INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA – INPA
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Grau de parentesco e relações sociais em ariranhas (*Pteronura brasiliensis*)

CAROLINA RIBAS

Manaus, Amazonas
Maio, 2012

CAROLINA RIBAS

Grau de parentesco e relações sociais em ariranhas (*Pteronura brasiliensis*)

Orientador: Dr. William Magnusson
Co-orientador: Dr. Guilherme Mourão

Tese apresentada ao Programa de Pós Graduação em Biologia Tropical e Recursos Naturais do Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de Doutor em Biologia (Ecologia).

Manaus, Amazonas
Maio, 2012

R482 Ribas, Carolina
Grau de parentesco e relações sociais em ariranhas (Pteronura brasiliensis) / Carolina Ribas.--- Manaus : [s.n.], 2012.
xi, 79 f.. : il.

Tese (doutorado) --- INPA, Manaus, 2012
Orientador : William Magnusson
Coorientador : Guilherme Mourão
Área de concentração : Ecologia

1. Ariranhas – comportamento. 2. Marcadores microssatélites. 3. Dardos de biópsia. 4. Parentesco. 5. Ecologia de mamíferos. I. Título.

CDD 19. ed. 599.744470415

Sinopse:

Estudei a estrutura social e grau de parentesco de uma população de ariranhas, *Pteronura brasiliensis*, Pantanal Sul, Brasil. Obtive a maior parte das amostras de tecido através de dardos de biopsia, desenvolvi marcadores de microssatélites específicos para a espécie e estabeleci os graus de parentesco em 52 indivíduos de 13 grupos.

Palavras- chave: dardos de biópsia, microssatélite, parentesco, estrutura social.

Relação da banca examinadora do trabalho escrito

Dr. Eduardo Eizirik
Pontífica Universidade Católica do Rio Grande do Sul
PARECER: Aprovada

Dr. Fernando Azevedo
Universidade Federal de São João del Rei
PARECER: Aprovada

Dra. Izeni Farias
Universidade Federal do Amazonas
PARECER: Aprovada

Dr. Matthew Gompper
University of Missouri, EUA
PARECER: Aprovada

Dr. Thomas E. Lacher, Jr.
Texas A&M University, EUA
PARECER: Aprovada

Relação da banca examinadora da defesa oral

Dr. Fernando Rosas
Instituto Nacional de Pesquisas da Amazônia (INPA)
PARECER: Aprovada

Dra. Izeni Farias
Universidade Federal do Amazonas
PARECER: Aprovada

Dra. Vera M. F. da Silva
Instituto Nacional de Pesquisas da Amazônia (INPA)
PARECER: Aprovada

Dedico esta Tese ao meu pai,

Alberto Marques Pereira, por ter aguentado firme

e se cuidado, para eu poder realizar este sonho com sanidade, e a

Gabriel Damasceno, pela cumplicidade.

Agradecimentos

À vida, por ser generosa, por estar sempre perto, por me conduzir e iluminar o caminho, por me deixar amá-la.

Muitas pessoas ajudaram, muitas participaram, mas sem Gabriel Damasceno, este trabalho não aconteceria. Não, **este** trabalho.

A minha banca de qualificação avisou, provavelmente não seria factível conseguir tecidos de ariranhas, desenvolver marcadores genéticos específicos para a espécie e analisar o set de dados em quatro anos. Tudo parecia difícil, mas talvez o mais duvidoso fosse conseguir acertar animais tão rápidos, inteligentes e "tridimensionais". Gabriel foi lá, enfrentou mosquitos, calor, desconforto, e muita, muita frustração, principalmente quando ainda éramos dois perdidos sem idéia de como resolveríamos aquele desafio. Desafio que passou a ser dele. Convivemos dias e horas, às vezes na mesma posição, sem poder comer, falar, mexer e claro, nem sempre foi bom, mas estávamos juntos, cúmplices. Choramos de surtos e de alegrias e a cada tecido novo era como se a gente se esquecesse da insanidade que era este projeto e o mundo ficasse colorido. Apaixonou-se pelo bicho, não teve jeito, e curtirmos muitos momentos únicos ao lado destas adoráveis criaturas. Encontramos onças-pintadas, soberanas, deitadas no barranco do rio ou nos rondando, que sabíamos por suas pegadas frescas, esturros ou dando de cara, quando denunciada pelos nossos amigos passarinhos. Morro do Azeite, 06 de outubro... Foram muitos momento inesquecíveis, e, além de tudo, A GENTE CONSEGUIU! Obrigada por estar comigo, obrigada por seu grande talento em acertar ariranhas. Você é o cara. Sempre serão!

Cronologicamente as informações aqui contidas são fruto de uma extrema curiosidade, nascida nas águas do rio Salobra. Agradeço à família Copetti de Moura por tão forte acolhida, no início de tudo.

Dividir um grande interesse é um bom passo para uma verdadeira amizade. Guilherme Mourão é primeiramente um amigo, que eu vou levar para sempre. Iniciamos, juntos, o programa de monitoramento de ariranhas no Pantanal que, em 2012, faz dez anos. Neste tempo, além do olhar científico sobre as ariranhas, eu aprendi valores, recebi carinho, dei muita risada e claro, discordamos um monte, mas sempre com muito respeito, de bons amigos. Passamos horas em nosso assunto preferido, sociabilidade, que nos levou a descobrir coisas sobre nós mesmos e, a esta Tese. Enfim, passei grande parte dos meus últimos anos em sua companhia e foi ótimo! Obrigada Gui, por me ensinar ciência, por me dar condições de ter convivido com as ariranhas todo este tempo e por você ser o cara que você é.

O sonho em responder sobre o parentesco de ariranhas nasceu no mestrado, mas para quem não tinha nenhuma experiência genética, precisava de alguém que lhe jogasse do precipício. Em meia hora de conversa com o Bill, olho no olho, no nosso primeiro encontro, ele me desafiou com um sorriso irônico: "você tem quatro anos para aprender e ensinar aos seus orientadores", como se fosse "simples, fácil, trivial". Além de tudo, naquela época, ele não queria de jeito nenhum, orientar com ariranhas. Eu saí da sua sala confusa, instigada e fui escrever o projeto. Muita água passou debaixo da ponte e enfim, ele aceitou o desafio, e foi inclusive, conhecê-las no Pantanal! E foi assim ao longo do tempo, pouco convívio, mas quando perto, te abre caminhos. Aprendi que não precisa de muito tempo com o Bill, ele é extremamente rápido em seu discernimento científico, basta você estar atento e deixar ele te ensinar. Obrigada!

À Claudia Keller, por me responder tudo o que eu precisava ouvir, em uma hora em que não se podia ter dúvida e, precisava coragem. Pelo apoio, firme e irrestrito, me auxiliando a conquistar um antigo desejo.

Ao Fernando Rosas por ter aberto seu laboratório para mim, pelas conversas sobre ariranhas e por ter me dado à oportunidade de cuidar da i/nyryny. À Galia de Mattos pelo apoio, no meu início de vida em Manaus.

À Vera da Silva, a quem eu admiro profundamente e que sempre me deu muita força, com boas conversas, novas ideias e exemplo.

A Nicole Cristina Lopes Dutra por ter me tirado "mil" dúvidas genéticas enquanto eu escrevia meu projeto de doutorado.

Ao Fabrício Santos por ter me aberto as portas de seu laboratório e Débora Garcia por me ensinar os primeiros passos em uma bancada genética.

Ao Antônio Sole-Cava, por ter me aceitado em seu laboratório e tornado o doutorado mais factível e agradável, me proporcionando o convívio com minha família. Por acreditar no trabalho e me fornecer condições de torná-lo possível. Por iniciar o desenvolvimento de microssatélites do laboratório com *Pteronura*! Pelo carinho "House" de ser. Sole, tenho muito orgulho de ser um membro do LBDM!

A Haydée Cunha, pela grande ajuda durante todo o processo, pela orientação genética, pela força nas conversas, pela acolhida e principalmente por ter feito este projeto, seu também.

A todos os parceiros do LBDM, que me ajudaram das mais diferentes formas, tirando dúvidas genéticas, de bancada ou simplesmente dando força. Um agradecimento especial à Aretusa G. A. Macedo e Anderson Vasconcellos, por terem posto a "mão na massa" comigo,

em momentos de desespero, e me ensinado a importância de normalizar as amostras! Aos divertidos almoços no bandejão.

Aos funcionários da Embrapa-Pantanal, que me apóiam desde o mestrado, principalmente nas pessoas de Wilson dos Santos Batista, Dênis Celin Tilcara, Odilza Soares C. e Sidnei José Benício. Em especial para Isac Teixeira de Carvalho pela parceria e trabalho em campo.

A Universidade Federal do Mato Grosso do Sul e toda equipe da Base de Estudos do Pantanal (BEP) pela base de apoio.

Ao Sr. João Júlio (in memorian), proprietário da Pousada Rio Vermelho, por me dar apoio logístico durante todo o tempo de trabalho, desde o mestrado.

Ao CNPq. pela bolsa de doutorado que me permitiu sobreviver aos quatros anos de estudo.

Ao Coronel Carlos Marsiglia (DFPC- Brasília), por me mostrar que basta uma pessoa de boa índole e que se importe para tudo que parecia difícil se transformar em simples. Com competência e atenção me auxiliou na importação dos dois projetores de CO_2 deste trabalho, mostrando que uma pessoa é capaz de fazer o sistema funcionar, mesmo com o descaso de alguns.

Ao Parque Zoológico Municipal Quinzinho de Barros, na pessoa de Rodrigo Teixeira, pela parceria no trabalho e amostra de *Lontra longicaudis* e ao Programa de Conservação Mamíferos do Cerrado, nas pessoas de Frederico Gemesio Lemos, Fernanda Cavalcanti de Azevedo e Ricardo Arrais, por ceder amostras de *Eira Barbara* e *Conepatus semistriatus*.

A Nicole Duplaix e Jorge Schweizer pelos dois grandes trabalhos com a espécie, que são fonte de conhecimento e inspiração e transbordam paixão.

Ao Arnaud Desbiez, por estar vivo.

À Patrícia Médici por ter me aberto os olhos para as ariranhas “marginais” da Estrada Parque Pantanal, por toda a força durante o trabalho e bons contatos.

Ao amigo Carlos André Zucco, amigo mesmo, daqueles que ajuda nas análises, formata sua tese, faz a sua figura da área de estudo, te da carona, enfim, salva a sua vida quando você mais precisa! Obrigada mesmo!

À Fabiana Lopes Rocha, minha amiga, que hoje também é minha “roomate”! Obrigada Bia por tudo, pela força, pela paciência em um ano chatinho, pela parceria, pelos mil papos sobre a vida, por sua ótima amizade!

Ao Ubiratan Piovezan, por sua amizade ao longo destes anos, pelos papos profundos sobre a vida e por ter ido ao campo comigo tentar a sorte com as ariranhas!

Ao Pedro Cordeiro Estrela por toda força, conselhos e “cover letter” norteadora.

À Raquel S. Juliano, por me deixar morar em sua casa, na reta final de fechamento de tese e me proporcionar um ambiente maravilhoso, com boas companhias de gatos (Estrupi!) e cachorro despertador Porã, vista para o rio Paraguai e sua divertida forma de ver a vida. Obrigada pelo carinho!

Ao Luiz Gustavo R. O. Santos pela ajuda nas analises.

À Caroline Leuchtenberger pela troca de informações durante o trabalho e amostras de muco e sangue.

As parceiras pantaneiras que torceram por mim: Alessandra Bertassoni, Carolina Carvalho Cheida, Rita Bianchi, Juliane Saab de Lima e Pâmela Antunes.

Às parceiras “manauaras” Camila Ferrara, Nívia Carmo, Maria Beatriz Nogueira Ribeiro, Dayse Campista, Nataly Pinto e Roberta Elise Silva e minha parceira de “pito” Fabiane Lima de Oliveira (in memorian). Camila foi perfeito ficar trabalhando a defesa na sua casa, as duas no mesmo momento, obrigada!

A Leslie Damasceno, que não tenho como expressar tudo o que me ajudou. Primeiro porque sempre sabia o que me dizer nas horas de desespero ou simplesmente de um conselho, com sua grande experiência e sabedoria acadêmica e com o carinho de quem sabe as dificuldades do processo. Por todas as vezes que veio cheia de encomendas de equipamentos para o projeto, sem os quais não teríamos feito o trabalho e por toda ajuda na revisão dos textos em inglês. Pela amizade e carinho.

Ao Sergio Damasceno, que atravessou o oceano com o primeiro projetor e passou momentos chatos na alfândega, por trazer vários equipamentos a cada viagem, pelo empréstimo do apt. da Lapa, pela força!

Ao meu pai, Alberto, por ser quem ele é; um homem forte, um leão, que sempre encheu a casa de exemplo e amor e nos ensinou, acima de tudo, os valores corretos de um bom cidadão. Ao grande amor e forte paixão mútua que nos envolve desde sempre, e que nos cria esta dependência. Por ter me ensinado, desde pequena, o gosto de ser “um detetive”, na época tentando me convencer a gostar de matemática, fui para os bichos. Não consigo imaginar o mundo sem ele. Meu alicerce, minha referência genética, um grande amor.

À minha mãe do coração Maria, por ser o ser humano mais iluminado que já conheci. Alguém, que de tão bela, me fez crescer com a sensação de ter sido tudo sempre certo e que eu era a pessoa mais sortuda do mundo, por tê-la. Seu coração é doce e puro e só melhora o mundo. À mãe Siumara Maria, que me faz empírico o poder da genética em sua forma de amar os bichos, os velhos e Hermann Hesse. Obrigada mãe, por me deixar tão preciosa

herança. Ao meu irmão, por sua sensibilidade e preocupação com as coisas do mundo, por ser meu irmão.

Ao tempo, sozinha comigo mesma, lendo, escrevendo, pensando, filosofando, dentro, profundo, sobre as coisas da vida, do mundo, de mim. Crescimento humano, além do científico. Sorte.

Este trabalho foi feito de paixão e curiosidade. Espero, de verdade, estar contribuindo, mesmo que de forma pequena, para o conhecimento, interesse e quem sabe o amor, a estes fascinantes animais.

Resumo

Análises moleculares têm revolucionado o conhecimento sobre os sistemas sociais das espécies. Ariranhas são animais sociais que apresentam uma forte cooperação entre os indivíduos dos grupos e observações de comportamento sugerem que são compostos por um par dominante reprodutivo e seus filhotes de anos subsequentes, que não reproduzem dentro do grupo de origem. Este trabalho teve como objetivos (1) estabelecer um método semi-invasivo de retirada de amostra genética (tecido) para ariranhas em vida livre; (2) desenvolver marcadores de microssatélites específicos para *Pteronura brasiliensis*; e (3) acessar o grau de parentesco dentro e entre grupos de ariranhas, em uma população do Pantanal Sul, Brasil. Dardos de biópsia foram capazes de coletar amostras de tecido de alta qualidade de DNA, de um grande número de ariranhas (n=41), há um custo menor e de forma menos invasiva do que a captura, além de possibilitar a escolha do indivíduo a ser amostrado. Oportunisticamente, amostras genéticas de outras fontes, que não dardos de biópsia, foram coletadas. Doze *loci* polimórficos de microssatélites específicos para *P. brasiliensis* foram isolados. Todos os 50 indivíduos de ariranhas genotipados pertenciam a uma única população, indicando substancial fluxo gênico na escala examinada. A variabilidade nuclear encontrada para a população do Pantanal estava na faixa observada para ariranhas em escalas geográficas maiores e para outras espécies de lontras. Os grupos de ariranhas foram geralmente compostos por um casal dominante não relacionado e seus parentes próximos. Entretanto, uma alta diversidade de graus de parentesco dentro dos grupos foi encontrada, contradizendo o conhecimento corrente de que grupos de ariranhas são exclusivamente formados por um casal dominante reprodutor e seus filhotes. Os mecanismos evolutivos que levam à alta variação de parentesco dentro dos grupos de ariranhas ainda não estão claros, mas alta taxa de migração de indivíduos entre os grupos, cópula extra-par e reprodução de subordinados podem desempenhar um papel importante, como acontece em outras espécies aparentemente monogâmicas.

Abstract

Relatedness and social relation of giant otters (*Pteronura brasiliensis*)

Molecular analysis has revolutionized knowledge of the social systems of species. Giant otters are social animals who demonstrate strong cooperation between individuals of the same group, and studies based on behavioral observation suggeste that the group is composed of a dominant reproductive pair and their offspring from subsequent years, which do not reproduce. This study had as objectives (1) to establish a semi-invasive method of retrieving genetic samples (tissue) from wild giant otters; (2) to develop specific microsatellite markers from *Pteronura brasiliensis*; (3) to access the degree of relatedness within and between groups of giant otters in the southern Pantanal, Brazil. Biopsy darts allowed us to collect tissue samples with high DNA quality from a large number of giant otters (n=41). This method cost less and was less invasive than capture, and allowed for the choice of individual to be sampled. Twelve polymorphic loci of microsatellites specific to *P. brasiliensis* with high resolution for paternal analysis were isolated. DNA samples were opportunistically collected by means other than biopsy darts, for a total of 50 genotyped giant otters. All examined animals belonged to a unique population, indicating substantial gene flow on the scale examined. The nuclear variability found in the population of the Pantanal was within the observed range for giant otters in larger geographic scales as well as for other otter species. The giant otter groups were usually composed of an unrelated dominant pair and their close relatives. However, the degree of relatedness varied within the groups, contradicting the current knowledge that giant otter groups are formed exclusively by a dominant pair and their offspring. The ecological mechanisms that lead to high relatedness variance within giant otters groups it is unclear, but we believe that high migration rate of individuals across groups, extra-pair copulation and subordinate reproduction can play important roles, as they do in other apparently monogamous species.

Sumário

Introdução Geral

As metodologias de estudo com animais silvestres têm se tornado cada vez mais dinâmicas e eficientes, evoluindo e aperfeiçoando-se junto às mudanças tecnológicas globais. Como consequência, questões antes nunca abordadas sobre a ecologia das espécies estão sendo respondidas, ampliando o conhecimento da vida silvestre e gerando bases mais seguras para o manejo destes recursos (Hughes, 1998; Worthington Wilmer *et al*., 1999; Ross, 2001; DeYoung e Honeycutt, 2005; De Woody, 2005).

Talvez o avanço mais recente nas técnicas de estudo dos animais silvestres seja o uso das ferramentas moleculares (DeYoung e Honeycutt, 2005). Estas técnicas têm revolucionado o conhecimento acerca da estrutura populacional das espécies (*i.e.* filogeografia) e de como estas espécies se organizam socialmente (*i.e.* relações de parentesco, paternidade; Coltman *et al*., 2003; Haynie *et al*., 2003; Cant, 2000), vislumbrando informações além das aparências.

Pesquisadores têm utilizado diferentes métodos de amostragem genética em mamíferos silvestres: a) não-invasivos através de fezes, pêlos e peles deixados no ambiente (*e.g.* Amos *et al*., 1992; Taberlet *et al*., 1997; Waits e Paetkau, 2005; Mowry *et al*., 2011), b) pouco-invasivos, através de dardos de biópsia, principalmente em cetáceos e grandes mamíferos terrestres africanos (*e.g.* Gemmel e Majluf, 1997; Spong *et al*., 2002; Muwanika *et al*., 2003), e c) invasivos, com a captura do animal, normalmente associada a estudos de telemetria (*e.g*. Girman *et al*., 1997; Griffin *et al*., 2003; Eizirik *et al*., 2008).

Ariranhas (*Pteronura brasiliensis*) são grandes mustelídeos semi-aquáticos (sub-família Lutrinae) e um dos poucos carnívoros sociais da América do Sul. Indivíduos podem ser reconhecidos através de padrões de pelagem individuais no pescoço e garganta (Duplaix, 1980).

A espécie é classificada como "Ameaçada" pela União Internacional para a Conservação da Natureza (IUCN, 2011) como consequência do histórico de caça (Carter e Rosas, 1997) e contínua destruição de habitat devido ao aumento da colonização das florestas tropicais e suas atividades econômicas, como mineração de ouro, hidrelétricas, desmatamento e pesca predatória (Schenck, 1999; IUCN, 2011; OSG IUCN, 2010). No Brasil, populações viáveis de ariranhas estão limitadas às bacias hidrográficas da Amazônia e Pantanal (Carter e Rosas, 1997). No Pantanal, grupos estão amplamente distribuídos ao longo dos rios e corixos da planície (Schweizer, 1992; Tomas *et al*., 2000; Ribas, 2004), e recentemente estão

ocupando ambientes marginais sub-ótimos, provavelmente por um aumento nas densidades populacionais nesta região (ver Apêndice).

Grupos de ariranhas geralmente variam de três a nove indivíduos e observações de comportamento sugerem que os grupos são compostos por um par dominante reprodutivo e seus filhotes de anos subsequentes, que não reproduzem dentro do grupo de origem (Duplaix, 1980; Schweizer, 1992; Kruuk, 2006). Indivíduos solitários são chamados de transeuntes e acredita-se que são jovens dispersores recentemente saídos do grupo familiar ou adultos que perderam o seu par (Duplaix, 1980; Schweizer, 1992).

Membros do grupo exibem comportamento altamente cooperativo, incluindo reprodução cooperativa (Duplaix, 1980; Schweizer, 1992; Rosas *et al*., 2009) e defesa de território, através de marcação e vocalização, assim como contra predadores e intrusos co-específicos (Duplaix, 1980; Schweizer, 1992; Ribas e Mourão, 2004). Por outro lado, grupos vizinhos claramente evitam-se (Duplaix, 1980; Kruuk, 2006), ocorrendo interações intra-agonísticas, incluindo canibalismo e brigas territoriais (Mourão e Carvalho, 2001; Ribas e Mourão, 2004).

A estrutura genética e a estrutura social estão indissociavelmente ligadas (DeWoody, 2005). Sociabilidade e comportamento cooperativo devem apenas evoluir e permanecer estáveis se todos os indivíduos envolvidos obtiverem benefícios adaptativos maiores do que o custo de viver em grupo (Hamilton, 1964). A seleção de parentesco favorece interações cooperativas entre indivíduos relacionados, aumentando suas aptidões indiretas. O parentesco entre os membros dos grupos sociais pode dar forma à evolução dos sistemas sociais (Dugdale *et al*., 2008).

Ariranhas defecam e urinam em latrinas comunais e as fezes de diferentes membros do grupo são misturadas durante o comportamento de marcação (Duplaix, 1980), impedindo a coletada de amostras de indivíduos específicos. Entretanto, algumas vezes as ariranhas depositam mucos isolados nas latrinas, o que permite a individualização da amostra (Garcia *et al*., 2007).

Para estudar estrutura social em ariranhas, é preciso uma amostragem genética do maior número de indivíduos por grupo possível, de forma segura e eficiente. Assim, embora algumas vezes têm se coletado amostras de sangue de ariranhas capturadas para estudo de telemetria (Silveira *et al*., 2011), uma estratégia alternativa à captura oportunista é requerida. Dardos de biópsia são bons candidatos para uma amostragem seletiva de tecido fresco e não contaminado (Karesh *et al*., 1987; Gemmel e Majluf, 1997).

