dogs
P = current dog population
S = sterilization rate of females
L = survival rate of puppies
Y = current puppy population

Y is further defined as

$$Y = B/2*0.75*P$$

Where B = birth rate

The final dog population T depends on the sterilization rate of females:

$$F = C + S/2*P-D*C$$

Where C = current sterile female popuation
F = projected number of sterile females

So that

$$T = F+A$$