Em geral, microssatélites são os marcadores moleculares mais informativos para acessar estrutura populacional, paternidade e parentesco e vêm sendo amplamente utilizados no estudo da ecologia da vida animal (De Woody, 2005; Randall *et al*., 2007; Dugdale *et al*., 2008). Microssatélites são sequências de nucleotídeos de DNA contendo de 1 a 6 repetições em *tandem* e estão amplamente distribuídos na maior parte do genoma dos eucariotos (Queller *et al*., 1993). Porque eles são encontrados milhares de vezes no genoma dos vertebrados e não são expressos, os microssatélites são considerados marcadores neutros, isto é, não estão sob a influência da seleção natural (De Woody, 2005). A diversidade genética é geralmente alta nos *loci* de microssatélites animais, sendo considerados muito mais polimórficos que outros marcadores moleculares (Moueix, 2006).

Ainda que marcadores de microssatélites desenvolvidos para lontra Européia e Norte Americana (*Lutra lutra* e *Lontra canadensis*, respectivamente) tenham sido testados em ariranhas (Pickles *et al*., 2009), o uso de marcadores heterólogos pode subestimar a heterozigosidade (Garner *et al*., 2005). Assim, é desejável o desenvolvimento de marcadores de microssatélites específicos para *Pteronura brasiliensis*, possibilitando o acesso a informações genéticas acuradas que possam lançar luz sobre o seu sistema social.

Este trabalho teve como objetivos gerais: (1) estabelecer um método semi-invasivo de retirada de amostra genética (tecido) para ariranhas em vida livre; (2) desenvolver marcadores de microssatélites específicos para *Pteronura brasiliensis*; e (3) acessar o grau de parentesco dentro e entre grupos de ariranhas, em uma população do Pantanal Sul, Brasil.

Sendo assim, a tese está organizada em três capítulos, cobrindo os objetivos descritos acima: Capítulo I- "Costs and benefits of semi-invasive genetic sampling of social aquatic carnivores (*Pteronura brasiliensis*)", Capítulo II- "Polymorphic microsatellite loci from the endangered Giant Otter (*Pteronura brasiliensis*)" e Capítulo III- "More than meets the eye: kinship and social organization in giant otters (*Pteronura brasiliensis*)".

Objetivos

(1) estabelecer um método semi-invasivo de retirada de amostra genética (tecido) para ariranhas em vida livre;

(2) desenvolver marcadores de microssatélites específicos para *Pteronura brasiliensis*;

(3) acessar o grau de parentesco dentro e entre grupos de ariranhas, em uma população do Pantanal Sul, Brasil.

1º Capítulo

Ribas, C., Damasceno, G., Magnusson, W., Cunha, H., Mourão, G. Costs and benefits of semi-invasive genetic sampling of a social aquatic carnivore (*Pteronura brasiliensis*). Manuscrito em preparação para *Wildlife Society Bulletin*

Costs and benefits of semi-invasive genetic sampling of a social aquatic carnivore (*Pteronura brasiliensis*)

Carolina Ribas[a,c,e,*], Gabriel Damasceno[b], William E. Magnusson[a], Haydée Cunha[c,d] and Guilherme Mourão[e].

[a]Graduate Program in Ecology, Instituto Nacional de Pesquisas da Amazônia - INPA,CP 478, 69011-970 Manaus, Amazonas, Brazil.
[b]C.C.G. Productions. Rio de Janeiro, RJ, Brazil.
[c]Laboratory of Molecular Biodiversity, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590, Brazil.
[d]Laboratory of Aquatic Mammals and Bioindicators, Universidade do Estado do Rio de Janeiro.
[e]Embrapa Pantanal, Brazilian Agricultural Research Corporation, CP 109, Corumbá-MS, 79320-900, Brazil

[*]Corresponding author: email address: ariranhapantanal@yahoo.com.br. Phone: +55-67-3234-5929, Fax: +55-67-3234-5815.

Abstract

Molecular tools have enabled wildlife researchers to access accurate information on genetic structure of wild populations and different genetic sampling strategies have been adopted. Our objective was to evaluate a semi-invasive genetic sampling method for free-ranging giant otters and evaluate the costs and benefits of acquisition of different biological samples, suitable for molecular genetic approaches. We used three CO2 biopsy dart projectors and attached a system of line and spinning reel to the end of the projector barrel to ensure biopsy dart recovery. We stalked the otters in areas with a clear view to a latrine or den entrance. Using biopsy darts in 105 days of fieldwork we obtained 45 skin samples from 41 adults in 12 groups. No behavioral changes were noted in the animals that had been darted in the following days. We opportunistically captured three cubs and removed a sample from each, and collected blood samples from two adults captured to tag with intraperitonial implanted transmitters. We also took a tissue sample from a dead cub and collected 23 mucus (anal secretion) deposits from latrines. Mean field costs were US$524 for dart samples and US$3200 for adult-capture samples. All tissue and blood samples amplified all 14 loci, but none of the 23 mucus samples amplified all loci and 18 did not amplify any locus. The use of biopsy darts was the most effective method to retrieve tissue samples with high-quality DNA, from a large number of giant otters and was less expensive and invasive than capture. Moreover, this methodology allowed direct sampling of known individuals, which is essential for studies of social structure.

KEY WORDS: biopsy dart, giant otter, parentage, Pantanal, relatedness, social system.

Introduction

Wildlife research has benefited from many technical advances. Molecular tools have enabled wildlife researchers to access accurate information on parentage, relatedness, mating system and dispersal of individuals, revolutionizing ideas about social structure, and providing critical information for the conservation and management of species (Hughes 1998, Worthington Wilmer et al. 1999, Ross 2001, DeYoung and Honeycutt 2005, De Woody 2005, Dugdale et al. 2007, Randall et al. 2007).

Non-invasive genetic sampling strategies that have been adopted in wild ranging mammals include DNA extraction from feces, fur and sloughed skin (e.g., Amos et al. 1992, Taberlet et al. 1997, Waits and Paetkau 2005, Mowry et al. 2011). Semi-invasive biopsy darting has been increasingly applied to cetaceans and free-ranging terrestrial mammals (e.g., Gemmel and Majluf 1997, Spong et al. 2002, Muwanika et al. 2003). The most invasive sampling method involves capture, usually associated with telemetry studies (e.g., Girman et al. 1997, Griffin et al. 2003, Eizirik et al. 2008).

Giant otters (*Pteronura brasiliensis*) are semi-aquatic mustelids (sub-family Lutrinae) that can reach a total length of 1.8meters and weigh 28-32kilos (Duplaix 1980). Endemic to South America, the species is classified as “Endangered" by the IUCN (2011) due to population decline in the historical range as a consequence of over-harvesting for the international pelt trade and habitat destruction (OSG IUCN 2010). Giant otters are social carnivores. Observational data suggest that the groups are formed by a dominant reproductive pair, and their offspring of previous years, which do not breed inside the original group (Duplaix 1980, Carter and Rosas 1997). Individuals frequently show cooperative behavior between members of the group (Duplaix 1980, Schweizer 1992, Davenport 2010) and marked agonistic interactions with neighboring groups (Mourão and Carvalho 2001, Ribas and Mourão 2004).

Giant otters that are members of groups defecate and urinate in communal latrines, and feces are mixed due to territorial marking behavior (Duplaix 1980), preventing collection of feces of known individuals. However, the individuals can also deposit isolated anal jelly secretions (mucus), containing intestine epithelial cells, which allow for individual sampling (Garcia et al. 2007). Most sources of samples for molecular genetic studies have been blood and tissue samples from captive or dead animals, mucus from latrines and museum samples (Franco-de-Sá et al. 2007, Garcia et al. 2007, Pickles et al. 2009, Pickles et al. 2011), though

samples have been collected opportunistically from giant otters captured for radio-telemetry studies (Silveira et al. 2011). Alternative strategies to opportunistic capture are required to sample the majority of animals in a group safely and effectively, and biopsy darting is a promising candidate for selective sampling of fresh and uncontaminated tissue (Karesh et al. 1987, Gemmel and Majluf 1997).

Here we present information on an adaptation of a semi-invasive method of genetic sampling for giant otters and evaluate the cost and benefits of different techniques for the acquisition of biological samples suitable for molecular-genetic studies of wild giant otters.

Material and Methods

Study Population

We studied giant otters in the Miranda and Vermelho rivers (UTM 21k 501897 7831480), in southern Brazilian Pantanal, and in water bodies around the *Estrada-Parque Pantanal* (EPP) highway (UTM 21k 0451800 7873700 to 21k 0496300 7831500), a 120km dirt road constructed 1-2m above the surrounding plains. The Miranda and Vermelho Rivers are considered to be favorable habitats long inhabited by giant otters, whereas the water bodies around the EPP road are considered sub-optimal habitats for this species (Ribas et al. 2012).

We conducted one field campaign in December 2008 and twelve campaigns in the dry seasons (June-December) of 2009 and 2010 to collect genetic samples from wild giant otters (license no. 12794-4/2012, granted by the Brazilian Federal Wildlife Agency - ICMBio), with the main goal of determining genetic relatedness within and among groups from this population. We surveyed the rivers for the giant otter groups, using a small boat with a 15horsepower outboard engine, and mapped their territories by locating tracks, active dens, and latrines on the river banks. Along the EPP highway, we sought animals and their tracks around the water bodies close to the road. Animals were filmed with a digital video recorder to identified individual otters through their throat and chest patterns before sampling.

We classified individuals as adults or cubs of the year, identified the alpha male and female in each group based on morphological characteristics, such as testicles and the larger width of the neck for males, and the presence of lactation in females and behaviors, such as greater activity in marking and defending territory by the dominants.

Semi-invasive genetic sampling

We used three types of CO_2 biopsy-dart projectors: Dist-inject model 35 dart gun (Zurich, Switzerland), Pneu-Dart 176b rifle and Pneu-Dart X-Caliber gauged rifle (Pneu-Dart, Inc. Williamsport, PA 17703 USA). To facilitate aiming, we attached a Professional 25 mW Green Military Astronomy Grade Laser Pointer to the 176b rifle. As the X-Caliber came equipped with a 3x9 adjustable scope, no laser was required to improve aim.

We attached a system of line and spinning reel (Apollo Spincast Reel) to the end of the projector barrel to ensure biopsy-dart recovery. The Power Pro-Red Multifilament (100% Spectra Fiber) 10 lb was more effective than the nylon lines, which, when stretched, wrapped around sticks and branches underwater. However, nylon lines seemed to have less friction leaving the reel.

Most of the darts used were Pneu-dart™ 2cc biopsy darts w/.400 cutter/.670 barb (Pneu-Dart, Inc. Williamsport, PA 17703 USA). A hole was drilled through the side of the rear of the biopsy-dart shaft to secure the line. However, as the line clearly affected flight trajectory and accuracy, we also tested floating darts (Pneu-Dart™ 5cc floating biopsy darts w/.400 cuter/.670barb). Besides being larger and heavier, the darts without the line were easily lost in the undergrowth or swept away by the current. The floating dart was apparently regarded as potential prey by grey-necked wood-rails (*Aramides cajanea*), kingfishers (*Ceryle torquata*) and caimans (*Caiman yacare*), as well as the giant otters, obliging us to leave the blind to recover them after every shot.

Only animals with their body totally above the water line were considered for sampling; only body parts posterior to the mid-shoulders were targeted, sampling was not attempted in the presence of cubs, and lactating females were only sampled when the rump could be targeted. Dart heads were sterilized with NaOH 1M and rinsed with distilled water before use, to avoid sample contamination and reduce the risk of infection.

Approximation was one of the most difficult aspects of the method, and our strategy changed through time. Before biopsy samples could be taken, each otter had to be identified, and the choice of individual to be sampled depended on its position and if it had been sampled before. Initially, we stalked otters in rivers from a boat in areas of heaviest use and anchored about 10 m from dens, or waited on the other side of the river. We approached animals by motor or rowing as soon as they appeared to be intent on climbing onto dry land, and on one occasion, two individuals were chased by boat to induce them to leave the water.

Subsequently, we changed our strategy. An observer and a camouflaged sniper hid on the bank, with a clear view to a latrine or den entrance, 5-10 m away. For such distances, the dart-gun pressure was adjusted to between 3.5 and 5.5 bar. We tried to sample the largest possible number from each social group, giving priority to the dominant pair and their putative sub-adult offspring.

Invasive genetic sampling

Capture of cubs for tissue samples

We captured cubs found at den entrances opportunistically by hand, and removed a layer of skin, 2-5mm in diameter from the tip of their tail with a sterile scalpel. We treated the small wound with "propolis" before returning the cub to its den. Latex gloves were used during the whole procedure to avoid contamination of the sample and to avoid leaving our scent on the animal. We waited in the vicinity until the adults returned to the den and we were sure that the cubs were safe.

Capture of adults

Concomitantly with our genetic survey, we conducted three capture campaigns in the area to install radio transmitters in adults (C. Leuchtenberger 'unpublished data'). These captures followed the method described in Silveira et al. (2011), and we collected whole-blood samples from two of animals, as one of them had already been dart-sampled.

Noninvasive genetic sampling

Every time we sighted solitary individuals excreting mucus, we collected it with a syringe without needle, as previously described by Garcia et al. (2007). Also, we often checked active latrines for fresh and individualized mucus.

DNA storage, extraction and analyses

Tissue and mucus samples were stored in 100% ethanol. Blood was stored in 1.5-mL microcentrifuge tubes. All samples were kept at -20^{o}C until DNA extraction. Hair samples were placed in paper envelopes and preserved dry, until the DNA extraction. DNA from tissue was first extracted using a phenol–chloroform protocol (Sambrook et al. 1989). Subsequently, DNA from both tissue and blood samples was isolated using the DNeasy Blood

& Tissue Kit (Qiagen®). DNA from mucus was recovered using the QIAamp DNA Stool Mini Kit (Qiagen®). We used DNeasy Blood & Tissue Kit (Qiagen®) and a CTAB protocol (Gusmão and Solé-Cava 2002) to extract DNA from hair samples. Samples with high concentration of DNA were normalized to final concentration of 30-50 $ng \cdot \mu l^{-1}$.

Twelve microsatellite loci, specifically developed from giant otters, were amplified and genotypes were determined following the protocol described by Ribas et al. (2011) and molecular sexing was used to determine the gender of the individuals that were not sexed in the field. The SRY gene was PCR amplified using the primers developed by Dallas et al. (2000), and the microsatellite loci were used as positive controls. Amplifications were carried out in 15µL reactions containing 60-100 ng of template DNA, 200µM dNTP, 2.5mM $MgCl_2$, 15µg BSA and 0.5µM of each primer. Thermal cycling was as follows: 3 min at 93°C, 30 cycles of 1 min at 92°C, 1 min at 50°C, and 1 min at 72°C, and 5 min of final extension at 72°C. PCR products were visualized after electrophoresis in 2% agarose gels.

Since none of the hair samples amplified more than a single microsatellite locus using the CTAB protocol, a whole genome amplification kit (Repli-g UltraFast Mini Kit - Qiagen®) was used prior to PCR of the microsatellite loci.

Results

Semi-invasive genetic sampling

In 105 days of fieldwork, we attempted to dart giant otters on 92 occasions, hitting the animals 59 times (64%). We recovered the dart on all occasions, except in one case, when the line broke and the dart sank. Most (76%, n=45) recovered darts retained a visible skin tissue sample of approximately 0.13 cm^3, 17% (n=10) retained only fur, and 7% (n=4) were empty. Fat tissue was also found in 86% of the darts with skin tissue.

We counted a total of 97 adult otters inhabiting the area during our study, and most animals were seen repeatedly. The 45 skin samples came from 41 adults (25 males and 16 females). Therefore we estimate that we sampled about 42% of the adult population. Sampled animals were from 12 different groups and 16 were considered dominant at the time of sampling. We darted animals after approaching by boat on 11 occasions, and 34 animals were darted from a camouflaged ambush point on the bank. Two individuals were sampled during boat chases, but we could not see their throats long enough to provide unambiguous identification. The average distance for shots was 9m (range from 5-23m). On 12 occasions

animals were in our sights, but their natural marks were not visible or recognized in time for a shot.

Animals were startled when punctured by the dart and the group reacted by growling and looking around for a few minutes, before returning to normal activities. Groups tracked by boat normally leave the area after the first shot on target, while sampled individuals from groups stalked by camouflage strategy remained at the local, with little reaction to the sampling, since they don't associate the "sting" with the projector. Animals that had been darted acted normally in the following days (Figure 1). Often the groups were sampled on different occasions, and no behavioral changes were noted.

The price of the dart projectors ranged from US$280 to US$1950, depending on the model. The total cost of the biopsy darts used during the study was US$114. The combined costs of fuel, scholarship for a field researcher (doctoral student), the salaries of a shooter and a field assistant plus living cost totaled US$21,637 for the 105 field days. The mean cost of dart samples was US$487 or US$524, depending on the projector (Table 1-1).

Capture of adults, opportunistic capture of cubs and mucus collection

Each adult capture campaign lasted for 10 days and resulted in one animal caught. For logistical and safety reasons, two boats were involved during capture attempts and at least two researchers and two field assistants were present. The cost of manufacturing the two traps used to capture the otters was US$749. Therefore the cost of each adult sample was about US$3200, including the trap, fuel, 10 days living costs, and 10 days scholarship for two field researchers (doctoral students) and salaries for two field assistants (Table 1-1).

We found cubs momentarily in absence of adults on three occasions and we caught them to collect small samples of skin tissue from their tails. On one occasion we found a dead cub partially buried in the den entrance and took a tissue sample from its remains.

We collected 23 isolated patches of mucus, but only on two occasions did we sight the individuals that had produced it, both solitary males.

Genetic analyses

Average DNA concentrations were 57 $ng.\mu L^{-1}$ (range from 12-350 $ng.\mu L^{-1}$, n=49), 22 $ng.\mu L^{-1}$ (n=2), and 21 $ng.\mu L^{-1}$ (5-47, n=12), from tissue, blood and mucus samples, respectively. The average DNA extract purity was 260/280 nm = 1.95; 260/230 nm = 1.15,

from tissue; 260/280 nm = 1.56; 260/230 nm = 1.08 from blood and 260/280 nm = 1.4; 260/230 nm = 0.85 from mucus.

All tissue (n=45) and blood samples (n=2) amplified all 12 loci, but none of the 23 mucus samples amplified all loci and 17 did not amplify any. None of the six hair samples amplified, probably because of the absence of hair follicles. Two of the six mucus samples that amplified were from the same male, which had already been sampled with a biopsy dart, and one mucus sample was excluded from analyses because less than half of the loci could be genotyped.

The cost for chemicals and kits for each molecular analysis of tissue or blood sample was US$16 and US$33 for each mucus sample, not including cost of permanent equipment.

Twenty eight of thirty-one adult individuals of known sex were correctly assigned to gender and 14 animals of previously unknown gender were sexed, for a total of nine males and five females.

Discussion

Efficacy of biopsy darting

Adult giant otters are good candidates for biopsy-dart sampling, since they are large, diurnal and conspicuous. Furthermore, they spend long periods of the day on land, in campsites, latrines, or dens, which facilitates setting ambushes. Giant otters are visually oriented (Duplaix 1980), and the use of camouflage allowed shots from shorter distances, improving the success rate of shots and minimizing the chance of hitting the animal in undesired body sections. Proximity to the animals allowed collection of detailed behavioral data. However, some concern with respect to the wind direction is advisable, as the otters apparently reacted to strong scents, such as deodorant, insect repellent and human food, and sometimes left before a shot could be taken.

Waiting in a boat for the otters to return to their den or latrine was only partially successful. The boat close to the den seemed to disturb the group and frequently the otter fled before the shooter got a clear shot. Boat chases were the worst strategy, because they appeared to severely stress the animals and made identification from throat markings difficult. In addition quickly identifying the animals proved to be difficult, because their throat markings can be hard to see depending on their position and are often similar to others within

the group. Therefore communication between the observer and the shooter must be precise, since the shot often must be taken within seconds of identifying the animal.

Success in acquiring skin samples depended on location and angle of impact of the dart. Shots perpendicular to the rump yielded best results. The pressure with which the dart is shot is also important, because it affects the chance of hitting the animal at the proper angle at longer distances, as well as the impact of the dart.

The use of a silent projector and camouflage strategy helped to minimize behavioral effects and allow multiple shots in the same event, showing itself to be the most efficient. The animals showed no adverse effects to sampling and we believe that the individuals are unlikely to be seriously harmed by the impact of a well-aimed biopsy dart.

Costs and Benefits of different sample types

The only published method to capture giant otters is the use of a funnel-shaped net fixed on an oval metal hoop with a door that opens into the net. The trap is installed before dawn, at the entrance of the den, while the giant otters are sleeping inside (Silveira et al. 2011). The animals leaving the den are captured, and it is not possible for the researcher to choose which one will be trapped. This method is invasive and requires sedation of the animal. It is also at least six times more costly than the use of biopsy darts and demands more complicated logistics. Furthermore, the Brazilian Institute of the Environment (IBAMA) currently prohibits capture of lactating females.

It is important to sample the maximum number of cubs of each litter for paternity analyses. Taking them from within dens is invasive and difficult since at least one adult usually remains with the cubs (Duplaix 1980, Rosas et al. 2009), and dens are deep and full of tree branches. We suggest identifying cubs from throat patterns, and sampling with biopsy darts months after, when they are larger.

Although opportunistic mucus collection during our study was inexpensive, DNA was amplified only from mucus collected within a few minutes after deposition, indicating that, mucus quality depends on the age of the sample, as also reported by Garcia et al. (2011). In our study, only 22% of mucus samples were suitable for DNA analysis. Moreover, non-invasive genetic samples are prone to genotyping errors and accurate results can only be obtained if similar pairs of genotypes are confirmed through data replication (Waits and Paetkau 2005, Hansen et al. 2008), making the laboratory costs far more expensive than those of tissue and blood samples. However, two of the six successfully genotyped mucus samples

were from solitary males, which were not sampled by the other methods. Solitary giant otters are known to be especially shy (Duplaix 1980) and it is uncommon to sight them on land or showing their throats markings above the water. Therefore, collection of mucus could supply genetic information about these difficult-to-sample individuals.

Although Pickles et al. (2009) concluded that non-invasive samples from giant otters could be used to reveal patterns of relatedness, it is unusual to find individual feces isolated in latrines, and only rarely can the researcher associate the sample to the animal, which is paramount for studies based on paternity and kinship. If an active latrine is found, with animals close by, we recommend an ambush to obtain tissue samples with a dart gun.

The most effective method to retrieve biological samples from wild giant otters, so far, is by using biopsy darts projected by a CO_2 rifle. Biopsy darting is capable of collecting tissue samples with high-quality DNA, from a large number of giant otters and is less expensive and invasive than capture. Moreover, this method allows direct sampling of known individuals, which is essential for studies of social structure.

Management Implications

Although biopsy darting seemed ideal for the selective, minimally invasive sampling of tissue for genetic analyses, there was little previous published information on the potential of remote biopsy systems in medium-sized mammals. To our knowledge, the only mammal of similar size to giant otters that had been biopsy darted was the African wild dog, *Lycaon pictus* (Moueix 2006). We believe that the adoption of biopsy darting may bolster studies on medium neotropical social mammals, especially in open areas, such as ungulates (i.e. *Blastoceros dichotomus*, *Ozotoceros bezoarticus* and *Tayassu pecari*), and large rodents (*Hydrochoerus hydrochaeris*).

Due to their aquatic and elusive behavior, giant otters demanded adaptations of biopsy gear and strategy, before successful sampling was achieved. For animals with a history of a drastically reduced area of occurrence and a complex social structure, the molecular tools may be the only sure way to access information about molecular ecology, sociability and population structure of the remaining populations.

The combination of methods and field strategies described here can have significant impacts, contributing in unique ways to the advancement of knowledge of the species; helping to support decisions on species management and conservation strategies, as well as the

delineation of protected areas, wildlife corridors, and reintroduction of this endangered otter species.

Acknowledgements

We thank Federal University of Mato Grosso do Sul for providing logistic support. CNPq granted a scholarship to CR, and a research grant to GM to support the field and some laboratory costs (# 476939/2008-9). We are indebted to I. T. Carvalho and U. Piovezan for fieldwork assistance and to C. Leuchtenberger for providing blood and mucus samples. We thank P. C. Estrela for helpful comments. L. Damasceno, S. Damasceno, Cel. C. Marsiglia and Cel. A. Rabello provided essential support with the equipment importation process.

References

Amos, W., H. Whitehead, M. J. Ferrari, D. A. Glockner-Ferrari, R. Payne, and J. Gordon. 1992. Restrictable DNA from sloughed cetacean skin; its potential for use population analysis. Marine Mammal Science 8:275-283.

Carter, S. K., and F. C. W. Rosas. 1997. Biology and conservation of the giant otter *Pteronura brasiliensis*. Mammal Review 27:1-26.

Dallas, J. F., D. N. Carss, F. Marshall, K. P. Koepfli, H. Kruuk, S. B. Piertney, and P. J. Bacon. 2000. Sex identification of the Eurasian otter *Lutra lutra* by PCR typing of spraints. Conservation Genetics 1:181–183.

Davenport, L. C. 2010. Aid to a declining matriarch in the giant otter (*Pteronura brasiliensis*). PLoS ONE 5:1-6.

DeYoung, R. W., and R. L. Honeycutt. 2005. The molecular toolbox: genetic techniques in wildlife ecology and management. Journal of Wildlife Management 69:1362-1384.

DeWoody, J. A. 2005. Molecular Approaches to the study of parentage, relatedness, and fitness: practical applications for wild animals. Journal of Wildlife Management 69:1400-1418.

Dugdale, H. L., D. W. Macdonald, L. C. Pope and T. Burke. 2007. Polygynandry, extra-group paternity and multiple-paternity litters in European badger (Meles meles) social groups. Molecular Ecology 16:5294–5306.

Duplaix, N. 1980. Observations on the Ecology and behavior of the giant otter *Pteronura brasiliensis* in Suriname. Revue d'Ecologie (la Terre et la Vie) 34:495-620.

Eizirik, E., T. Haag, A. S. Santos, F. M. Salzano, L. Silveira, F. C. C. Azevedo, and M. M. Furtado. 2008. Jaguar conservation genetics. CAT News, Special Issue 4:31-34.

Hansen, H., M. Ben-David, and D. B. McDonald. 2008. Effects of genotyping protocols on success and errors in identifying individual river otters (*Lontra canadensis*) from their faeces. Molecular Ecology Resources 8:282–289.

Hughes, C. 1998. Integrating molecular techniques with field methods in studies of social behavior: a revolution results. Ecology 79:383–399.

IUCN – International Union for Conservation of Nature and Natural Resources (2011) IUCN Red List of Threatened Species. <http://www.iucnredlist.org.html>. Accessed 25 Sep 2011.

Franco-de-Sá, J. F. O., F. C. W. Rosas, and E. Feldberg. 2007. Cytogenetic study of the giant otter *Pteronura brasiliensis* Zimmermann 1780 (Carnivora, Mustelidae, Lutrinae). Genetics and Molecular Biology 30:1093-1096.

Garcia, D.M., M. Marmontel, F. W. Rosas, and F. R. Santos. 2007. Conservation genetics of the giant otter (*Pteronura brasiliensis* (Zimmerman, 1780)) (Carnivora, Mustelidae). Brazilian Journal of Biology 67:631–637.

Gemmel, N. J., and P. Majluf. 1997. Projectile biopsy sampling of fur seals. Marine Mammal Science 13: 512-516.

Girman, D. J., M. G. L. Mills, E. Geffen, and R. K. Wayne. 1997. A molecular genetic analysis of social structure, dispersal, and interpack relationships of the African wild dog (*Lycaon pictus*). Behavioral Ecology and Sociobiology 40:187-198.

Griffin, A. S., J. M. Pemberton, P. N. M. Brotherton, G. McIlrath, D. Gaynor, R. Kansky, J. O'Riain, and T. H. Clutton-Brock. 2003. A genetic analysis of breeding success in the cooperative meerkat (*Suricata suricatta*). Behavioral Ecology 14:472–480.

Gusmão, J. and A. M. Solé-Cava. 2002. Um sistema de diagnóstico molecular para a identificação de espécies comerciais de camarões marinhos brasileiros. *Comunicaciones y Foros de Discusión in. Primer Congreso Iberoamericano Virtual de Acuicultura* (eds Blas I), pp 754–764. Zaragosa.

Karesh, W. D. V. M., F. Smith, and H. Frazier-Taylor. 1987. A remote method for obtaining skin biopsy samples. Conservation Biology 1:261-262.

Moueix, C. 2006. Genetic verification of multiple paternity in two free-ranging isolated populations of African wild dogs (*Lycaon pictus*). PhD thesis, University of Pretoria, Onderstepoort.

Mourão, G. and L. Carvalho. 2001. Cannibalism among giant otters (*Pteronura brasiliensis*). Mammalia 65:225-227.

Mowry, R. A., M. E. Gompper, J. Beringer, and L. Eggert. 2011. River otter population size estimation using noninvasive latrine surveys. The Journal of Wildlife Management 75:1625–1636.

Muwanika, V. B., H. R. Siegismund, J. B. A. Okello, C. Masembe, P. Arctander, and S. Nyakaana. 2003. A recent bottleneck in the warthog and elephant populations of Queen Elizabeth National Park, revealed by a comparative study of four mammalian species in Uganda national parks. Animal Conservation 6:237–245.

Otter Specialist Group (OSG-IUCN) (2010) *Pteronura brasiliensis* (Gmelin, 1788), the Giant Otter. <http://www.otterspecialistgroup.org/Species/Pteronura_brasiliensis.html>. Accessed 13 Mar 2011.

Pickles, R. S. A., J. J. Groombridge, V. D. Zambrana Rojas, and W. C. Jordan. 2009. Cross-species characterisation of polymorphic microsatellite loci in the giant otter (*Pteronura brasiliensis*). Molecular Ecology Resources 9:415–417.

Pickles, R. S. A., J. J. Groombridge, V. D. Zambrana Rojas, P. Van Damme, D. Gottelli, S. Kundu, R. Bodmer, C. V. Ariani, A. Iyengar, and W. C. Jordan. 2011. Evolutionary history and identification of conservation units in the giant otter, *Pteronura brasiliensis*. *Molecular Phylogenetics and Evolution*. doi:10.1016/j.ympev.2011.08.017.

Randall, D. A., J. P. Pollinger, R. K. Wayne, L. A. Tallents, P. J. Johnson, D. W Macdonald. 2007. Inbreeding is reduced by female-biased dispersal and mating behavior in Ethiopian wolves. Behavioral Ecology 18:579–589.

Ribas, C., G. Mourão. 2004. Intraspecific agonism between giant otter groups. IUCN Otter Specialist Group Bulletin 21:89-93.

Ribas, C., A. V. Vasconcellos, G. Mourão, W. Magnusson, A. M. Sole-Cava, and H. A. Cunha. 2011. Polymorphic microsatellite loci from the endangered giant otter (*Pteronura brasiliensis*). Conservation Genetics Resources 3:769-771.

Ribas, C., G. Damasceno, W. Magnusson, C. Leuchtenberger, and G. Mourão. 2012. Giant otters feeding on caiman: evidence for an expanded trophic niche of recovering populations. Studies on Neotropical Fauna and Environment 47:19-23.

Rosas, F., M. Cabral, G. De Mattos, and R. Silva. 2009. Parental and alloparental care of giant otters (*Pteronura brasiliensis*) (Carnivora, mustelidae) in Balbina hydroelectric lake, Amazonas, Brazil. Sociobiology 54:919-924.

Ross, K. G. 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. Molecular Ecology 10:265–284.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning a laboratory manual. Cold Spring Harbor Laboratory Press, New York, USA.

Schweizer, J. 1992. Ariranhas no Pantanal: ecologia e comportamento da *Pteronura brasiliensis*. Edibran-Editora Brasil Natureza Ltda, Curitiba, BR.

Silveira, L., M. M. Furtado, F. C. W. Rosas, L. C. L. C Silva, M. M. M. Cabral, R. S. Tôrres, A. Kouba, and A. T. A. Jácomo. 2011. Tagging giant otters (*Pteronura brasiliensis*) (Carnivora, Mustelidae) for Radio-Telemetry Studies. Aquatic Mammals 37:208-212.

Spong, G., J. Stone, S. Creel, and M. Bjorklund. 2002. Genetic structure of lions (*Panthera leo* L.) in the Selous Game Reserve: implications for the evolution of sociality. Journal of Evolutionary Biology 15:945–953.

Taberlet, P., J. J. Camarra, S. Griffin, E. Uhres, O. Hanotte, L. P. Waits, C. Dubois-Paganon, T. Burke, and J. Bouvet. 1997. Noninvasive genetic tracking of the endangered Pyrenean brow bear population. Molecular Ecology 6:869-876.

Waits, L. P., and D. Paetkau. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 69:1419-1433.

Worthington Wilmer, J., P. J. Allen, P. P. Pomeroy, S. D. Twiss, W. Amos. 1999. Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (Halichoerus grypus). Molecular Ecology 8:1417- 1429.

Table

Table 1-1- Summary of different potential methods to collect genetic samples from free-ranging giant otters, and some of its characteristics and recommended use.

	n	Average DNA Concentration ($ng \cdot \mu l^{-1}$)	Amplification success (%)	Cost (US$) of each sample		Disturbance	Potential use
				field	laboratory		
Mucus	23	21	22	.	57.5	negligible	Opportunistic to assess solitary individuals difficult to sample with other methods*
Carcass Tissue	1	57	100	.	27.8	negligible	Opportunistic to complement relatedness or paternity studies
Skin of Pups	3	57	100	.	27.8	medium	Opportunistic to complement relatedness or paternity studies
Tissue biopsy darts	45	57	100	524	27.8	low	Feasible as the main method to collect high quality samples
Blood of adults	2	22	100	3200	27.8	high	Too expensive as the main method for studies of relatedness or paternity, opportunistic, in case of the capture was demanded by other studies (e.g. telemetry)
Hair samples	6	xx^{t**}	0		Undetermined	negligible	Although none of the hair samples could be analyzed, this method may work if hair traps collect hair with follicle.

* See text.

** No DNA determined

2º Capítulo

Ribas, C., Vasconcellos, A., Mourão, G., Magnusson, W. E., Solé-Cava, A., Cunha, H. 2011. Polymorphic microsatellite loci from the endangered Giant Otter (*Pteronura brasiliensis).* *Conservation Genetics Resources* 3:769-771.

Polymorphic microsatellite loci from the endangered Giant Otter (*Pteronura brasiliensis)*

*Carolina Ribas[1,2,3], Anderson Vasconcellos[2], Guilherme Mourão[3], William Magnusson[1], Antonio Solé-Cava[2], Haydée Cunha[2,4].

[1]Coordenação de Pesquisas em Ecologia, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil.
[2]Laboratório de Biodiversidade Molecular, Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590, Brazil.
[3]Embrapa Pantanal, Corumbá, Mato Grosso do Sul, Brazil.
[4]Laboratório de Mamíferos Aquáticos e Bioindicadores (MAQUA), Faculdade de Oceanografia, Universidade do Estado do Rio de Janeiro.
*Corresponding author: email address: ariranhapantanal@yahoo.com.br. Phone: +55-67-32345929, Fax: +55-67-32345842.

Word count: 1489

Abstract

We describe the first microsatellite loci isolated from the giant otter (*Pteronura brasiliensis*), an endangered mustelid endemic to South America. Fourteen di- and trinucleotide polymorphic loci were characterised in fourteen individuals from the Pantanal wetlands, Central Brazil. Number of alleles per locus ranged from 2 to 5, and average observed heterozygosity was 0.577. Two loci were in linkage disequilibrium, and one further locus deviated from Hardy Weinberg equilibrium, probably due to the presence of null alleles. The transferability of these markers to two other mustelids (*Lontra longicaudis* and *Eira barbara*) and to the mephitid *Conepatus semistriatus* was also evaluated. These loci are useful to study the ecology and evolution of these species.

Keywords: Lutrinae, Mustelidae, Mephitidae, Kinship, Social system, Population structure.

Giant otters (*Pteronura brasiliensis*) are large semi-aquatic mustelids (sub-family Lutrinae) and one of the few social carnivores endemic to South America. They are considered the most threatened otter species in the world (IUCN 2010) as a consequence of historical hunting (Carter and Rosas 1997), and past and present anthropogenic habitat destruction (e.g. mining and hydroelectric dams, river and land pollution, and over-fishing) (Schenck 1999; OSG IUCN 2010).

Microsatellites are ideal markers to address population structure and kinship issues, thus they are able to provide crucial information for the elaboration of conservation plans for this endangered species. Although microsatellite primers developed for Eurasian and North American River otters (*Lutra lutra* and *Lontra canadensis*, respectively) have been tested in giant otters (Pickles et al. 2009), the use of heterologous primers may lead to underestimation of heterozygosity ("ascertainment bias", Garner et al. 2005). Therefore, we isolated microsatellite loci for *P. brasiliensis* (which are the first microsatellites characterised in otters from Latin America). We also tested their applicability in the mustelids *Lontra longicaudis* (neotropical river otter) and *Eira barbara* (tayra), and in the mephitid *Conepatus semistriatus* (striped hog-nosed skunk).

Genomic DNA was extracted from giant otter skin samples collected with biopsy darts (Ribas et al., *in prep.*), using a phenol–chloroform protocol (Sambrook et al. 1989). Microsatellites were isolated from an enriched partial genomic library, following the protocol of Bloor et al. (2001). A pool of high-quality genomic DNA (4µg) was digested with SauIIIA, ligated to phosphorylated double-stranded linker oligonucleotides and size selected (between 500 and 1000 bp). DNA fragments were hybridised with biotinylated $(CA)_{12}$, $(CAA)_8$ and $(GATA)_5$ probes, and isolated using streptavidin-coated magnetic beads. The forward linker oligo was used as a primer for enrichment of DNA containing microsatellites. Enriched fragments were then cloned using pGEM-T vectors (Promega) and OneShot TOP10 competent cells (Invitrogen). Recombinant clones were screened for the presence of microsatellite inserts, which was confirmed by two or more amplified products after a PCR primed with the forward linker oligo and (non-biotinylated) microsatellite oligos. Forty-eight positive clones were sequenced in both directions in an ABI3500 sequencer. Sequences were edited using SeqMan (DNAStar).

Twenty-five primer pairs flanking microsatellite regions were designed using WebSat (Martins et al. 2009). We used the tailed primer method (Schuelke 2000), hence, PCR reactions contained three primers: tailed (forward with M13 tail), labelled (M13 with either

VIC, NED, PET or 6-FAM fluorescent dyes), and reverse. PCR consisted of 1U GoTaq (Promega), 0.20 mM dNTPs, 2.5 mM $MgCl_2$, 15µg BSA, 0.2µM of tailed primer, 0.4µM of labelled primer, and 0.8µM of reverse primer, in 15 µL reactions with approximately 20ng of DNA template. Cycling conditions were: 94°C, 4 min, 30X (92°C, 45 seg; T_a, 45 seg; 72°C, 45 seg), 8X (92°C, 45 seg; 53°C, 45 seg; 72°C, 45 seg), 72°C, 30 min. T_a for all primer pairs was 60°C, except for *Pbra01*, which was 52°C. PCR products were pooled, separated in an ABI3500 sequencer and sized using GeneMapper and GS500-LIZ (Applied Biosystems).

During optimisation attempts, five primer pairs were discarded due to PCR failure. The remaining 20 were evaluated for polymorphisms in fourteen giant otters from the Miranda/Vermelho River (UTM- 21K 502060, 7831592), Pantanal wetlands, Brazil. Six loci were monomorphic, while 14 loci resolved between 2 and 5 alleles and had observed and expected heterozygosities varying between 0-0.857, and 0.138-0.775, respectively (Table 2-1). Deviations from Hardy-Weinberg and linkage equilibrium conditions were tested using FSTAT (Goudet et al. 1995) and the online version of Genepop (Raymond and Rousset 1995). Locus *Pbra16* presented a clear heterozygote deficiency, possibly caused by the presence of null alleles, as suggested by the high null allele frequency estimated by the software Cervus (Kalinowski et al. 2007). All loci pairs were in linkage equilibrium, except for markers *Pbra21* and *Pbra23*, which were strongly linked ($P<0.00055$, which remains significant after sequential Bonferroni correction - Rice 1989). The probability of non-exclusion of a false parent was estimated for each locus (Table 2-1) and also combining the twelve selected markers (i.e. excluding *Pbra16* and *Pbra21*). The estimated proportions of type II errors were small (5.7% without knowing the parents and 0.4% knowing one of them), indicating that the selected loci provide sufficient power for paternity analyses in the species.

All 14 markers were tested for cross-amplification in two individuals each of neotropical otter and tayra, and in one striped hog-nosed skunk, using the optimized conditions detailed in Table 2. Five loci could not be amplified in any of these species (*Pbra01*, *Pbra10*, *Pbra20*, *Pbra23* and *Pbra24*), but seven of them were successfully amplified in the two other mustelids (Table 2-2).

These markers will contribute to elucidate the giant otter social system and population structure, providing information that will be useful in the elaboration of management and conservation plans for this endangered species.

Acknowledgements

We thank Federal University of Mato Grosso do Sul for providing logistic support. This study was part of the Ph.D. dissertation of CR (at INPA, Brazil). CNPq granted a scholarship to CR, and a research grant to GM to support the field and some of Laboratory costs (# 476939/2008-9). We are indebted to Isac Teixeira de Carvalho for his fieldwork assistance, to Parque Zoológico Municipal Quinzinho de Barros and Programa de Conservação Mamíferos do Cerrado, for providing Lontra longicaudis, Eira barbara and Conepatus semistriatus tissue samples, respectively. We thank Fabiana Lopes Rocha for all her help and contacts. All collections were authorized by the Environment Ministry (IBAMA 12794-4/2012) and the dart projector was imported under International Import Certificate n° 5834/DFPC from the Brazilian Government. We are especially grateful to Gabriel Damasceno for his great aim with the biopsy dart projector.

References

Bloor PA, Barker FS, Watts PC, Noyes HA, Kemp SJ (2001) Microsatellite libraries by enrichment. Protocol available at: http://www.genomics.liv.ac.uk/animal/Protocol1.html

Carter SK, Rosas FCW (1997) Biology and conservation of the giant otter *Pteronura brasiliensis*. Mammal Review 27:1-26

Garner A, Rachlow JL, Hicks JF (2005) Patterns of genetic diversity and its loss in mammalian populations. Conservation Biology 19: 1215–1221

Goudet J (1995) FSTAT (version 1.2): a computer program for calculating F-statistics. Journal of Heredity 86:485-486

IUCN Red List of Threatened Species (2010) http://www.iucnredlist.org/apps/redlist/details/18711/0. Accessed 13 March 2011

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099-1006

Martins WS, Lucas DCS, Neves KFS, Bertioli DJ (2009) WebSat - A web software for microsatellite marker development. Bioinformation 3:282-283

OSG-IUCN Otter Specialist Group (2010) http://www.otterspecialistgroup.org/Species/Pteronura_brasiliensis.html. Accessed 13 March 2011

Pickles RSA, Groombridge JJ, Zambrana Rojas VDZ, Jordan WC (2009) Cross-species characterisation of polymorphic microsatellite loci in the giant otter (*Pteronura brasiliensis*). Molecular Ecology Resources 9:415–417

Raymond M, Rousset F (1995) GENEPOP, Version 1.2. Population genetics software for exact tests and ecumenicisms. Journal of Heredity 86:248-249

Rice WR (1989) Analysing tables of statistic tests. Evolution 43:223-225

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning a Laboratory Manual, Cold Spring Harbor Laboratory Press, New York

Schenck C (1999) Lobo de Río (*Pteronura brasiliensis*): Presencia, uso de hábitat y protección en el Perú. Zoological Society of Frankfurt, Germany

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233-234

Tables

Table 2-1. Levels of variability of 14 polymorphic microsatellite loci in the giant otter (*Pteronura brasiliensis*) from Pantanal, Brazil (*n*=14). N_a indicates number of alleles observed; H_o, observed heterozygosity; H_e, expected heterozygosity; *Null freq.*, estimated null allele frequency; *PExcl1* and *2*, probability of non-exclusion of a parent, unknowing both or one of them, respectively.

Table 2-2. Results of cross-amplification tests. Failure of amplification is indicated by a minus. Loci that were successfully amplified are indicated by the corresponding annealing temperature (^oC).

Table 2.1.

Locus	GenBank Acession no.	Primer sequence(5'-3')	Motif	Size range*(bp)	N_a	H_O H_E	Null freq.	PExcl1	PExcl2
Pbra01	JF712852	F: ACCACAAGGGGTTCACTCTAAA	AC (18)	219-225	4	0.357	0.1875	0.871	0.749
		R: TGACCTACTGTCCATTCTGCTG				0.521			
Pbra02	JF712853	F: TCTCCCCATTTTCACTCTGG	AC(9)_AC(16)	401-413	5	0.714	0.0034	0.687	0.509
		R: ACTTTCAGCCTTTGGTGCTC				0.746			
Pbra05	JF712854	F: GGAAAGGGTTGCTGAATGAA	CA(18)	363-375	4	0.714	-0.0159	0.743	0.578
		R: GAGGGTCCTGATGATGGAAG				0.706			
Pbra08	JF712855	F: TACTCTTTTCAGATGCCCCACT	GT(16)	181-191	3	0.571	-0.0051	0.843	0.734
		R: AATATGATGTCTCCCGCACG				0.582			
Pbra09	JF712856	F: CACCTTTCCCTCACTTTTGC	CA(20)	394-400	3	0.429	0.0769	0.899	0.763
		R: TCATCCTTCAGTTATGCCGA				0.466			
Pbra10	JF712857	F: GCCTGACAAGTGATTGCGTA	TG(14)	319-327	3	0.500	-0.1328	0.92	0.798
		R: CCGAACCAGAGGCATAAGAA				0.415			
Pbra11	JF712858	F: GGTTGCCTATGGCTGAGAGA	(TG)(GA)	339-343	3	0.714	-0.1355	0.831	0.685
		R: GGAGCATGTCTTCCGTGATT				0.603			
Pbra14	JF712859	F: AGAAACACACACGGGACACA	AC(10)CA(11)	136-160	3	0.500	-0.1366	0.924	0.818
		R: TTGCTAATGCTGTAGGGGCT				0.405			
Pbra16	JF712860	F: CAGTGCGGGTCATACAAAGA	CTT(8)	327-336	2	**0**	0.8315	0.991	0.938
		R: ACAGAACCAGTCCCTGTTGG				**0.138**			
Pbra17	JF712861	F: AACACCAAAGCAAACCCTTG	TG	336-350	4	0.643	0.0757	0.675	0.499
		R: CCACCACAGAAAGCACAAAA				0.775			
Pbra20	JF712862	F: GCCAGACCATCCAACAAAGT	CA	358-370	4	0.714	0.0144	0.701	0.526

		R: TTTCCTTTCTCCATCCTCCA				0.749			
Pbra21[a]	JF712863	F: GGAAACAACACAGCGGAACT	AC(19)	195-205	4	0.857	-0.078	0.693	0.52
		R: CTGAATGAGACACGCAGGAA				0.759			
Pbra23[a]	JF712864	F: AGATGTTCAGAGAGGCGGAA	TG(17)	171-181	4	0.857	-0.0735	0.686	0.512
		R: GGGTGAGTTGTCGGTTTGTT				0.765			
Pbra24	JF712865	F: GGTGTCTTTGAAGTGGTTAT	TG(13)	313-335	4	0.786	-0.072	0.743	0.578
		R: AGTGGCTTAACGGACTGAGC				0.706			

*Allele sizes discounting the tailed extension of the primers.

Table 2.2

Species	*N*	*Pbra02*	*Pbra05*	*Pbra08*	*Pbra09*	*Pbra11*	*Pbra14*	*Pbra16*	*Pbra17*	*Pbra21*
Lontra longicaudis	2	60	54	-	60	60	58	58	60	52
Eira barbara	2	-	54	54	58	60	60	58	54	52
Conepatus semistriatus	1	-	-	-	-	-	-	-	-	56

3º Capítulo

Ribas, C., Damasceno, G., Cunha, H., Magnusson, W., Solé-Cava, A., Mourão, G. 2011. More than meets the eye: kinship and social organization in giant otters (*Pteronura brasiliensis*). Manuscrito formatado para *Molecular Ecology*.

More than meets the eye: kinship and social organization in giant otters (*Pteronura brasiliensis*)

Carolina Ribas[a,b,c,*], Gabriel Damasceno[b], Haydée Cunha[c,d], William Magnusson[a], Antonio Solé-Cava[c] and Guilherme Mourão[e].

[a]Graduate Program in Ecology, Instituto Nacional de Pesquisas da Amazônia - INPA,CP 478, 69011-970 Manaus, Amazonas, Brazil.

[b]C.C.G. Productions. Rio de Janeiro, RJ, Brazil.

[c] Laboratory of Molecular Biodiversity, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590, Brazil.

[d]Laboratory of Aquatic Mammals and Bioindicators, Universidade do Estado do Rio de Janeiro.

[e]Embrapa Pantanal, Brazilian Agricultural Research Corporation, CP 109, Corumba´-MS, 79320-900, Brazil

[*]Corresponding author: email address: ariranhapantanal@yahoo.com.br. Phone: +55-67-3234-5929, Fax: +55-67-3234-5815.

Keywords: dispersal, microsatellite, mustelidae, relatedness, sociality, social system

Abstract

Behavioral observations suggest that groups of giant otters are composed of a dominant reproductive pair, and their offspring of previous years, which do not breed inside the original group. Our study combines genetic data and long-term behavioral information from two river systems to determine genetic relatedness within and between social groups and describe dispersal patterns of the species. We obtained the samples from 41 adults using biopsy darts projected by a CO_2 rifle. Opportunistically, we also collected skin samples from cubs, blood from two individuals that were captured to implant radio transmitters, and mucus (anal secretions). We genotyped a total of 50 giant otters, distributed in 13 social groups or as transients individuals (n=2), for twelve polymorphic loci. Our genetic results show a single population throughout the study area, and the nuclear variability found was within the observed range for giant otters in larger geographic areas. The average relatedness within groups (r = 0.23) was high and the giant otter groups were generally composed of an unrelated alpha pair and their close relatives. However, the degree of relatedness varied within the groups, including groups of completely unrelated individuals, contradicting the current knowledge that giant otter groups are formed exclusively by a dominant pair and their offspring. Our data are conflicting in relation to sex-biased dispersal of giant otters. On one hand, sex ratio was biased toward males and males were more related within groups, suggesting that groups are retaining more males. On the other hand, the negative correlation between kinship and distance between territories were higher in females than males and solitary transients individuals were known to be males, suggesting that males were the disperser sex. The social system of giant otters is much more complex than anticipated. The evolutionary mechanisms that lead to the high relatedness variance within giant otters groups it is unclear, but we believe that high migration rate of individuals across groups, extra-pair copulation and subordinate reproduction can play an important role, as they do in other apparently monogamous species.

Introduction

Genetic and social structure are inextricably linked (DeWoody 2005). Sociality and cooperative behavior should only evolve and remain stable if all individuals involved obtain net fitness benefit relative to the costs (Hamilton 1964). The relative importance of indirect vs. direct fitness benefits of group living have been debated (Gompper 1997; Clutton-Brock 2002). Although relatedness between group members will usually facilitate the evolution of cooperation (West *et al*. 2002), direct benefits derived from natal philopatry are probably important mechanisms influencing group formation within many groups, including the carnivores (Spong 2002; Van Horn *et al*. 2004).

Philopatry may result in kin cooperation and lower mortality rate due to familiarity with the natal area, while dispersal offers the possibility of finding a new territory, reduces kin competition, and helps in avoiding inbreeding (Lawson Handley & Perrin 2007). It has been suggested that male social mammals generally disperse to breed, whereas females remain in their natal group (Greenwood 1980), although genetic studies suggest a variety of dispersal strategies (Gompper & Wayne 1996), like female-biased dispersal in a background of predominantly philopatric males (Randall *et al*. 2007), dispersal systems independents of sex (Girman *et al*. 1997), and even philopatry of both sexes (Amos *et al.* 1993) (reviewed in Lawson Handley & Perrin 2007).

Giant otters (*Pteronura brasiliensis*) are large social semi-aquatic carnivores (Mustelidae, sub-family Lutrinae) (Duplaix 1980) endemic to South America. This species was heavily hunted in the past due to a high demand for their fur on the international market. This has resulted in a drastic reduction in its populations (Carter & Rosas 1997). Currently, the species is threatened by multiple anthropogenic influences arising from increased human settling in tropical lowland rainforests, and is classified as “Endangered" by the IUCN (2011).

Groups of giant otters generally vary in size from three to nine individuals and behavioral observations suggest that groups are composed of a dominant reproductive pair, and their offspring of previous years, which do not breed inside the original group (Duplaix 1980; Schweizer 1992; Kruuk 2006). Solitary individuals are regarded as transients and are usually subadult animals dispersing from a family group or a member of a pair who has lost its mate (Duplaix 1980; Schweizer 1992). Individuals within groups are highly cooperative, and show behaviors such as cooperative breeding (Duplaix 1980; Schweizer 1992; Rosas *et*

al. 2009), territory defense through scent marking and vocalizations, as well as active defense against predators and conspecific intruders (Duplaix 1980; Schweizer 1992; Ribas & Mourão 2004), and long-term assistance of elderly family member (Davenport 2010). In contrast, neighboring groups of giant otters show mutual avoidance (Duplaix 1980; Kruuk 2006), exhibiting marked agonistic interactions, including cannibalism and territorial fights (Mourão & Carvalho 2001; Ribas & Mourão 2004).

Dispersal strategies of giant otters are unknown. Factors such as age, sex, size and breeding structure of the group (i.e. presence of cubs and if the dominant female is pregnant), availability of territory or potential mates, and time of the year may influence dispersal in giant otters (Ribas 2004; Leuchtenberger & Mourão 2008). Our study combines genetic data and long-term behavioral information to determine genetic relatedness within and between social groups and describe dispersal patterns of the species. Here, we examine if the most accepted social model for giant otters agreed with empirical data. The features of the hypothesized model include reproductive suppression (i.e. groups would be formed by a non-promiscuous alpha couple and their brood) and a male-biased dispersion. Also we asked if kinship selection is enough to explain the evolution and maintenance of the sociability in giant otters. The results indicate that giant otters have a very complex social structure, and that none of the present hypotheses as to how giant otter social systems evolved and are maintained is entirely satisfactory.

Materials and methods

Study population

The Pantanal is a large Neotropical wetland, covers 160,000 km^2 of lowland terrain in Bolivia, Paraguay and Brazil, and is characterized by low altitude plains subject to seasonally alternating periods of flood and drought (Alho *et al*. 1988; Harris *et al*. 2005).

We have been using digital video to monitor the ecology and social relations of giant otter groups from Miranda and Vermelho Rivers (UTM 21k 501897 7831480), in the Southern Pantanal, since 2002 (Ribas 2004). In 2008, we also began monitoring giant otter groups in pools and water bodies around the *Estrada-Parque Pantanal* (EPP) highway (UTM 21k 0451800 7873700 to 21k 0496300 7831500). The Miranda and Vermelho Rivers are considered to be favorable habitats long inhabited by giant otters (Schweizer 1992; Ribas 2004), whereas the water bodies around the EPP road are considered sub-optimal habitats,

since the water gradually diminishes during the dry season (June to December), and the aquatic fauna that is the main prey of giant otters becomes scarce until the next rainy season (Ribas *et al*. 2012) (Figure 3-1).

We mapped the territories of giant otters by locating tracks, active dens, and latrines on the banks of rivers and other water bodies. We identified individual otters through their throat and chest patterns and catalogued them in a photogram compiled in a database, including the general information of each individual (i.e. gender, hierarchy, social group, territory, behavior, geographic coordinates, etc.).

We classified individuals as adults or cubs of the year, identified the alpha male and female in each group based on morphological characteristics, such as testicles and the larger width of the neck for males, and the indicators of lactation in females and behaviors, such as greater activity in marking and defending territory by the dominants.

Sample collection

We collected genetic samples (license no. 12794-4/2012, granted by the Brazilian Federal Wildlife Agency - ICMBio) during the dry seasons, from November 2008 to October 2010. We obtained a 41 skin samples from different adults by using biopsy darts projected by a CO_2 rifle. We also opportunistically, collected skin from the tails of three live cubs, and a dead cub found buried in a den. We collected blood from two individuals that were captured to implant radio transmitters. We also collected 23 fresh mucus (anal jelly secretions) samples from active latrines.

DNA extraction, amplification and genotyping

DNA from tissue and blood samples was extracted using the DNeasy Blood & Tissue Kit (Qiagen®), and from mucus using the QIAamp DNA Stool Mini Kit (Qiagen®). Samples with high concentration of DNA were normalized to final concentration of 30-50 $ng \cdot \mu l^{-1}$.

Twelve polymorphic loci, specifically developed from giant otters (Ribas *et al*. 2011), were PCR amplified in 15µL reactions containing approximately 1U GoTaq (Promega), 0.20 mM dNTPs, 2.5 mM $MgCl_2$, 15µg BSA, 0.2µM of forward, M13-tailed primer, 0.4µM of labelled M13-primer, and 0.8µM of reverse primer. Thermal cycling conditions were: 94°C, 4 min; 30X (92°C, 45 seg; T_a, 45 seg; 72°C, 45 seg), 8X (92°C, 45 seg; 53°C, 45 seg; 72°C, 45 seg); and 72°C, 30 min. T_a for all primer pairs was 60°C, except for *Pbra01*, which was 52°C.

PCR products were pooled in two panels, and sized in an ABI3500 sequencer using GS500-LIZ and the software GeneMapper (Applied Biosystems).

All tissue (n=45) and blood samples (n=2) amplified all 12 loci, but most (17 out 23) of the mucus samples completely failed to amplify. Two of the mucus samples that amplified were from the same male, which had already been sampled with a biopsy dart, and one mucus sample was excluded from analyses because less than half of the loci could be genotyped, leaving three mucus samples for analyses.

Molecular sexing was used to confirm or determine the gender of the individuals. The SRY gene was PCR amplified using the primers developed by Dallas *et al*. (2000), and the microsatellite loci were used as positive controls. Amplifications were carried out in 15µL reactions containing 60-100 ng of template DNA, 200µM dNTP, 2.5mM $MgCl_2$, 15µg BSA and 0.5µM of each primer. Thermal cycles were as follows: 3 min at 93^{o}C, 30 cycles of 1 min at 92^{o}C, 1 min at 50^{o}C, 1 min at 72^{o}C, and 5 min of a final extension at 72^{o}C. PCR products were visualized after electrophoresis in 2% agarose gels.

Population analyses

Behavioral data collected during eight years of monitoring enabled the assignment of six cubs to two dominant unsampled females, each in a different group, which were the only possible mothers at the cubs' time of birth in each group. Following the genetic confirmation that in both groups the alpha males were the fathers of the cubs (Ribas, unpublished data), the mothers' genotypes were reconstructed using GERUD (Jones 2005) and used in the analyses.

Deviations from Hardy-Weinberg and linkage equilibrium conditions were tested using FSTAT (Goudet 1995), and the existence of null alleles was evaluated using Micro-Checker (van Oosterhout *et al*. 2004). The combined non-exclusion probability of the twelve loci was estimated using program Cervus (Kalinowski *et al*. 2007).

Since samples were collected in two areas approximately 52 km apart (minimum straight-line distance), the Bayesian clustering analysis implemented in Structure (Pritchard *et al*. 2000) was used to investigate the existence of fine-scale geographic population structure. Analyses were run using the admixture and correlated allele frequencies model, and no information on individuals' geographic origin. Ten independent MCMC analyses for each K population scenarios (K = 1 and 2) were run, each with 500.000 steps following 100.000 burn-in steps.

Due to the indication of severe population decline caused by hunting in the recent past, three analytical approaches were used to search for evidence of demographic bottlenecks in giant otters from the Pantanal. First, the occurrence of a recent bottleneck was investigated using the Bottleneck program (Piry *et al*. 1999). All three mutation models (Infinite Alleles Model, IAM; Two-Phase Model, TPM; and Stepwise Mutation Model, SMM) were used for coalescent simulations. The TPM model was set to accommodate 95% of single step mutations, and variance of 12 among multiple step mutations, as recommended by Piry *et al*. (1999). Significance of deviations from equilibrium heterozygosity was evaluated using the Wilcoxon signed rank test (Luikart & Cornuet 1998). The qualitative "mode-shift" test of Luikart *et al*. (1998) was also used. Finally, we tested whether the M-Ratio was significantly below the critical M-Ratio (M_c) of a stable population of similar size, using programs M_P-Val and Critical M (Garza & Williamson 2001). As recommended by Garza & Williamson (2001), pre-bottleneck theta was set to 10, the proportion of multi-step mutations was set to 0.10, and the average number of repeats involved in non-stepwise mutations was set to 3.5. In addition, the analysis was also run assuming a pre-bottleneck theta ten times smaller.

The extent of inbreeding was estimated by computing the Internal Relatedness (IR) coefficient, a measure of how genetically related were the parents of each individual (Amos *et al*. 2001). IR was estimated using the R package Rhh (Alho *et al*. 2010). IR values usually follow a normal distribution with zero mean for individuals born to unrelated parents, outbred individuals with high negative values and inbred individuals with high positive values (Amos *et al*. 2001). Additionally, inbreeding was also evaluated by estimating FIS, using FSTAT (Goudet 1995).

The program ONeSAMP (Tallmon *et al*. 2008) was used to estimate the effective population size (N_e) through approximate Bayesian computation. Three independent analyses were run, changing the priors on minimum and maximum N_e (2 and 200, 10 and 300, and 4 and 100).

Relatedness analyses

The relatedness coefficient (*r*) for all pairs of individuals (dyads) was estimated using ML-Relate (Kalinowski *et al*. 2006). We use the Kolmogorov-Smirnov test to compare the distributions of relatedness of all possible dyads between and within groups.

Permutations were used to test if the mean relatedness within groups differed from that expected from random association. We compared the observed mean *r* within groups with those calculated from simulated groups, assigning at random one dominant male, one dominant female and subordinates to "groups". In each of 1000 simulations, we preserved the basic structure of the original groups (i.e. each group have at least one pair and its original size).

To test predictions based on the null hypotheses concerning the giant otter social structure, differences in values of *r* were compared within and among groups, and within and among social strata. To minimize the lack of independence of data associated with the pairwise comparisons, we used a bootstrap procedure to resample data 1000 times, using sample sizes compatible with the objects being compared (i.e. n=50, for comparisons involving individuals, n=20 when stratified by sex, and n=10, when groups were compared).

As the *r* distributions were non-normal, we used Kruskal-Wallis analyses associated with the bootstrap procedure. Probabilities were calculated as the proportion of calculated $\chi^2 < \chi^2_{critical}$ with an alpha=0.05.

We assessed if the relatedness in dyads were correlated with the distance between the animals being compared using the Mantel test (with 5000 permutations), using the R-package "ade4" (Dray & Dufour 2007). The distance was measured as the Euclidian distance between the center of the parental territory of the individuals in the comparison. The analyses were repeated after stratifying by sex, to test if relatedness in dyads within females and within males were correlated with distance. The probability (P) is presented as the proportion of simulated correlations higher than the observed value i.e., when the observed correlation is negative, high values of P means significant.

Results

The study population

We counted a total of 97 adult otters inhabiting the area during our study, and most animals were seen repeatedly. Of these, 84 were distributed in 17 groups (mean=5, range 2 to 12 individuals per group) and 13 were solitary. Twenty eight adult individuals of known sex were correctly assigned to gender, and 14 animals of previously unknown gender were genetically sexed. The sex ratio was 46:31 ≈1.5 males per female. The sex of 20 unsampled individuals sighted in the area could not be determined.

Genetic data set

We genotyped 50 giant otters, distributed in either one of 13 social groups or as transients individuals (n=2). We also reconstructed the genotypes of two alpha females based on their known brood and used the reconstructed genotypes in analyses. Therefore, our sample totaled 32 male and 20 female giant otters. Nine of these were alpha males and eight were alpha females. Of the 31 subordinates, four were yearling cubs. The number of individuals sampled from each group varied from one to eight and we sampled about 70% of group members (Table 3-1). Considering all individuals seen, we estimate that we sampled about 54% of the adult population.

The number of alleles per locus varied from 3 to 6, with expected and observed heterozygosities ranging between 0.312 and 0.766 (average 0.608), and 0.314 and 0.827 (average 0.619), respectively. No loci showed Hardy-Weinberg and linkage disequilibrium. In addition, we did not find evidence for scoring error due to stuttering or large allele dropout. The combined non-exclusion probability for all loci was 5% without knowing the parents and 0.3% knowing one of them (Table 3-2).

Fine-scale geographic population structure

The Structure analysis did not detect population differentiation between giant otters from the Vermelho and Miranda Rivers and the *EPP* (K=1, LnP(D)=-1355.13, SD=0.63; K=2, LnP(D)=-1357.98, SD=25.78). Therefore, all analyses assumed that the sampled individuals belong to a single population.

Effective population size and investigation of bottlenecks

All three independent analyses of N_e, each with different minimum and maximum thresholds as priors, yielded similar results: 24.7 (N_e between 2-200: 95% credible limits: 19.6 – 34.9), 29.0 (N_e between 10-300: 95% credible limits: 24.8 – 41.8) and 24.7 (N_e between 4-100: 95% credible limits: 20.7 – 34.6).

The Wilcoxon test was only significant for the IAM model (P=0.001), which is more likely to indicate heterozygosity excess for microsatellite data (Luikart & Cornuet 1998). The two other models that conform better to microsatellite evolution failed to reveal any pattern consistent with a recent bottleneck (TPM, P=0.17; SMM, P=0.31). The “mode-shift” test also

suggested that the observed allele frequency distributions fit mutation-drift equilibrium expectations (having L-shaped distributions).

Our estimate of the M value for giant otters in the Pantanal was 0.66 (P=0.004), irrespective of the pre-bottleneck theta assumed. The critical M (higher than 95% of equilibrium values) was 0.71 (theta = 10) or 0.79 (theta = 0.1). The M value was similar to values observed in mammalian species known to have undergone demographic declines (between 0.599 and 0.693, Table 2 in Garza & Williamson 2001). Therefore, the M-Ratio test indicated that the population has experienced a bottleneck.

Relatedness

The relatedness of giant otter dyads ranged from 0 to 0.739 and averaged 0.100 (median=0.005) and its distribution was clearly non-normal (Figure 3-2a). The distribution of relatedness between and within groups differed in shape (D=0.373, P<0.001,Figure 3-2b,c). Although *r* between groups had an L-shaped distribution, the distribution of *r* within groups was bimodal, with values of $r \geq 0.25$ being frequent. The average *r* within groups (0.229; n=10) was higher than the mean *r* of groups generated by random (mean=0.100 range 0.083-0.124, P<0.001). Although six of 10 groups showed high overall relatedness ($r > 0.2$), two had mean coefficients of relatedness equivalent to 3rd order kin relationship categories (e.g. first cousins, $r \approx 0.125$, Blouin 2003) and two groups were formed by completely unrelated individuals (Table 3-1).

Five of eight groups had unrelated alpha pairs, but two pairs were relatively closely related (r=0.160 and 0.192) and one pair was 1st order kin related (r=0.5, Table 3-1). Subordinates fell equally into the three relationship categories: in two of six groups they were 1st kin relatives, in two they were 2nd kin relatives ($r \approx 0.25$) and in two they were unrelated (r<0.09; Table 3-1). In three of seven groups, the alpha pairs were closely related on average to the subordinates (r>0.4), in one they were 2nd kin related (r=0.283), in two were 3rd kin related and in one group, the alpha pair was unrelated to the sampled subordinate (Table 3-1).

Mean *r* of females within groups varied from unrelated (three of six groups), 2nd order relatives ($r \approx 0.3$, two groups) and 3rd order relatives (r=0.142, one group). In general, males were genetically more related than females within groups, with three groups having closely related males ($r \approx 0.5$), three groups with *r* equivalent to 2nd order kin relationships, one with a 3rd kin relationship, and one with unrelated males (Table 3-1). The mean differences between

sexes within groups tended to be different, although not significantly (t=1.926, df=12, P=0.078). Also, in eight of ten cases females were related with males within groups (0.45>*r*>0.15), although in two groups they were unrelated (Table 3-1). Average *r* between all possible male dyads (0.095, range 0-0.637, n=496) and between female dyads (0.106, range 0-0.598, n=190) did not differ significantly (P=0.941). The average *r* between dominant males was 0.069 (range 0-0.5, n=36), and did not differ from that between dominant females (0.116 range 0-0.5, n= 36, P=0.925).

Mean relatedness (*r*) between all possible dyads were negatively correlated with the distances between the centers of the territories of the dyads (Mantel analyses, r_{obs}=-0.135, P=1), and females were more negatively related with distance (r_{obs}=-0.279, P=0.999) than males (r_{obs}= -0.124, P=0.996). However, when we excluded the dyads formed by animals of the same group (i.e. distance = 0), the slope of the regression line for males becomes negligible ($-0.15*10^{-3}$) whereas the slope for females were still meaningful ($-0.16*10^{-2}$); Figure 3-3).

Dispersal and group dynamics

During the eight-year study, we recorded the formation of five new pairs, which attempted to establish their own territories. Three were composed of individuals from neighboring groups and partially overlapped at least one of the parental territories. A fourth pair was of unknown origin, but the male was 1st order related with at least one of the subordinates of a neighboring group. These new formed pairs moved 6-18 km trying to establish their territories and at least one (EXC, Table 3-1) successfully raised an offspring. One group (GD4) seems comprise a coalition of unrelated young dispersers of both sexes (Table 3-1). We also observed 13 solitary transient individuals, six of which were male and seven for which sex was not determined. Those that we observed more than once (n=5) moved an average of 12 km (4.5-30 km), overlapping territories occupied by groups. One of them was slightly related (*r*=0.159) to the individuals from the group with the overlapping territory.

On two occasions dominant females were replaced by subordinates of the same group. In both cases, the subordinates were 1st order relatives (*r*=0.5) to the alpha female, and shared in the nursing of the cubs for up to three reproductive cycles, before the matriarchs finally

disappeared. The estimated minimum age of these matriarchs, at the time of the replacement, was eight and nine years, and the subordinates were at least six years old.

In one case, after a territorial fight, the winning group incorporated a female and a male subordinate of the other group. These subordinates were unrelated to each other (r=0), but the female was related to the original group (r=0.433) and unrelated to the winner group. The male was unrelated to the original group (r=0.040) but 2nd order related to the winner group (r=0.220).

Evaluation of inbreeding

The internal relatedness coefficient (IR) indicated that individuals were equally divided into those born to unrelated (IR = -0.08 to 0.08; N = 15), outbred (IR = -0.41 to -0.13; N = 20) and inbred (IR = 0.10 to 0.62; N = 17) parents. Therefore, 67% of the giant otters analyzed showed no sign of inbreeding. The inbreeding coefficient F_{IS} was not significantly different from zero (F_{IS} = - 0.02), also indicating that the population is not inbred.

Discussion

Population Structure

Our genetic results show that giant otters comprised a single population throughout the study area, indicating substantial gene flow on the scale examined. In the wet season, almost the whole floodplain is connected by water pathways and giant otters can disperse long distances. The Rio Vermelho and Rio Negro, both known to support high numbers of giant otters (this study, Mourão & Carvalho 2001), become connected by a large swamp which would also facilitate the movements of the otters (Figure 3-1). Total population size should be around 250 otters, as we our estimated effective population size of 25-30 individuals. We counted 97 adult otters in at least 17 groups, which would correspond to 38.8% of the census population.

Microsatellite variability in the study population (H_e = 0.61) was within the range observed by Pickles *et al*. (2011b) in the three other giant otter phylogroups (H_e = 0.61-0.64). Those heterozygosity levels are similar to those seen in other otters species, such as the sea otter (*Enhydra lutris,* H_e=0.47-0.49, Larson *et al*. 2002, Aguilar *et al*. 2008), Eurasian otter

(*Lutra lutra*, H_e=0.66, Lanszki *et al*. 2010, H_e = 0.37-0.71, Mucci *et al*. 2010) and river otter (*Lontra canadensis*, H_e=0.65, Latch *et al*. 2008).

Recent bottleneck

Recent bottlenecks result in a faster decrease in allele numbers than in expected heterozygosity (H_e), due to the loss of rare alleles. An excess of heterozygosity (H_e) compared to the expected equilibrium heterozygosity (H_{eq}) is therefore suggestive of a bottleneck, because H_{eq} is calculated from allele numbers. For our data, the IAM model showed significant heterozygosity excess, but since the other two models had non-significant results, and the IAM is more likely to produce false positives when microsatellite data are used (Luikart & Cornuet 1998), we conclude that those analyses provide no evidence of a bottleneck effect on the study population. That conclusion is also supported by the mode-shift test.

On the other hand, the M-Ratio test detected a bottleneck signal, as the estimated value of M (0.66) was below the critical threshold obtained from simulated equilibrium populations (M_c = 0.71 and 0.79). The M ratio test is expected to have a longer recovery time than heterozygosity excess and allele frequency (i.e. mode-shift) tests. Thus, those methods have different temporal detection windows (Cornuet & Luikart 1996; Garza & Williamson 2001). H_e-based analyses are able to detect severe demographic decline within the last $2N_e$ - $4N_e$ generations (Piry *et al*. 1999). Since the N_e of our population was estimated at 25 – 30, and considering that giant otters have generation lengths of seven years (Groenendijk *et al*. 2004), the detection window of Bottleneck for our data would be 350-815 years ago. The bottleneck detected by the M Ratio test for our population would be more than 700 years ago, because simulations showed that this statistic takes about one hundred generations to recover after a demographic decline (Garza & Williamson 2001).

Those estimates for the time of a bottleneck far predate the extensive hunting of the species during the 1950's and 1960's (Carter & Rosas 1997). By the end of the 1960's, more than 50 thousands pelts were exported from Brazil (Harris *et al*. 2005) and giant otters were not recorded in the Miranda and Vermelho Rivers (Schweizer 1992). Sequence data from giant otters distributed among four drainage basins in South America found a high overall haplotype and nucleotide diversity (h=0.93, π=0.015), except for the Pantanal phylogroup (h=0.44, p=0.0015) (Pickles *et al*. 2011a). Those authors suggest that the reduced variability

could be a consequence of hunting, but a second alternative hypothesis is that Pantanal was colonized later than the other basins and the lower genetic diversity could reflect a founder effect. That scenario is supported by our data, as the historical bottleneck detected through the M ratio test reflects such a founding event. In that case, the colonization of the area by founders of the present day giant otter population could have happened even before this period, because simulations have suggested that the bottleneck signal may last thousands of generations if the population is not completely isolated (Swatdipong *et al*. 2010). A third non exclusive explanation is that the population experienced a bottleneck during past climatic changes, as the Pantanal had at least two dry events in the Holocene period: a 40,000-8000 BP cool and dry and 3500-1500 BP warm and dry period. (Junk *et al*. 2006).

The social structure of giant otters

The giant otter groups were generally composed of an unrelated alpha pair and their close relatives. Also, the average relatedness within groups was higher than the mean relatedness of randomly generated, and close genetic relatedness was more frequent for dyads within groups than in dyads between groups. Those data suggest that kin selection is an important mechanism structuring social cohesion and cooperation among giant otters. The average relatedness within groups (r = 0.23) was high and similar to other carnivores that have a social system based on one reproductively dominant pair, such as African wild dogs (*Lycaon pictus*) (r = 0.27, Girman *et al*. 1997), Ethiopian wolves (*Canis simensis*) (r = 0.30, Randall *et al*. 2007) and meerkats (*Suricata suricatta*) (r = 0.28, Dugdale *et al*. 2008).

Despite the high average relatedness within groups, mean coefficient of relatedness of dyads of subordinates within groups was more variable. Groups averaging 1st, 2nd and 3rd degree relatives were evenly distributed in our sample, indicating that some groups were not composed of just one reproductive pair and their offspring. In two cases the subordinates were closely related to each other, but 3rd degree related to the alpha pair (Table 3-1) indicating that the kin composition of groups is variable and does not always correspond to the parent-brood model of Duplaix (1980) and Schweizer (1992). One group composed of unrelated individuals was clearly formed by young dispersers, and none of their members could be assigned as dominant based on behavior. Moreover, we recorded individual members of this group performing different activities at the same time, suggesting lower cohesion, although they shared dens, latrines and most of the time swam and fished together. This type of social

structure resembles packs of young adult dispersers, found in mixed-sex groups of some social carnivores, such as Ethiopian wolves (*Canis simensis*, Randall *et al*. 2007), striped hyena (*Hyaena hyaena*) (Wagner *et al*. 2007), and in coalitions of male lions *(Panthera leo*, Packer *et al*. 1991, Spong *et al*. 2002). In those species, the coalitions are usually, but not always, composed by close relatives. Molecular genetic studies of carnivores indicate that cooperation between unrelated individuals may be common (Gompper & Wayne 1996) and high variance in within-group relatedness is found in other social carnivores, such as coastal river otters (*Lontra canadensis*, Blundell *et al*. 2009), white-nosed coatis (*Nasua narica*, Gompper *et al*. 1997), Ethiopian wolves (Randall *et al*. 2007) and lions (Spong *et al*. 2002).

The existence of groups composed of unrelated giant otters suggests that in some instances group formation could be non-kin based and that kinship selection alone is not enough to explain the evolution and maintenance of the sociability in giant otter. (Clutton-Brock 2002; West *et al*. 2006). The diversity of relatedness coefficients found within groups emphasizes that quantitative information from different categories of relatedness and cooperative behavior will be important to reveal different aspects of the evolution and maintenance of social behaviors in carnivores (Gompper & Wayne 1996) such as giant otters.

Dispersal and group dynamics

Mating system and competition for resources can explain sex-biased dispersal (Greenwood 1980) resulting in the philopatric sex being more related within groups (Dugdale 2008). In our study, although males within groups tended to be genetically more related than females, the difference was not significant. Also, the average relatedness between dyads of males and between dyads of females did not differ, nor was there a significant difference in the relatedness of dyads of alpha males and dyads of alpha females. None of these results suggests strong asymmetry in dispersal between sexes. However, the relatedness of dyads within each sex decreased with distance between the individuals, for both sexes when dyads of animals within the same group were included in the analysis, but not for males when dyads within groups were excluded from the analysis. This suggests some asymmetry in distance dispersal between sexes with females being more philopatric or at least having more limited dispersal distances.

On the few occasions when we witnessed new formed pairs trying to establish territories, it happened in the vicinity of the territory of at least one of the original groups.

However, these observations could be biased, by a focus on already monitored areas and probably does not reflect all possibilities for dispersal and foundation of new groups.

We found a male-biased sex ratio of 40:31, considering just the individuals composing groups. However, previous studies carried out in the same area reported balanced (8:9, Ribas 2004) or female biased (10:19, Leuchtenberger & Mourão 2008) sex ratios. A male-biased sex ratio suggests more retention of males than females within the groups. However, we observed at least 13 transient individuals in the study area. Transients are thought to represent dispersing males (Schweizer 1992), and the six transients otters for which we could determine sex were all males, which would support a male-biased dispersal model for giant otters. In monogamous systems, philopatric males can increase their chances of acquiring a high-quality territory with which to maintain a female, especially those systems with parental care (Randall 2007), especially if they inherit the parental territory. However, in the two cases of replacement of dominants we witnessed, it was females that inherited the territory. Therefore, our data are conflicting in relation to philopatry and sex-biased dispersal of giant otters.

Membership exchanges are known to happen among giant-otter groups (Evangelista 2004; Ribas 2004; Leuchtenberger & Mourão 2008). Also, territorial agonism is common in the study area (e. g. Ribas & Mourão 2004; Leuchtenberger & Mourão 2009; this study) and they are frequently followed by major changes in group membership (this study). Those changes could have important implications. The advantages for the group receiving individuals may be related to the increase in the number of cooperative individuals vs. the cost of lowering the internal kinship (West *et al*. 2006). Another obvious advantage is to promote exogamous mating, thus reducing inbreeding and its deleterious consequences. For non-kin individuals, direct benefits may be related to increased probability of survival, mating success, successfully raising offspring, and successful dispersal (Clutton-Brock 2002).

Inbreeding avoidance

Our results suggest that giant otters have some inbreeding avoidance strategy, because inbreeding was not verified despite the indication of natal philopatry. It is unclear how giant otters avoid inbreeding., but some animals actively avoid mating with close kin via olfaction (DeWoody 2005) and the existence of female mate choice is suggested for giant otters (Schweizer 1992; Carter & Rosas 1997). Moreover, copulations outside the group apparently

allow outbreeding without dispersal (Keane *et al*. 1996) and short distance dispersal may be probably sufficient to avoid inbreeding (Lawson Handley & Perrin 2007).

Except for one couple, all dominant pairs were unrelated or distantly related (two couples). The only dominant pair strongly related (r=0.5) was living in a marginal habitat and under stressful environmental conditions. Additionally, cubs were not present during the time we monitored this group and therefore it is possible that they did not mated.

The evolution of sociability in giant otters

Most mustelids live alone or in pairs (Gittleman 1989). For giant otters the advantages of living in conspicuous and tight groups are unclear (Kruuk 2006). Assistance in hunting large prey and defense against predators are traditionally considered the main evolutionary constraints to explain sociality in carnivores (Macdonald 1983). Giant otters feed mainly on small prey (mainly fish) and participate in little cooperative feeding (Kruuk 2006). However, acquisition, inheritance, and/or defense of a high-quality territory could be an important evolutionary force selecting for group living in giant otters. Avoidance of predation by larger predators such as black caimans (*Melanosuchus niger*) and jaguar (*Panthera onca*), has been suggested to explain why giant otters form groups (Kruuk 2006; Brecht-Munn & Munn 1988), and defense against aggression from adjacent groups and/or solitary conspecifics has been proposed for giant otters in the Pantanal (Leuchtenberger & Mourão 2009).

Alloparental care may also be a factor determining the giant otter social system. In cooperatively breeding vertebrates, nonbreeding helpers raise young produced by dominant breeders (Clutton-Brock 2002). This type of social system is characterized by reproductive suppression (Wilson 1980; Keane *et al*. 1994). Indirect fitness benefits have been posited as a primary reason for the evolution of cooperative behavior (Hamilton 1964), although there is increasing evidence that helpers can be unrelated to the young they are raising (Clutton-Brock 2002). Moreover, subordinates can also breed, though at lower frequency, therefore obtaining some direct fitness benefits (e.g. *Lycaon pictus*, Girman *et al*. 1997; *Suricata suricata*, Griffin *et al*. 2003; *Vulpes vulpes*, Iossa *et al*. 2009). These conflicting studies suggest that different evolutionary mechanisms may be acting to maintain carnivore societies (Gompper & Wayne 1996; Clutton-Brock 2002).

For giant otters, the possibility of substituting an alpha individual and the benefits of inheriting a group with an established territory may compensate for periods of reproductive

suppression. Moreover, the presence of two lactating females in the same group does not seem to be uncommon (Rosas & Mattos 2003; Leuchtenberger & Mourão 2009; this study), and paternity studies are needed to confirm the degree of reproductive suppression in giant otters subordinate individuals.

Finally, territory availability and quality probably affect giant otter social dynamics. For a giant otter, the decision to disperse and attempt to establish its own territory comes at the cost of increase in the likelihood of serious injuries or death, especially in environments where all potential territories are occupied. Kin cooperation is a selective pressure that may provide further competitive advantages to residents and thereby promote philopatry (Lawson Handley & Perrin 2007).

The social system of giant otters is much more complex than anticipated. Groups are not formed exclusively by dominant pairs and their offspring, although they are mostly composed of relatives. The evolutionary mechanisms that lead to the high relatedness variance within giant otters groups is unclear, but we believe that high migration rate of individuals across groups, extra-pair copulation and subordinate reproduction can play an important role, as they do in other apparently monogamous species.

Acknowledgements

We thank Federal University of Mato Grosso do Sul for providing logistic support. CNPq granted a scholarship to CR, and a research grant to GM to support the field and some of Laboratory costs (# 476939/2008-9). We are indebted to I. T. Carvalho for fieldwork assistance, to C. Leuchtenberger for providing blood and mucus samples and C.A.Zucco and L.G.Oliveira-Santos for analysis procedures. We thank L. Damasceno, S. Damasceno, Cel. and C. Marsiglia provided essential support with the equipment importation process.

References

Aguilar A, Jessup DA, Estes J, Garza JC (2008) The distribution of nuclear genetic variation and historical demography of sea otters. *Journal Compilation*, **11**, 35–45.

Alho CJR, Lacher TEJr, Gonçalves HC (1988) Environmental degradation in the Pantanal ecosystem of Brazil. *BioScience*, **38**, 164-171.

Alho JS, Välimäki K, Merila J (2010) Rhh: an R extension for estimating multilocus heterozygosity and heterozygosity–heterozygosity correlation. *Molecular Ecology Resources*, **10**, 720-722.

Amos B, Schlktterer C, Tautz D (1993) Social structure of pilot whales revealed by analytical DNA profiling. *Science*, **260**, 670-672.

Amos W, Worthington Wilmer J, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T (2001) The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **268**, 2021-2027.

Balloux F (2001) EASYPOP (Version 1.7): a computer program for population genetics simulations. *Journal of Heredity*, **92**, 301-302.

Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *TRENDS in Ecology and Evolution*, **18**, 503-511.

Blundell GM., Ben-David M, Groves P, Bowyer RT, Geffen E (2009) Kinship and sociality in coastal river otters: are they related? *Behavioral Ecology*, **15**, 705–714.

Brecht-Munn M, Munn CA (1988) The Amazon's gregarious giant otters. *Animal Kingdom*, **91**, 34-41.

Carter SK, Rosas FCW (1997) Biology and conservation of the giant otter *Pteronura brasiliensis*. *Mammal Review*, **27**, 1-26.

Clutton-Brock T (2002) Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, **296**, 69-72.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001-2014.

Dallas JF, Carss DN, Marshall F, Koepfli KP, Kruuk H, Piertney SB, Bacon PJ (2000) Sex identification of the Eurasian otter *Lutra lutra* by PCR typing of spraints. *Conservation Genetics*, **1**, 181–183.

Davenport, LC (2010) Aid to a declining matriarch in the giant otter (*Pteronura brasiliensis*). *PLoS ONE*, **5**, 1-6.

DeWoody JA (2005) Molecular approaches to the study of parentage, relatedness, and fitness: practical applications for wild animals. *Journal of Wildlife Management*, **69**, 1400-1418.

Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, **22**, 1-20.

Dugdale HL, Macdonald DW, Popo LC, Johnson P (2008) Reproductive skew and relatedness in social groups of European badgers, *Meles meles*. *Molecular Ecology*, **17**, 1815–1827.

Duplaix N (1980) Observations on the ecology and behavior of the giant otter *Pteronura brasiliensis* in Suriname. *Revue d'Ecologie (la Terre et la Vie)*, **34**, 495-620.

Evangelista E (2004) Change of partners in a giant otter alpha couple. *IUCN Otter Specialist Group Bulletin*, **21**, 47–51.

Garner A, Rachlow JL, Hicks JF (2005) Patterns of genetic diversity and its loss in mammalian populations. *Conservation Biology*, **19**, 1215–1221.

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**, 305–318.

Girman DJ, Mills MGL, Geffen E, Wayne RK (1997) A molecular genetic analysis of social structure, dispersal, and interpack relationships of the African wild dog (*Lycaon pictus*). *Behavioral Ecology and Sociobiology*, **40**, 187–198.

Gompper ME, Wayne RK (1996) Genetic relatedness among individuals within carnivore societies. In: *Carnivore Behavior, Ecology, and Evolution. Volume 2* (eds Gittleman JL), pp. 429-452. Cornell University Press, New York.

Gompper ME, Gittleman JL, Wayne RK (1997) Genetic relatedness, coalitions and social behaviour of white-nosed coatis, *Nasua nasua*. *Animal Behaviour*, **53**, 781-797.

Goudet J (1995) FSTAT (version 1.2): a computer program for calculating F-statistic. *Journal of Heredity*, **86**, 485-486.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Griffin AS, Pemberton JM, Brotherton PNM *et al*. (2003) A genetic analysis of breeding success in the cooperative meerkat (*Suricata suricatta*). *Behavioral Ecology*, **14**, 472–480.

Groenendijk J, Hajek F, Duplaix N *et al*. (2005) Surveying and monitoring distribution and population trends of the giant otter (*Pteronura brasiliensis*). Guidelines for a standardisation of survey methods as recommended by the giant otter section of the IUCN/SSC Otter Specialist Network. *Habitat*, 16, 1–100.

Hamilton WD (1964) The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, **7**, 1–16.

Harris MA, Tomas W, Mourão G, Da Silva CJ, Guimarães E, Sonoda F, Fachim E (2005) Safeguarding the Pantanal wetlands: threats and conservation initiatives. *Conservation Biology*, **19**, 714–720.

Iossa G, Soulsbury CD, Baker PJ, Edwards KJ, Harris S (2009) Behavioral changes associated with a population density decline in the facultatively social red fox. *Behavioral Ecology*, **20**, 385–395.

IUCN – International Union for Conservation of Nature and Natural Resources (2011) IUCN Red List of Threatened Species. URL http://www.iucnredlist.org.

Jones AG (2005) GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. *Molecular Ecology*, **5**, 708-711.

Junk WJ, Cunha CN, Wantzen KM, Petermann P, Strussmann C, Marques M. Adis J (2006) Biodiversity and its conservation in the Pantanal of Mato Grosso, Brazil. *Aquatic Sciences*, **68**, 278-309.

Kalinowski ST, Wagner AP, Taper ML (2006) ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, **6**, 576–579.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099-1106.

Keane B, Waser PM, Creel SR, Cree NM, Elliott LF, Minchella DJ (1994) Subordinate reproduction in dwarf mongooses. *Animal Behaviour*, **47**, 65-75.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**, 230-241.

Kruuk H (2006) *Otters: ecology, behaviour and conservation*. Oxford University Press, New York.

Lanszki J, Hidas A, Szentes K, Révay T, Lehoczky I, Jeney Zs, Weiss S (2010) Genetic structure of otter (*Lutra lutra*) populations from two fishpond systems in Hungary. *Mammalian Biology*, **75**, 447-450.

Larson S, Jameson R, Etnier M, Fleming M, Bentzen P (2002) Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. *Molecular Ecology*, **11**, 1899-1903.

Latch E, Scognamillo DG, Fike JA, Chamberlain MJ, Rhodes JR OE (2008) Deciphering ecological barriers to North American river otter (*Lontra canadensis*) gene flow in the Louisiana landscape. *Journal of Heredity*, **99**, 265–274.

Lawson Handley LJ, Perrin N (2007) Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology*, **16**, 1559–1578.

Leuchtenberger C, Mourão G (2008) Social organization and territoriality of giant otters (Carnivora: Mustelidae) in a seasonally flooded savanna in Brazil. *Sociobiology*, **52**, 257-270.

Leuchtenberger C, Mourão G (2009) Scent-marking of giant otter in the Southern Pantanal, Brazil. *Ethology*, **115**, 210-216.

Luikart G, Cornuet JM (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology*, **12**, 228-237.

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238–247.

Macdonald DW (1983) The ecology of carnivore social behaviour. *Nature*, **301**, 379-384.

Mourão G, Carvalho L (2001) Cannibalism among Giant Otters (*Pteronura brasiliensis*). *Mammalia*, **65**, 225-227.

Mourão G, Ribas C, Magnusson W (2006) Manejo de fauna silvestre no Brasil. In: Biologia da Conservação- Essências (eds Rocha CFD, Bergallo HG, Sluys MV, Alves MAS), pp. 459-477. RiMa, São Paulo.

Mucci N, Arrendal J, Ansorge H *et al.* (2010) Genetic diversity and landscape genetic structure of otter (*Lutra lutra*) populations in Europe. *Conservation Genetics*, **11**, 583–599.

Packer C, Gilbert DA, Pusey AE, O'Brien SJ (1991) A molecular genetic analysis of kinship and cooperation in African lions. *Nature*, **351**, 562–565.

Pickles RSA, Groombridge JJ, Zambrana Rojas VD, van Damme P, Gottelli D, Kundu S, Bodmer R, Ariani CV, Iyengar A, Jordan WC (2011a) Phylogeography and identification of evolutionary significant units in the giant otter. *Molecular Phylogenetics and Evolution*, **61**, 616-627.

Pickles RSA, Groombridge JJ, Zambrana Rojas VD, Van Damme P, Gottelli D, Ariani CV, Jordan WC (2011b) Genetic diversity and population structure in the endangered giant otter, *Pteronura brasiliensis*. *Conservation Genetics*, DOI 10.1007/s10592-011-0279-9.

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.

Randall DA, Pollinger JP, Wayne RK, Tallents LA, Johnson PJ, Macdonald DW (2007) Inbreeding is reduced by female-biased dispersal and mating behavior in Ethiopian wolves. *Behavioral Ecology*, **18**, 579–589.

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, URL: http://www.R-project.org/.

Ribas C (2004) *Desenvolvimento de um programa de monitoramento em longo prazo das ariranhas (Pteronura brasiliensis) no Pantanal brasileiro*. MSc dissertation. Universidade Federal de Mato Grosso do Sul, Brazil.

Ribas C, Mourão G (2004) Intraspecific agonism between giant otter groups. *IUCN Otter Specialist Group Bulletin*, **21**, 89-93.

Ribas C, Vasconcellos AV, Mourão G, Magnusson W, Sole-Cava AM, Cunha HA (2011) Polymorphic microsatellite loci from the endangered Giant Otter (*Pteronura brasiliensis*). *Conservation Genetics Resources*, **3**,769-771.

Ribas C, Damasceno G, Magnusson W, Leuchtenberger C, Mourão G (2012) Giant otters feeding on caiman: evidence for an expanded trophic niche of recovering populations. *Studies on Neotropical Fauna and Environment*, **47**,19-23.

Rosas FCW, de Mattos GE (2003b) Notes on giant otter (*Pteronura brasiliensis*) behavior in the lake of Balbina Hydroelectric Power Station, Amazonas, Brazil. *The Latin American Journal of Aquatic Mammals*, **2**, 127-129.

Rosas F, Cabral M, De Mattos, G, Silva R (2009) Parental and alloparental care of giant otters (*Pteronura brasiliensis*) (Carnivora, mustelidae) in Balbina hydroelectric lake, Amazonas, Brazil. *Sociobiology*, **54**, 919-924.

Schweizer J (1992) *Ariranhas no Pantanal: Ecologia e Comportamento da Pteronura brasiliensis*. Edibran-Editora Brasil Natureza Ltda, Curitiba.

Spong G, Stone J, Creel S, Bjorklund M (2002) Genetic structure of lions (*Panthera leo L*.) in the Selous Game Reserve: implications for the evolution of sociality. *Journal of Evolutionary Biology*, **15**, 945–953.

Swatdipong A, Primmer CR, Vasemägi A (2010) Historical and recent genetic bottlenecks in European grayling, Thymallus thymallus. *Conservation Genetics*, **11**, 279-292.

Tallmon DA, Koyuk A, Luikart G, Beaumont MA (2008) ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. *Molecular Ecology Resource*, **8**, 299–301.

Van Horn RC, Engh AL, Scribner KT, Funk SM, Holekamp KE (2004) Behavioural structuring of relatedness in the spotted hyena (*Crocuta crocuta*) suggests direct fitness benefits of clan-level cooperation. *Molecular Ecology*, **13**, 449–458.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535-538.

Wagner AP, Creel S, Frank LG, Kalinowski ST (2007) Patterns of relatedness and parentage in an asocial, polyandrous striped hyena population. *Molecular Ecology*, **16**, 4356–4369.

West SA, Pen I, Griffin AS (2002) Cooperation and competition between relatives. *Science*, **296**, 72-75.

West SA, Griffin AS, Garder A (2006) Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *Journal of Evolutionary Biology*, **20**, 415-432.

Wilson EO (1980) Sociobiology. The Abridged Edition. The Belknap Press of Harvard University Press. Cambridge, Massachusetts and London, England.

Figures and Tables

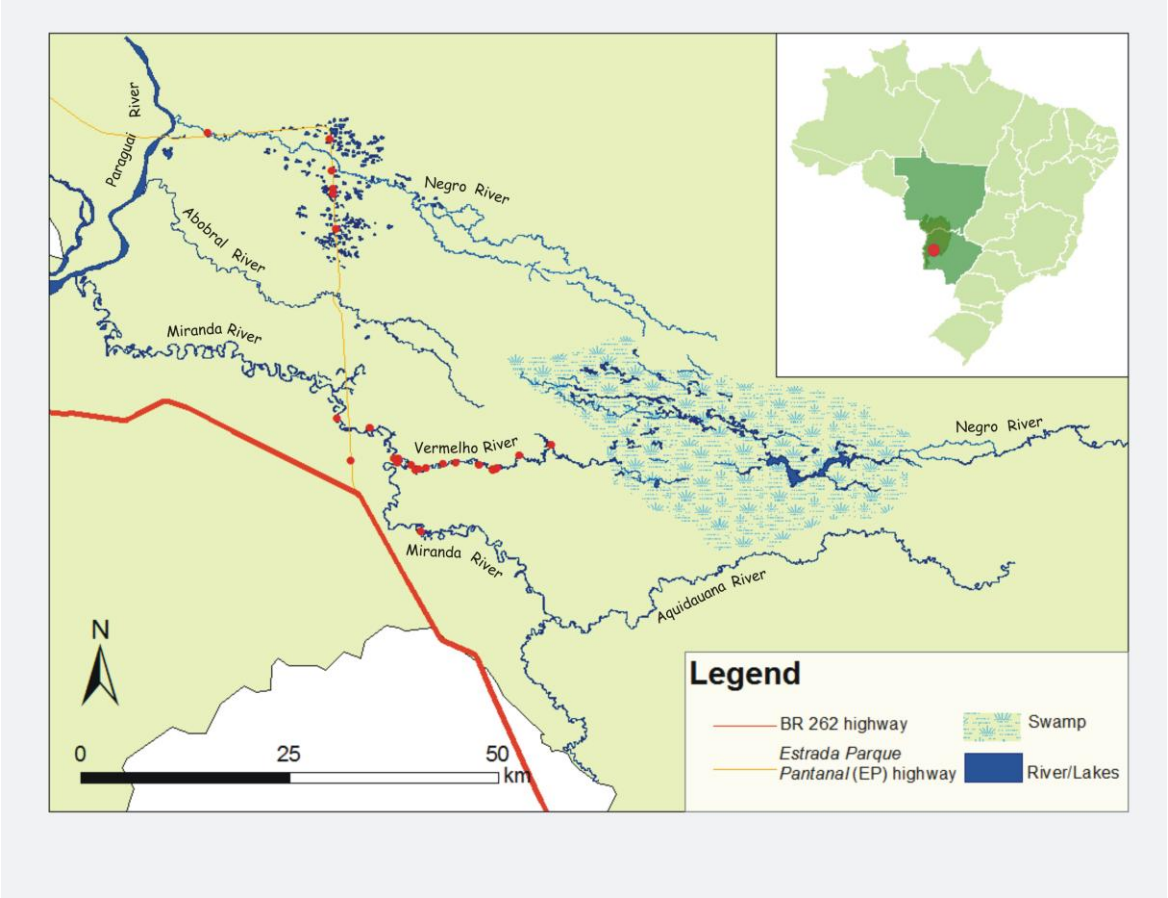


Figure 3-1 -Map of the study area showing the sites where genetic samples were collected (red dots), from November 2008 to October 2010.

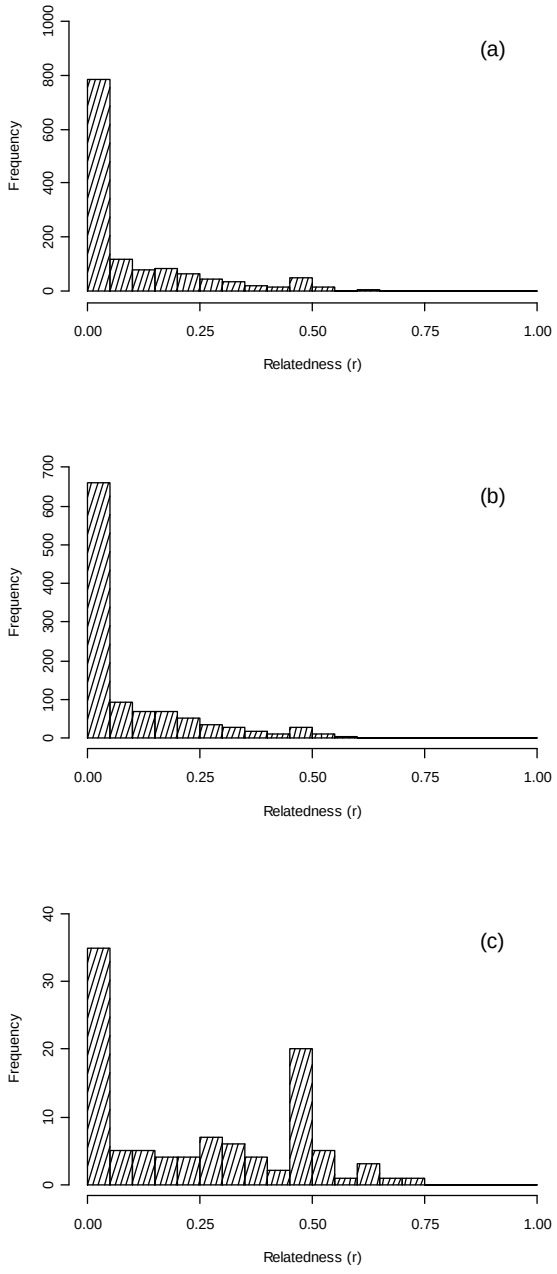


Figure 3-2 (a) Relatedness (*r*) of all possible dyads for 52 giant otters in 13 groups; (b) *r* of dyads of otters from different groups; (c) *r* of 103 dyads within groups. Samples were collected from November 2008 to October 2010 in the Miranda-Vermelho Rivers and Estrada Parque Pantanal highway, in the southern Pantanal of Brazil.

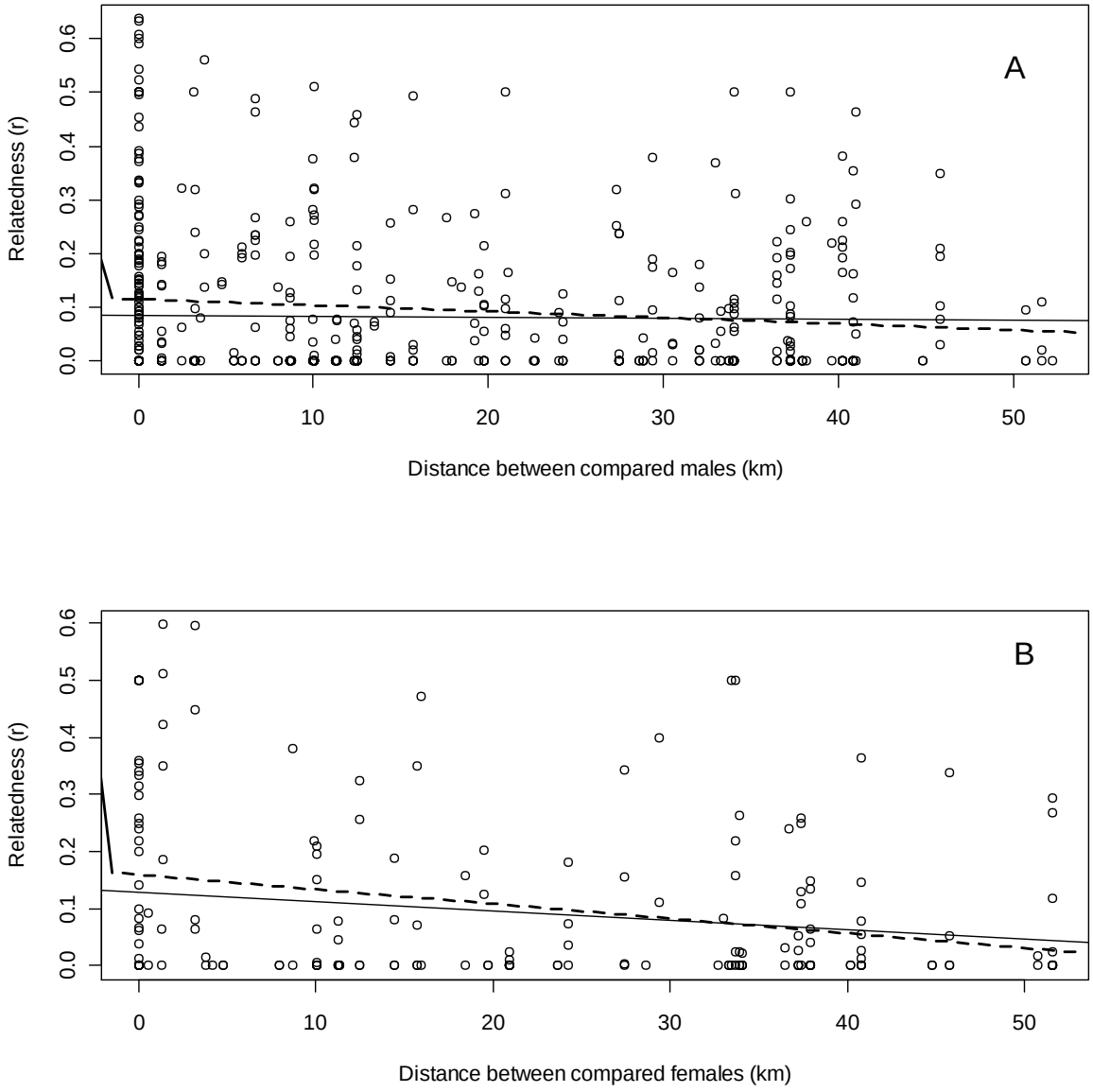


Figure 3-3 - Pairwise relatedness estimates for (A) all sampled males and (B) all sampled females plotted against the distance between the centers of group territories of the compared giant otters. Mantel tests indicate that both sexes were negatively related with distance, but this effect is stronger in females (r_{obs}=-0.279, P=1) than in males (r_{obs}= -0.124, P= 0.998). Dashed lines are simple linear fits of all data, and continuous lines are simple linear fits after excluding the dyads within groups (i.e. distance=0).

Table 3-1 -Mean relatedness (r) within groups of giant otter, sex and social status, in stretches of the Miranda and Vermelho Rivers, in the Pantanal of Brazil, from November 2008 to October 2010. Numbers within parentheses indicate the number of individuals included in genetic analyses.

Group[1]	size	All members	Alpha pair	Subordinate	Alpha-to-subordinate	Female	Males	Females-to-males
BAB	10	0.304 (8)	≤ 0.033 (3)[2]	0.239 (6)	0.411 (8)	0.332 (5)	0.326 (3)	0.281 (8)
NOV	9	0.187 (8)	0	0.251 (6)	0.124 (8)	0 (2)	0.154 (6)	0.245 (8)
GEP	7	0.220 (6)	0.5	0.089 (4)	0.283 (6)	0 (2)	0.273 (4)	0.207 (6)
BMi	6	0.217 (5)	0	0.470 (3)	0.127 (5)	0.354 (2)	0.307 (3)	0.149 (5)
EXC	6	0.439 (4)	0	0.632 (2)	0.500 (4)	-	0.544 (3)	0.333 (4)
AAZ	6	0.402 (4)	-	-	-	0.142 (2)	0.497 (2)	0.443 (4)
BOT	8	0.357 (3)	0.192	-	0.439 (3)	-	0.500 (2)	0.285 (3)
GD4	4	0 (3)	-	0 (3)	-	-	0 (2)	0 (3)
PMA	4	0.001 (3)	0.003	-	0 (3)	0 (2)	-	0.002 (3)
EP1	2	0.160 (2)	0.160	-	-	-	-	0.160 (2)
Mean	5.5	0.229 (46)	0.099 (17)[2]	0.280 (24)	0.269 (37)	0.138 (15)	0.325 (25)	0.211 (46)

[1]Groups with only one sampled individual were not included.

[2]One alpha female died and was replaced

Table 3-2. Variability levels in 52 giant otters from Pantanal. N indicates sample size, N_a number of alleles observed, H_o observed heterozygosity, H_e expected heterozygosity, NE-1P and NE-2P probability of non-exclusion of a parent, unknowing both or one of them, respectively, Null estimated null allele frequency.

	N	N_a	H_o	H_e	NE-1P	NE-2P	Null
Pbra01	48	4	0.438	0.531	0.857	0.726	+0.1123
Pbra02	52	5	0.827	0.766	0.640	0.459	-0.0457
Pbra05	52	4	0.750	0.702	0.728	0.559	-0.0382
Pbra08	46	3	0.500	0.519	0.868	0.762	+0.0212
Pbra09	51	4	0.529	0.511	0.870	0.732	-0.0139
Pbra10	51	4	0.314	0.312	0.951	0.834	+0.0151
Pbra11	51	3	0.608	0.567	0.842	0.698	-0.0552
Pbra14	52	4	0.500	0.464	0.893	0.772	-0.0415
Pbra17	51	4	0.784	0.736	0.696	0.523	-0.0430
Pbra20	51	4	0.706	0.757	0.673	0.497	+0.0297
Pbra21	50	6	0.820	0.707	0.710	0.537	-0.0857
Pbra24	51	5	0.647	0.728	0.690	0.512	+0.0469

Síntese

Pesquisadores da vida animal têm se beneficiado de diversos avanços tecnológicos. Análises moleculares possibilitam o acesso à informação acurada sobre paternidade, parentesco, padrões de dispersão, entre outros, revolucionando o conhecimento sobre os sistemas sociais das espécies e embasando medidas de conservação e manejo para estes animais (DeYoung e Honeycutt, 2005; De Woody, 2005).

Ariranhas são animais sociais que apresentam uma forte cooperação entre os indivíduos dos grupos (Duplaix, 1980) e interações agonísticas com grupos vizinhos (Mourão e Carvalho, 2001; Ribas e Mourão, 2004). Seu sistema social tem sido descrito como monogâmico e observações de comportamento sugerem que os grupos são compostos por um par dominante reprodutivo e seus filhotes de anos subseqüentes, que não reproduzem (Duplaix, 1980; Schweizer, 1992).

Ariranhas defecam e urinam em latrinas comunais e misturam os dejetos durante o comportamento de marcação (Duplaix, 1980). Assim, mucos isolados são o único tipo de amostra não invasiva disponível para estudos sociais. Entretanto, raramente o pesquisador consegue associar a amostra ao animal excretor, o que é imprescindível para estudos de parentesco e paternidade. Neste estudo, análises de DNA de amostras de mucos tiveram uma baixa taxa de sucesso, e apenas 17% das amostras foram adequadas para as análises. Além disso, amostras genéticas não invasivas são propensas a erros de genotipagem, associados à pequena quantidade ou baixa qualidade de DNA disponível na amostra (Taberlet *et al*., 1997) e resultados acurados podem apenas ser obtidos através da confirmação dos pares dos genótipos através da replicação das análises (Waits e Paetkau, 2005; Hansen *et al*., 2008), tornando os custos de laboratório mais caros do que aqueles de amostras de tecido e sangue.

Recentemente, ariranhas estão sendo capturadas para estudos de radiotelemetria e amostras genéticas têm sido coletadas oportunisticamente (Silveira *et al*., 2011). Nesta metodologia, os animais são capturados com redes na entrada da loca e não é possível para o pesquisador escolher o animal a ser amostrado. Este método é invasivo, com a necessidade de sedação dos animais, além de demandar uma logística complicada e cara.

O método de retirada de tecido de animais silvestres através de dardos de biópsia lançados por um projetor de Co_2 vem sendo utilizado em grandes vertebrados terrestres africanos (Spong *et al*., 2002; Muwanika *et al*., 2003; Moueix 2006), mas não há precedente

de seu uso em vertebrados terrestres Neotropicais. Ariranhas adultas são bons candidatos para amostragem com dardos de biópsias, uma vez que são animais grandes, diurnos e conspícuos. Além disso, ariranhas passam grandes períodos do dia em terra, utilizando latrinas, subidas para as locas ou pegando sol nos barrancos, o que facilita as emboscadas.

Dardos de biópsia foram capazes de coletar amostras de tecido de alta qualidade de DNA, de um grande número de ariranhas (n=41), há um custo menor e de forma menos invasiva do que a captura. A outra grande vantagem do método foi à possibilidade de amostrar indivíduos específicos, imprescindível em estudos de parentesco. Os animais não mostraram efeito adverso à amostragem e dardos com pressão regulada e bem direcionados não são capazes de prejudicar seriamente os indivíduos. Eu estou convencida de que o método mais eficaz para obter amostras biológicas de ariranhas selvagens, até agora, é através de dardos de biópsia.

Microssatélites são os marcadores ideais para acessar estrutura populacional e parentesco das populações selvagens (Queller *et al*., 1993). Neste trabalho, 14 *loci* polimórficos de microssatélites específicos para *P. brasiliensis* foram isolados, sendo 12 deles apropriados para as análises e todos com alta resolução para análises de paternidade (Ribas, *et al.*, 2011).

Todos os 50 indivíduos de ariranhas genotipados pertenciam a uma única população, indicando substancial fluxo gênico na escala examinada. A variabilidade nuclear encontrada neste estudo para a população do Pantanal estava na faixa observada para ariranhas em escalas geográficas maiores (Pickles *et al*., 2011b) e para outras lontras (Aguilar *et al*., 2008; Mucci *et al*. 2010; Latch *et al*. 2008).

Os grupos de ariranhas foram geralmente compostos por um casal dominante não relacionado e seus parentes próximos. Entretanto, uma alta diversidade de graus de parentesco dentro dos grupos foi encontrada, desde grupos formados por pais e filhotes, passando por grupos de subordinados com diferentes graus de parentesco entre si e em relação aos dominantes, até grupos de indivíduos completamente não relacionados, sugerido neste trabalho como grupo de dispersores. Estes resultados contradizem o conhecimento corrente de que grupos de ariranhas são exclusivamente formados por um casal dominante reprodutor e seus filhotes de anos subseqüentes (Duplaix, 1980; Schweizer, 1992; Carter e Rosas, 1997).

Nossos dados sobre dispersão em ariranhas foram conflitantes. A razão sexual encontrada foi enviesada para machos e o grau de parentesco foi maior entre machos dentro dos grupos, sugerindo a maior retenção deste sexo nos grupos. Ao mesmo tempo, todos os

solitários dispersores que tiveram o sexo conhecido (6 de 13) eram machos, sugerindo que os machos são os dispersores (Schweizer, 1992). Duas fêmeas subordinadas foram vistas substituindo as dominantes na hierarquia do grupo, e em ambos os casos as subordinadas eram parentes em primeiro grau das matriarcas, indicando que pode haver uma pressão para a filopatria entre as fêmeas. Além disso, houve uma correlação negativa entre parentesco e distância entre territórios mais forte entre as fêmeas do que entre os machos, sugerindo um maior grau de filopatria para fêmeas.

O sistema social em ariranhas é mais complexo do que o previsto. Grupos não são formados exclusivamente por um casal reprodutor e seus filhotes, ainda que geralmente sejam compostos por parentes próximos. Os mecanismos evolutivos que levam à alta variação de parentesco dentro dos grupos de ariranhas ainda não estão claros, mas alta taxa de migração de indivíduos entre os grupos, cópula extra-par e reprodução de subordinados podem desempenhar um papel importante, como acontece em outras espécies aparentemente monogâmicas.

Referências Bibliográficas

Aguilar, A.; Jessup, D.A.; Estes, J.; Garza, J.C. 2008. The distribution of nuclear genetic variation and historical demography of sea otters. *Journal compilation*, 11: 35–45.

Alho, C.J.R.: Lacher, T.E. Jr:, Gonçalves, H.C. 1988. Environmental degradation in the Pantanal ecosystem of Brazil. *BioScience*, 38: 164-171.

Alho, J.S.; Välimäki, K.; Merila, J. 2010. Rhh: an R extension for estimating multilocus heterozygosity and heterozygosity–heterozygosity correlation. *Molecular Ecology Resources*, 10: 720-722.

Amos, W.; Whitehead, H.; Ferrari, M.J.; Glockner-Ferrari, D.A.; Payne, R.; Gordon, J. 1992 Restrictable DNA from sloughed cetacean skin; its potential for use population analysis. *Marine Mammal Science*, 8(3): 275-283.

Amos, B.; Schlktterer, C.; Tautz, D. 1993. Social Structure of Pilot Whales Revealed by Analytical DNA Profiling. *Science*, 260: 670-672.

Amos, W.; Worthington Wilmer, J.; Fullard, K.; Burg, T.M.; Croxall, J.P.; Bloch, D.; Coulson, T. 2001. The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 268: 2021-2027.

Balloux, F. 2001. EASYPOP (Version 1.7): a computer program for population genetics simulations. *Journal of Heredity*, 92(3): 301-302.

Bloor, P.A.; Barker, F.S.; Watts, P,C,; Noyes, H.A.; Kemp, S.J. 2001. Microsatellite libraries by enrichment. (www.genomics.liv.ac.uk/animal/Protocol1.html). Acesso: 19/04/2011.

Blouin, M.S. 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *TRENDS in Ecology and Evolution*, 18(10): 503-511.

Blundell, G.M.; Ben-David, M.; Groves, P.; Bowyer, R.T.; Geffen, E. 2009. Kinship and sociality in coastal river otters: are they related? *Behavioral Ecology*, 15(5): 705–714.

Brecht-Munn M.; Munn C.A. 1988. The Amazon's Gregarious Giant Otters. *Animal Kingdom*, 91: 34-41.

Cabral, M.M.M.; Zuanon, J.; de Mattos, G.E.; Rosas, F.C.W. 2010. Feeding habits of giant otters *Pteronura brasiliensis* (Carnivora: Mustelidae) in the Balbina hydroelectric reservoir, Central Brazilian Amazon. *Zoologia*, 27(1): 47–53.

Cant, M.A. 2000. Social control of reproduction in banded mongooses. *Animal Behaviour*, 59: 147-158.

Carter S.K.; Rosas F.C.W. 1997. Biology and Conservation of the Giant Otter *Pteronura brasiliensis*. *Mammal Review*, 27(1): 1-26.

Clavero, M.; Prenda, J.; Blanco-Garrido, F.; Delibes, M. 2008. Hydrological stability and otter trophic diversity: a scale-insensitive pattern? *Canadian Journal of Zoology*, 86(10): 1152–1158.

Clutton-Brock, T. 2002. Breeding Together: Kin Selection and Mutualism in Cooperative Vertebrates. *Science*, 296: 69-72.

Coltman, D.W.; Pilkington, J.G.; Pemberton, J.M. 2003. Fine-scale genetic structure in a free-living ungulate population. Molecular Ecology, 12: 733-742.

Cornuet, JM.; Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, 144: 2001-2014.

Creel, S.R.; Creel, N.M. 1995. Communal hunting and pack size in Africa wild dogs, *Lycaon pictus*. *Animal Behaviour*, 50: 1325-1339.

Dallas, J.F.; Carss, D.N.; Marshall, F.: Koepfli, K.P.; Kruuk, H.; Piertney, S.B.; Bacon, P.J. 2000. Sex identification of the Eurasian otter *Lutra lutra* by PCR typing of spraints. *Conservation Genetics*, 1: 181–183.

Davenport, L.C. 2010. Aid to a Declining Matriarch in the Giant Otter (*Pteronura brasiliensis*). *PLoS ONE*, 5(6)**:** 1-6.

DeYoung, R.W.; Honeycutt, R.L. 2005. The molecular toolbox: genetic techniques in wildlife ecology and management. *Journal of Wildlife Management*, 69(4): 1362-1384.

DeWoody, J.A. 2005. Molecular Approaches to the study of parentage, relatedness, and fitness: practical applications for wild animals. *Journal of Wildlife Management*, 69(4): 1400-1418.

Díaz H.J.; Sánchez I.M. 2002. Historical and Actual Presence of the Giant Otter (*Pteronura brasiliensis*) on the Lower Meta River, Department of Casanare – Colombia Orinoquia. *IUCN Otter Specialist Group Bulletin*, 19(2): 97–102.

Dray, S.; Dufour, A.B. 2007. The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22(4): 1-20.

Dugdale, H.L.; Macdonald, D.W.; Pope, L.C.; Buke, T. 2007. Polygynandry, extra-group paternity and multiple-paternity litters in European badger (Meles meles) social groups. Molecular Ecology, 16: 5294–5306.

Dugdale, H.L.; Macdonald, D.W.; Popo, L.C.; Johnson, P. 2008. Reproductive skew and relatedness in social groups of European badgers, Meles meles. *Molecular Ecology*, 17: 1815–1827.

Duplaix, N. 1980. Observations on the Ecology and behavior of the giant otter *Pteronura brasiliensis* in Suriname. *Revue d'Ecologie (la Terre et la Vie)*, 34: 495-620.

Eizirik, E.; Haag, T.; Santos, A.S.; Salzano, F.M.; Silveira, L.; Azevedo, F.C.C.; Furtado, M.M. 2008. Jaguar Conservation Genetics. *CAT News, Special Issue*, 4: 31-34.

Evangelista, E. 2004. Change of partners in a giant otter alpha couple. *IUCN Otter Specialist Group Bulletin*, 21(1): 47–51.

Franco-de-Sá, J.F.O.; Rosas, F.C.W.; Feldberg, E. 2007. Cytogenetic study of the giant otter Pteronura brasiliensis Zimmermann 1780 (Carnivora, Mustelidae, Lutrinae). *Genetics and Molecular Biology*, 30(4): 1093-1096.

Garcia, D.M.; Marmontel, M.; Rosas, F.W.; Santos, F.R. 2007. Conservation genetics of the giant otter (Pteronura brasiliensis (Zimmerman, 1780)) (Carnivora, Mustelidae). *Brazilian Journal of Biology*, 67(4): 631–637.

Garner, A.; Rachlow, J.L.; Hicks, J.F. 2005. Patterns of genetic diversity and its loss in mammalian populations. *Conservation Biology* 19(4): 1215–1221.

Garza, J.C.; Williamson, E.G. 2001. Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, 10: 305–318.

Gemmel, N.J.; Majluf, P. 1997. Projectile biopsy sampling of fur seals. *Marine Mammal Science*, 13: 512-516.

Girman, D.J.; Mills, M.G.L.; Geffen, E.; Wayne, R.K. 1997. A molecular genetic analysis of social structure, dispersal, and interpack relationships of the African wild dog (*Lycaon pictus*). *Behavioral Ecology and Sociobiology*, 40: 187-198.

Gompper, M.E.; Wayne, R.K. 1996. Genetic relatedness among individuals within carnivore societies. *In*: Gittleman J.L. (Eds) *Carnivore Behavior, Ecology, and Evolution*. Vol. 2. Cornell University Press, New York. p. 429-452.

Gompper, M.E.; Gittleman, J.L.; Wayne, R.K. 1997. Genetic relatedness, coalitions and social behaviour of white-nosed coatis, *Nasua nasua*. *Animal Behaviour*, 53: 781-797.

Goudet, J. 1995. FSTAT (version 1.2): a computer program for calculating F-statistics. *Journal of Heredity* 86(6): 485-486.

Greenwood, P.J. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, 28: 1140–1162.

Griffin, A.S.; Pemberton J.M.; Brotherton, P.N.M., McIlrath, G.; Gaynor, D.; Kansky, R.; O'Riain, J; Clutton-Brock, T.H. 2003. A genetic analysis of breeding success in the cooperative meerkat (*Suricata suricatta*). *Behavioral Ecology*, 14(4): 472–480.

Groenendijk, J.; Hajek, F.; Duplaix, N. *et al*. 2005. Surveying and monitoring distribution and population trends of the giant otter (*Pteronura brasiliensis*). Guidelines for a standardisation of survey methods as recommended by the giant otter section of the IUCN/SSC Otter Specialist Network. *Habitat*, 16: 1–100.

Gusmão, J.; Solé-Cava, A.M. 2002. Um sistema de diagnóstico molecular para a identificação de espécies comerciais de camarões marinhos brasileiros. *Comunicaciones y Foros de Discusión n. Primer Congreso Iberoamericano Virtual de Acuicultura* (eds Blas I), pp 754–764. Zaragosa.

Hamilton, W.D. 1964. The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, 7: 1–16.

Hansen, H.; Ben-David, M.; McDonald, D.B. 2008. Effects of genotyping protocols on success and errors in identifying individual river otters (*Lontra canadensis*) from their faeces. *Molecular Ecology Resources*, 8(2): 282–289.

Harris, M.B.; Tomas, W.M.; Mourão, G.; Da Silva, C.J.; Guimarães, E.; Sonoda, F.; Fachim, E. 2005. Desafios para proteger o Pantanal brasileiro: ameaças e iniciativas em conservação. *Megadiversidade*, 1: 156-164.

Harris, M.A.; Tomas, W.; Mourão, G.; Da Silva, C.J.; Guimarães, E.; Sonoda, F.; Fachim, E. 2005. Safeguarding the Pantanal wetlands: threats and conservation initiatives. *Conservation Biology*, 19: 714–720.

Haynie, M.L.; Van Den Bussche, R.A.; Hoogland, J.L.; Gilbert, D.A. 2003. Parentage, multiple paternity, and breeding success in Gunnison's and Utah Prairie Dogs. *Journal of Mammalogy*, 84(4): 1244-1253.

Hughes, C. 1998. Integrating molecular techniques with field methods in studies of social behavior: a revolution results. *Ecology*, 79 (2): 383-399.

Iossa, G.; Soulsbury, C.D.; Baker, P.J.; Edwards, K.J.; Harris, S. 2009. Behavioral changes associated with a population density decline in the facultatively social red fox. *Behavioral Ecology*, 20: 385–395.

IUCN – International Union for Conservation of Nature and Natural Resources. 2011. IUCN Red List of Threatened Species. (www.iucnredlist.org). Acesso: 25/09/2011

Jones, A.G. 2005. GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. *Molecular Ecology*, 5: 708-711.

Junk, W.J.; Cunha, C.N.; Wantzen, K.M.; Petermann, P.; Strussmann, C.; Marques, M.; Adis, J. 2006. Biodiversity and its conservation in the Pantanal of Mato Grosso, Brazil. *Aquatic Sciences*, 68: 278-309.

Kalinowski, S.T.; Wagner, A.P.; Taper, M.L. 2006. ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, 6: 576–579.

Kalinowski, S.T.; Taper, M.L.; Marshall, T.C.; 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*,16(5): 1099-1006.

Karesh, W.D.V.M.; Smith, F.; Frazier-Taylor, H. 1987. A remote method for obtaining skin biopsy samples. *Conservation Biology*, 1(3): 261-262.

Keane, B.; Waser, P.M.; Creel, S.R.; Creel, N.M.; Elliott, L.F.; Minchella, D.J. 1994. Subordinate reproduction in dwarf mongooses. *Animal Behaviour*, 47: 65-75.

Keller, L.F.; Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, 17: 230-241.

Kruuk, H. 2006. *Otters: ecology, behaviour and conservation*. Oxford University Press Inc, New York. 263pp.

Laidler, L. 1984. *The behavioral ecology of the giant otter in Guyana*. Dissertação de Mestrado, Universidade de Cambridge, Cambridge.

Lanszki, J.; Hidas, A.; Szentes, K.; Révay, T.; Lehoczky, I.; Jeney, Zs.; Weiss, S. 2010. Genetic structure of otter (Lutra lutra) populations from two fishpond systems in Hungary. *Mammalian Biology*, 75: 447-450.

Larson, S.; Jameson, R.; Etnier, M.; Fleming, M.; Bentzen, P. 2002. Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. *Molecular Ecology*, 11: 1899-1903.

Latch, E.; Scognamillo, D.G.; Fike, J.A.; Chamberlain, M.J.; Rhodes, JR. O.E 2008. Deciphering ecological barriers to North American river otter (*Lontra canadensis*) gene flow in the Louisiana landscape. *Journal of Heredity*, 99(3): 265–274.

Lawson Handley, L. J.; Perrin, N. 2007. Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology*, 16: 1559–1578.

Leuchtenberger, C.; Mourão, G. 2008. Social organization and territoriality of giant otters (Carnivora:Mustelidae) in a seasonally flooded savanna in Brazil. *Sociobiology*. 52(2): 257-270.

Leuchtenberger, C.; Mourão, G. 2009. Scent-marking of giant otter in the Southern Pantanal, Brazil. *Ethology*, 115: 210-216.

Luikart, G.; Cornuet, J.M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology*, 12: 228–237.

Luikart, G.; Allendorf, F.W.; Cornuet, J.M.; Sherwin, W.B. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, 89: 238–247.

Macdonald, D.W. 1983. The ecology of carnivore social behaviour. *Nature*, 301: 379-384.

Martins, W.S.; Lucas, D.C.S.; Neves, K.F.S.; Bertioli, D.J. 2009. WebSat - A web software for microsatellite marker development. *Bioinformation* 3: 282-283.

Moueix, C. 2006. *Genetic verification of multiple paternity in two free-ranging isolated populations of African wild dogs (Lycaon pictus)*. Master of Science. University of Pretoria, Onderstepoort.

Mourão, G.; Carvalho, L. 2001. Cannibalism among Giant Otters (*Pteronura brasiliensis*). *Mammalia*, 65(2): 225-227.

Mourão, G.; Ribas, C.; Magnusson, W. 2006. Manejo de Fauna Silvestre no Brasil. *In*: Rocha, C.F.D.; Bergallo, H.G.; Sluys, M.V.; Alves, M.A.S. (Eds). *Biologia da Conservação-Essências*. RiMa, São Paulo. p. 459-477.

Mowry, R.A.; Gompper, M.E.; Beringer, J; Eggert, L. 2011. River Otter Population Size Estimation Using Noninvasive Latrine Surveys. *The Journal of Wildlife Management*, 75(7): 1625–1636.

Mucci, N.; Arrendal, J.; Ansorge, H.; *et al*. 2010. Genetic diversity and landscape genetic structure of otter (*Lutra lutra*) populations in Europe. *Conservation Genetics*, 11: 583–599.

Muwanika, V.B.; Siegismund, H.R.; Okello, J.B.A.; Masembe, C.; Arctander, P.; Nyakaana, S. 2003. A recent bottleneck in the warthog and elephant populations of Queen Elizabeth National Park, revealed by a comparative study of four mammalian species in Uganda national parks. *Animal Conservation*, 6(3): 237–245.

Otter Specialist Group (OSG-IUCN) 2010. *Pteronura brasiliensis* (Gmelin, 1788), the Giant Otter (www.otterspecialistgroup.org/Species/Pteronura_brasiliensis.html). Acesso: 13/03/2011.

Packer, C.; Gilbert, D.A.; Pusey, A.E.; O'Brien, S.J. 1991. A molecular genetic analysis of kinship and cooperation in African lions. *Nature*, 351: 562–565.

Pickles, R.S.A.; Groombridge, J.J.; Zambrana Rojas, V.D.; Jordan, W.C. 2009. Cross-species characterisation of polymorphic microsatellite loci in the giant otter (*Pteronura brasiliensis*). *Molecular Ecology Resources*, 9(1): 415–417.

Pickles, R.S.A.; Groombridge, J.J.; Zambrana Rojas, V.D.; Van Damme, P.; Gottelli, D.; Kundu, S.; Bodmer, R.; Ariani, C.V., Iyengar, A.; Jordan, W.C. 2011. Evolutionary history and identification of conservation units in the giant otter, *Pteronura brasiliensis*. *Molecular Phylogenetics and Evolution*, 61(3): 603-615.

Pickles, R.S.A.; Groombridge, J.J.; Zambrana Rojas, V.D.; Van Damme, P.; Gottelli, D.; Ariani, C.V.; Jordan, W.C. 2011b. Genetic diversity and population structure in the endangered giant otter, *Pteronura brasiliensis*. *Conservation Genetics*, DOI 10.1007/s10592-011-0279-9.

Piry, S.; Luikart, G.; Cornuet, J.M. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, 90: 502–503.

Pritchard, J.K.; Stephens, M.; Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155(2): 945-959.

Queller, D.C.; Strassmann, J.E.; Hughes, C.R. 1993. Microsatellites and kinship. *Trends in Ecology and Evolution*, 8: 285–288.

Randall, D.A.; Pollinger, J.P.; Wayne, R.K.; Tallents, L.A.; Johnson, P.J.; Macdonald, D.W. 2007. Inbreeding is reduced by female-biased dispersal and mating behavior in Ethiopian wolves. *Behavioral Ecology*, 18: 579–589.

Raymond, M.; Rousset, F. 1995. GENEPOP, Version 1.2. Population genetics software for exact tests and ecumenicisms. *Journal of Heredity* 86(3): 248-249.

R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, (http://www.R-project.org/). Acesso: 15/11/11.

Ribas, C.P. 2004. *Desenvolvimento de um programa de monitoramento em longo prazo das ariranhas (*Pteronura brasiliensis*) no Pantanal brasileiro*. Dissertação de Mestrado, Universidade Federal do Mato Grosso do Sul, Campo Grande, 68pp.

Ribas, C.; Mourão, G. 2004. Intraspecific agonism between giant otter groups. *IUCN Otter Specialist Group Bulletin*, 21(2): 89-93.

Ribas, C.; Vasconcellos, A.V.; Mourão, G.; Magnusson, W.; Sole-Cava, A.M.; Cunha, H.A. 2011. Polymorphic microsatellite loci from the endangered Giant Otter (*Pteronura brasiliensis*). *Conservation Genetic Resources*, 3(4): 769-771.

Ribas C, Damasceno G, Magnusson W, Leuchtenberger C, Mourão G. 2012. Giant otters feeding on caiman: evidence for an expanded trophic niche of recovering populations. Stud Neotrop Fauna E. 47(1): 19-23.

Rice, W.R. 1989. Analysing tables of statistic tests. *Evolution*, 43: 223-225.

Román, J. 2010. What do otters eat where there is no fish? *Mammalian Biology*, 76(2): 237-239.

Rosas F.C.W.; Zuanon, J.A.S.; Carter, S.K. 1999. Feeding ecology of the giant otter, *Pteronura brasiliensis*. *Biotropica*, 31(3): 502-506.

Rosas, F.C.W.; de Mattos, G.E. 2003. Notes on giant otter (*Pteronura brasiliensis*) behavior in the lake of Balbina Hydroelectric Power Station, Amazonas, Brazil. *The Latin American Journal of Aquatic Mammals*, 2: 127-129.

Rosas, F.C.W.; Waldemarin, H.; de Mattos G.E. 2008. Ariranha, *Pteronura brasiliensis* (Zimmermann, 1780). *In:* Machado, A.B.M.; Drummond, G.M.; Paglia, A.P. *Livro Vermelho da Fauna Brasileira Ameaçada de Extinção*. Ministério do Meio Ambiente e Fundação Biodiversitas, Belo Horizonte. p.800-801.

Rosas, F.; Cabral, M., De Mattos, G.; Silva, R. 2009. Parental and alloparental care of giant otters (*Pteronura brasiliensis*) (Carnivora, mustelidae) in Balbina hydroelectric lake, Amazonas, Brazil. *Sociobiology*, 54, 919-924.

Rosas-Ribeiro P.F. 2009. Conflitos entre pescadores e ariranhas (*Pteronura brasiliensis*) na Reserva de Desenvolvimento Sustentável Uacari, rio Juruá, Amazonas. Dissertação de Mestrado, Instituto Nacional de Pesquisas da Amazônia, Manaus.

Ross, K.G. 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Molecular Ecology*, 10: 265-284.

Sambrook, J.; Fritsch, E.F.; Maniatis, T. 1989 *Molecular Cloning a Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York

Schenck, C. 1999. *Lobo de Río (*Pteronura brasiliensis*). Presencia, uso de hábitat y protección en el Perú.* Agencia de Cooperación Técnica Alemana (GTZ), Sociedad Zoológica de Frankfurt, Lima, Peru.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, 18: 233-234

Schweizer, J. 1992. *Ariranhas no Pantanal: Ecologia e Comportamento da Pteronura brasiliensis*. Edibran-Editora Brasil Natureza Ltda, Curitiba. Brasil. 200 pp.

Silva, R.E. 2010. Ecologia alimentar da ariranha (*Pteronura brasiliensis*) e da lontra neotropical (*Lontra longicaudis*) no Parque Nacional do Jaú, Amazonas, Brasil. Dissertação de Mestrado, Instituto Nacional de Pesquisas da Amazônia, Manaus.

Silveira, L.; Furtado, M.M.; Rosas, F.C.W.; Silva, L.C.L.C.; Cabral, M.M.M.; Tôrres, R.S.; Kouba, A.; Jácomo, A.T.A. 2011. Tagging Giant Otters (*Pteronura brasiliensis*) (Carnivora, Mustelidae) for Radio-Telemetry Studies. *Aquatic Mammals*, 37(2): 208-212.

Spong, G.; Stone, J.; Creel, S.; Bjorklund, M. 2002. Genetic structure of lions (*Panthera leo* L.) in the Selous Game Reserve: implications for the evolution of sociality. *Journal of Evolutionary Biology*, 15: 945–953.

Stander, P.E. 1992. Cooperative hunting in lions: the role of the individual. *Behavioral Ecology and Sociobiology*, 21(6): 445-454.

Swatdipong, A.; Primmer, C.R.; Vasemägi, A. 2010. Historical and recent genetic bottlenecks in European grayling, Thymallus thymallus. *Conservation Genetics*, 11: 279-292.

Taberlet, P.; Camarra, J.J.; Griffin, S.; Uhres, E.; Hanotte, O.; Waits, L.P.; Dubois-Paganon, C.; Burke, T.; Bouvet, J. 1997. Noninvasive genetic tracking of the endangered Pyrenean brow bear population. *Molecular Ecology*, 6(9): 869-876.

Tallmon, D.A.; Koyuk, A.; Luikart, G.; Beaumont, M.A. 2008. ONeSAMP: a program to estimate effective population size using approximate Bayesian computation. *Molecular Ecology Resource*, 8: 299–301.

Tomas, W.M.; Lima Borges, P.A.; Rocha, H.J.F.; Sa Filho, R.; Kutchenski Jnr, F.; Villafaine Udry, T. 2000. *Potencial dos rios Aquidauana e Miranda, no Pantanal de Mato Grosso do Sul, para a conservação da ariranha (Pteronura brasiliensis)*. Anais do III Simpósio sobre recursos naturais e sócio-econômicos do Pantanal. Empresa Brasileira de Pesquisa Agropecuaria (Embrapa)- Pantanal, Corumbá, Brasil.

Uscamaita, M.R.; Bodmer. R. 2009. Recovery of the endangered giant otter *Pteronura brasiliensis* on the Yavarí-Mirin and Yavarí Rivers: a success story for CITES. *Oryx*. 44(1): 83-88.

Van Damme, P.; Wallace, R.; Swaenepoel, K.; Painter, L.; Ten, S.; Taber, A.; Gonzalez, J.; Saravia, I.; Fraser, A.; Vargas, J. 2002. Distribution and population status of the Giant

Otter *Pteronura brasiliensis* in Bolivia. *IUCN Otter Specialist Group Bulletin*, 19(2): 87-96.

Van Horn, R.C.; Engh, A.L.; Scribner, K.T.; Funk, S.M.; Holekamp, K.E. 2004. Behavioural structuring of relatedness in the spotted hyena (*Crocuta crocuta*) suggests direct fitness benefits of clan-level cooperation. *Molecular Ecology*, 13: 449–458.

Van Oosterhout, C.; Hutchinson, W.F.; Wills, D.P.M.; Shipley, P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4: 535-538.

Wagner, A.P.; Creel, S.; Frank, L.G.; Kalinowski, S.T. 2007. Patterns of relatedness and parentage in an asocial, polyandrous striped hyena population. *Molecular Ecology*, 16: 4356–4369.

Waits, L.P.; Paetkau, D. 2005. Noninvasive genetic sampling tools for wildlife biologists: A review of applications and recommendations for accurate data collection. *Journal of Wildlife Management*, 69(4): 1419-1433.

West, S.A.; Pen, I.; Griffin, A.S. 2002. Cooperation and competition between relatives. *Science*, 296: 72-75.

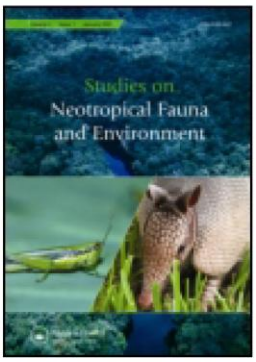
West, S.A.; Griffin, A.S.; Garder, A. 2006. Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *Journal of Evolutionary Biology*, 20: 415-432.

Wilson, E. O. 1980. *Sociobiology*. The Abridged Edition. The Belknap Press of Harvard University Press. Cambridge, Massachusetts and London, England. 697pp.

Worthington Wilmer, J.; Allen, P.J.; Pomeroy, P.P.; Twiss, S.D.; Amos, W. 1999. Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (Halichoerus grypus). Molecular Ecology, 8: 1417- 1429.

Apêndices

Giant otters feeding on caiman: evidence for an expanded thophic niche of recovering populations



Studies on Neotropical Fauna and Environment
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/nnfe20

Giant otters feeding on caiman: evidence for an expanded trophic niche of recovering populations

Carolina Ribas [a b] , Gabriel Damasceno [c] , William Magnusson [a] , Caroline Leuchtenberger [a b] & Guilherme Mourão [b]

[a] Instituto Nacional de Pesquisas da Amazônia - INPA Manaus, AM, Brazil

[b] Embrapa Pantanal, Brazilian Agricultural Research Corporation, Corumbá, MS, Brazil

[c] CCG Productions, Rio de Janeiro, RJ, Brazil

Available online: 13 Mar 2012

To cite this article: Carolina Ribas, Gabriel Damasceno, William Magnusson, Caroline Leuchtenberger & Guilherme Mourão (2012): Giant otters feeding on caiman: evidence for an expanded trophic niche of recovering populations, Studies on Neotropical Fauna and Environment, 47:1, 19-23

To link to this article: http://dx.doi.org/10.1080/01650521.2012.662795

Studies on Neotropical Fauna and Environment
Vol. 47, No. 1, April 2012, 19–23

SHORT COMMUNICATION

Giant otters feeding on caiman: evidence for an expanded trophic niche of recovering populations

Carolina Ribas[a,b]*, Gabriel Damasceno[c], William Magnusson[a], Caroline Leuchtenberger[a,b] & Guilherme Mourão[b]

[a]*Instituto Nacional de Pesquisas da Amazônia – INPA Manaus, AM, Brazil;* [b]*Embrapa Pantanal, Brazilian Agricultural Research Corporation, Corumbá, MS, Brazil;* [c]*CCG Productions, Rio de Janeiro, RJ, Brazil*

(Received 2 November 2011; accepted 30 January 2012)

As water along dirt roads in the Pantanal floodplains diminishes, aquatic fauna becomes restricted to shallow pools. At the end of the 2009 dry season, we filmed giant otters living in pools predating on yacare caimans. Such predation has not been recorded in giant otters inhabiting the Pantanal. Individual otters captured sub-adult caimans. The otters did not share the prey, but conspecifics stole it after conflicts. Caiman predation could be related to resource scarcity in these marginal environments. Information on diet and interactions of endangered populations may underestimate their trophic niche when they recover from over-hunting and expand into sub-optimal habitat.

À medida que as águas ao longo das estradas de terra na planície pantaneira diminuem, a fauna aquática se torna restrita às poças rasas do entorno. No final da estação seca de 2009, nós filmamos ariranhas predando jacarés em baías temporárias. Este tipo de predação não foi descrito antes para ariranhas do Pantanal. As ariranhas capturaram jacarés sub-adultos, individualmente. A presa não foi dividida voluntariamente, mas foi roubada por outros indivíduos do grupo, após conflitos. Esta predação pode estar relacionada com a escassez de alimento nestes ambientes marginais. Informações de dieta e interações em populações ameaçadas talvez subestimem seu nicho trófico quando estas se recuperam, após período sem caça, e expandem para ambientes sub-ótimos.

Keywords: Ariranha; *Pteronura brasiliensis*; predation; *Caiman crocodilus yacare*; Pantanal floodplains; Brazil

Introduction

Giant otters (*Pteronura brasiliensis*) are large social semi-aquatic mustelids endemic to South America. Predominantly piscivores throughout the geographical distribution of the species, their preferred fish are from the orders Characiformes, Perciformes and Siluriformes (Duplaix 1980; Laidler 1984; Rosas et al. 1999; Rosas-Ribeiro 2009). Giant otters take quite large fish (> 30 cm), especially slow-moving species, according to their availability and ease of capture (Duplaix 1980; Schweizer 1992; Kruuk 2006). While some reports indicate that they consume varied foods, such as crustaceans, mollusks, reptiles, amphibians, water birds, and mammals, these represent less than 1% of their diet (Duplaix 1980; Schweizer 1992; Rosas et al. 1999; Rosas-Ribeiro 2009; Cabral et al. 2010; Silva 2010).

Giant otters suffered a drastic reduction in distribution as a consequence of excessive hunting to supply the fur trade during the 1950s and 1960s (Carter & Rosas 1997) and habitat destruction (Schenck 1999). The decrease in demand on the international market, as well the establishment and enforcement of protective laws, allowed recuperation of some giant-otter populations, noticeably in Colombia (Díaz & Sánchez 2002), Peru (Uscamaita & Bodmer 2009), Bolivia (Van Damme et al. 2002), and Brazil (Schweizer 1992; Ribas 2004; Leuchtenberger & Mourão 2008; Rosas et al. 2008). In Brazil, well established giant-otter populations are limited to the Amazonian and Pantanal basins, and in the latter, groups are widely distributed along the rivers and creeks of the floodplain (Schweizer 1992; Carter & Rosas 1997; Ribas 2004; Tomas et al. 2011).

We present the first record of giant-otter predation on caimans in the Pantanal, which is likely to be due to food limitation of peripheral populations of giant otters that have been excluded from larger rivers by other groups. We also describe behaviors which indicate competitive interactions among group members, rather than cooperative hunting of a preferred prey.

Materials and methods

Study area

Pantanal, the largest South American wetland, covers 160,000 km^2 of lowland terrain extending from Bolivia to Paraguay and Brazil. The Pantanal is

*Corresponding author. Email: ariranhapantanal@yahoo.com.br

ISSN 0165-0521 print/ISSN 1744-5140 online

http://dx.doi.org/10.1080/01650521.2012.662795
http://www.tandfonline.com

recognized by its flatness and low altitude, and suffers alternating periods of flood and drought with strong seasonality (Alho et al. 1988; Harris et al. 2005). The Estrada-Parque Pantanal (EPP) highway is a 120 km dirt road constructed on a causeway 1–2 m above the surrounding plains across a section of the southern Pantanal. It crosses a diverse range of water bodies and marshlands, including streams, lakes, and ponds along the roadside, and trenches originating from the construction of the road (borrow pits). In the dry season (June to December), the availability of water gradually diminishes, and much of the aquatic fauna is restricted to shallow pools in sub-optimal habitat until the next rainy season.

Monitoring of giant otter behavior and vocalization

We started monitoring giant otters in the southern Pantanal (Mato Grosso do Sul, Brazil) in 2002, and we became aware of marginal populations of giant otters in pools and water bodies around the EPP highway (UTM 21k 0451800 7873700 to 21k 0496300 7831500) in 2008. We do not have records of giant otters occupying borrow pits along roads during the dry season before then. We recorded behavior of giant otters with a digital camera (Sony model DCR-TRV340), and used the sound-tracks of the records to extract the sounds produced by the animals in digital format. Spectrogram and analysis of acoustic variables (frequency of highest energy [FM], and time duration of sound [DT]) were performed using Bioacustic Raven 1.2 (Cornell Lab of Ornithology, Ithaca, NY, USA), and the vocalizations were classified according to Duplaix (1980).

Results

We accompanied a group of giant otters from October to December 2009. In the first month, the five adults that composed the group changed their territory and traveled about 6 km through savannas, drying lakes and creeks. In early November, the group won a fight against another large group and conquered a lake of about 0.2 km^2, incorporating a male and a female of the eight individuals from the other group. During October and November, they preyed almost only on small loricariid catfish (< 20 cm), characteristic of benthic environments. By the end of November, most fish were scarce and the otters dug deep into the mud to catch marbled swamp eels (*Synbranchus marmoratus*, Synbranchidae). In December, at the end of the dry season, we twice registered the group feeding on large sub-adult caimans (*Caiman crocodilus yacare*, Alligatoridae) estimated to be 1.2 and 1.4 m total length, with an estimated mass of 6 kg and 9 kg. The caimans were captured by individual giant otters, although more than one giant otter was present during the captures. On each occasion, the giant otters began feeding on the back of the caiman's neck (Figure 1). Capture and consumption of the first caiman lasted 53 minutes. The giant otters did not voluntarily share the prey with other members of the group. However, in the first event three individuals fed on the same caiman, which was successively stolen after vocal and/or physical conflicts. The subordinate that hunted and first fed on the caiman, vocalized a growl sound ($n = 19$) when other adults approached him and his prey. The growls had an average FM of 465.6 Hz ($\pm$ 267.7) and an average DT of 2.25s ($\pm$ 1.33) (Figure 2a). During the last 6 minutes of feeding, the dominant male approached the second subordinate feeding on the caiman and produced long wavering screams ($n = 37$; FM = 2411.9 kHz $\pm$ 492.39; DT = 0.96 s $\pm$ 1.23), apparently soliciting the prey. The average DT of these screams was 1.49 s ($\pm$ 1.39), at times interspersed with the HAH call (Duplaix 1980) of a higher frequency ($n = 23$; FM = 3067.29 kHz $\pm$ 520.94; DT = 0.54 s $\pm$ 0.79) (Figure 2b). Vocalization lasted until the theft of the prey.

When we made these observations, the individuals in the group were visibly debilitated, thin, and with the mucous membranes of their anus, mouth and eyes apparently inflamed. One of the lower canines of the dominant male was missing and his carnassials were visibly worn, probably due to the friction with the sand and mud. Despite the fact that they could catch caimans, which were common in the area, we noted that the overall health of the group deteriorated as the dry spell intensified. The otters had taken caimans before. Analysis of feces from the group's latrine collected on November 19 revealed the presence of osteoderms and other remains of caimans, and local residents reported that giant otters had been seen feeding on caiman along the highway since November.

Discussion

Otters, in general, are highly specialized carnivores with a diet dominated by fish (Kruuk 2006). Nevertheless, there is evidence that high levels of water instability lead to decreased fish consumption by Eurasian otters (Clavero et al. 2008; Román 2010). In eight years and 3000 h of giant-otter observation on rivers with some hydrological predictability, we have seen consumption of prey other than fish only three times, and those prey were crabs (Trichodactylidae).

Even in optimum conditions, caimans compete with giant otters for fish and can be potential predators of their cubs (Schweizer 1992). During the

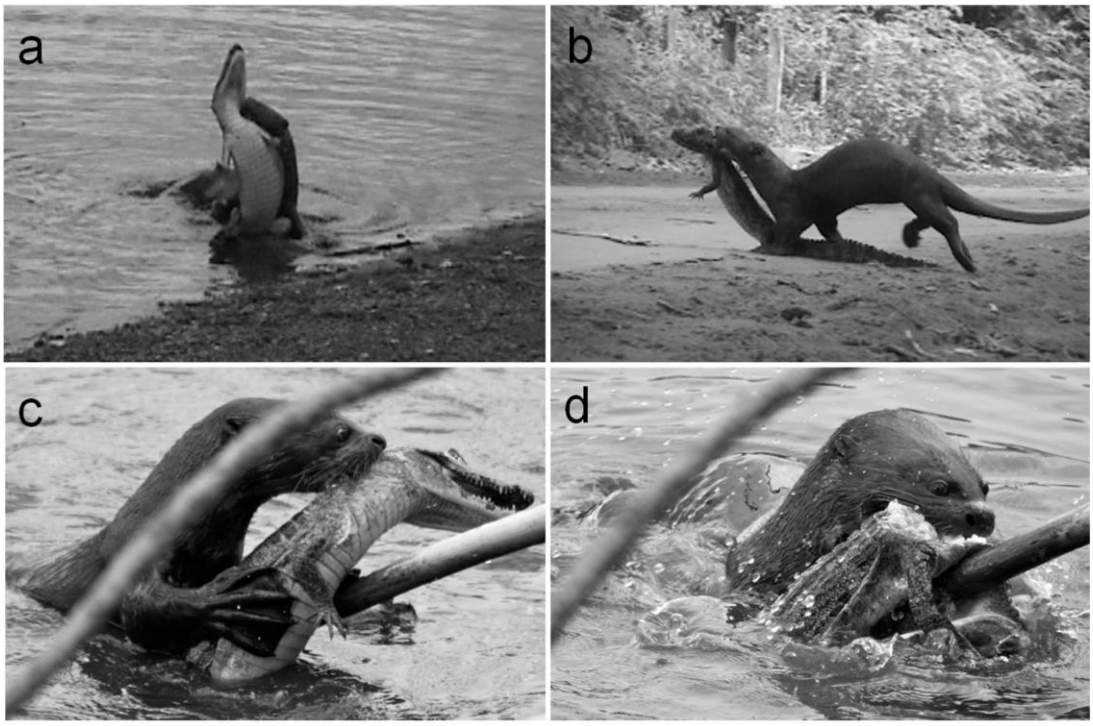


Figure 1. A subordinate giant otter (a) brings the still alive caiman to the shore, (b) crosses the highway, (c) begins feeding on the back of the caiman's neck and (d) eats the caiman.

last two years we registered 12 agonistic encounters between yacare caiman and giant otters in the southern Pantanal. Giant otters generally surround and fence off caimans that get too close to their den entrances and campsites. In six instances, only one giant otter was the aggressor, despite the presence of other members of group. Only once a caiman initiated the aggression, attacking a giant otter about to leave water to go to a latrine. The otters never killed or ate caimans in these cases.

Brecht-Munn and Munn (1988) reported that giant otters hunting cooperatively can kill large prey including anacondas (*Eunectes* sp.) and juvenile black caimans (*Melanosuchus niger*). This did not occur in our observations; each group member hunted its own prey and did not share the prey voluntarily, as do other social carnivores that have cooperative hunting, such as lions and wild dogs (Stander 1992; Creel & Creel 1995).

Because of their high metabolic rate, giant otters need to eat up to 3 kg of fish per day to maintain their activities (Duplaix 1980; Kruuk 2006). By the end of the dry season, the quantity of fish in the borrow pits along roads is drastically reduced, mainly from the high rate of predation by piscivorous animals, such as birds and caimans. At the same time, the densities of caimans increases as they congregate in diminishing water pools in the flood plains.

Our data suggest that predation on caimans by giant otters along the Estrada Parque Pantanal highway was due to the drought and resource scarcity in these marginal environments. That some groups are occupying sub-optimal habitats, and feeding on prey that normally is not taken, is probably related to the increase in otter density in the Pantanal and suggests that giant otter populations might be reaching their carrying capacity in the southern Pantanal. As otter density increases, and more groups are pushed into marginal habitat, it is likely that their interactions with other species will increase in ways not predictable from data collected from low-density populations in primary habitat.

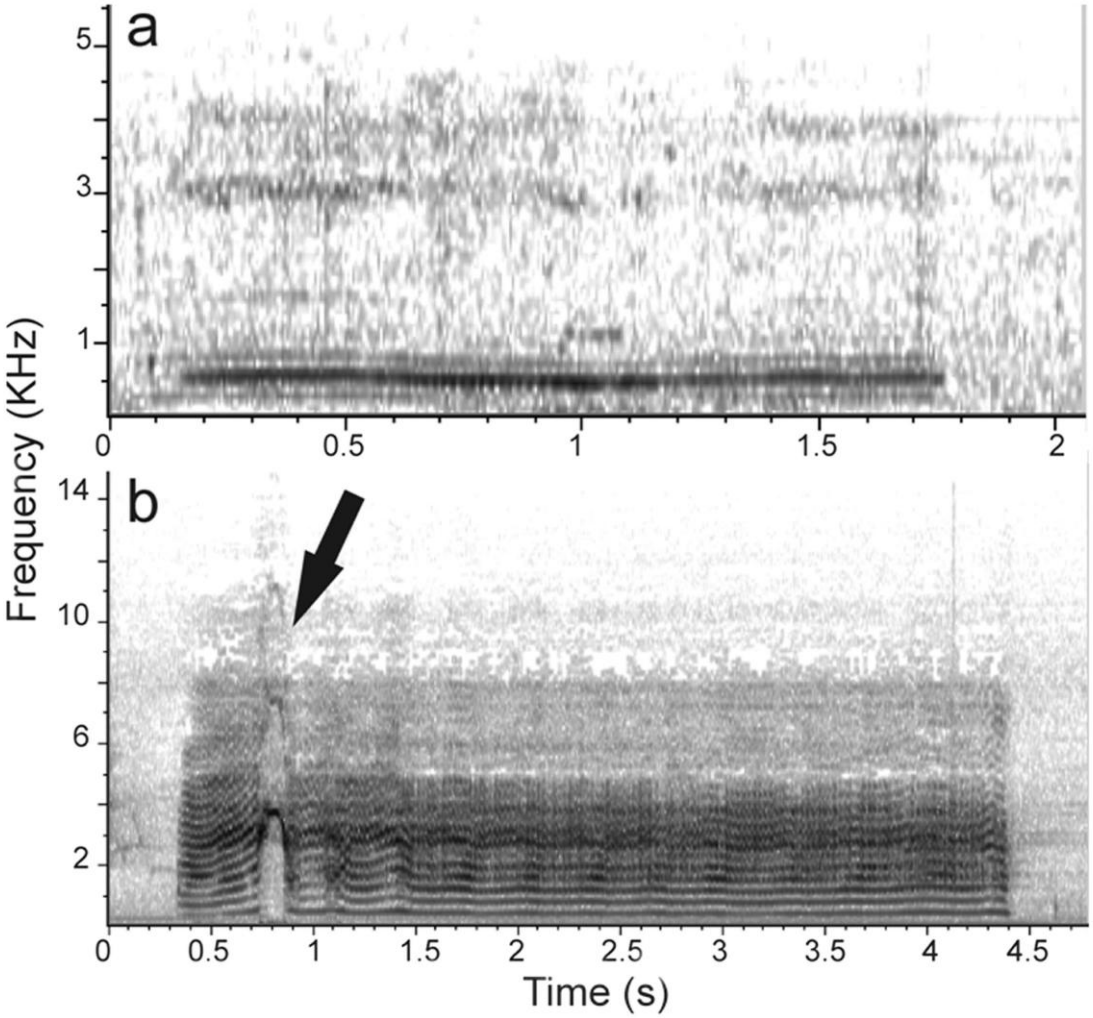


Figure 2. Spectrograms of giant otter sounds vocalized during caiman predation; (a) growl, (b) wavering scream interspersed with the HAH call (arrow).

Acknowledgments

We thank Federal University of Mato Grosso do Sul to provide logistical support. This study formed part of the INPA's Ph.D. dissertation, which received funding from the CNPq (scholarship and grant # 476939/2008-9). We thank Isac Teixeira de Carvalho for assistance in the field, and Dr. Leslie Damasceno for revision of the English expression of the manuscript. We are especially grateful to Patrícia Medici for inspiring our research in the Estrada Parque Pantanal. We also acknowledge the two reviewers who presented relevant comments on the manuscript.

References

Alho CJR, Lacher TEJr, Gonçalves HC. 1988. Environmental degradation in the Pantanal ecosystem of Brazil. BioScience. 38:164–171.

Brecht-Munn M, Munn CA. 1988. The Amazon's gregarious giant otters. Animal Kingdom. 91:34–41.

Cabral MMM, Zuanon J, de Mattos GE, Rosas FCW. 2010. Feeding habits of giant otters *Pteronura brasiliensis* (Carnivora: Mustelidae) in the Balbina hydroelectric reservoir, Central Brazilian Amazon. Zoologia. 27(1):47–53.

Carter SK, Rosas FCW. 1997. Biology and conservation of the giant otter *Pteronura brasiliensis*. Mammal Review. 27(1):1–26.

Clavero M, Prenda J, Blanco-Garrido F, Delibes M. 2008. Hydrological stability and otter trophic diversity: a scale-insensitive pattern? Can J Zool. 86(10):1152–1158.

Creel SR, Creel NM. 1995. Communal hunting and pack size in Africa wild dogs, *Lycaon pictus*. Anim Behav. 50:1325–1339.

Díaz HJ, Sánchez IM. 2002. Historical and actual presence of the Giant Otter (*Pteronura brasiliensis*) on the Lower Meta River, Department of Casanare – Colombia Orinoquia. IUCN Otter Specialist Group Bulletin. 19(2):97–102.

Duplaix N. 1980. Observation on the ecology and behavior of the giant otter *Pteronura brasiliensis* in Suriname. Rev Ecol (Terre Vie). 34:495–620.

Harris MB, Tomas WM, Mourão G, Da Silva CJ, Guimarães E, Sonoda F, Fachim E. 2005. Desafios para proteger o Pantanal brasileiro: ameaças e iniciativas em conservação. Megadiversidade. 1:156–164.

Kruuk H. 2006. Otters: ecology, behaviour and conservation. New York: Oxford University Press.

Laidler L. 1984. The behavioral ecology of the giant otter in Guyana. [PhD dissertation] [Cambridge; UK]: University of Cambridge.

Leuchtenberger C, Mourão G. 2008. Social organization and territoriality of giant otters (Carnivora: Mustelidae) in a seasonally flooded savanna in Brazil. Sociobiology. 52(2):257–270.

Ribas CP. 2004. Desenvolvimento de um programa de monitoramento em longo prazo das ariranhas (*Pteronura brasiliensis*) no Pantanal brasileiro. [master's thesis] [Campo Grande]: Universidade Federal do Mato Grosso do Sul.

Román J. 2010. What do otters eat where there is no fish? Mamm Biol. 76(2):237–239.

Rosas FCW, Waldemarin H, de Mattos GE. 2008. Ariranha, *Pteronura brasiliensis* (Zimmermann, 1780). In: Machado ABM, Drummond GM, Paglia AP, editors. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. Belo Horizonte: Fundação Biodiversitas. p. 800–801.

Rosas FCW, Zuanon JAS, Carter SK. 1999. Feeding ecology of the giant otter, *Pteronura brasiliensis*. Biotropica. 31(3):502–506.

Rosas-Ribeiro PF. 2009. Conflitos entre pescadores e ariranhas (*Pteronura brasiliensis*) na Reserva de Desenvolvimento Sustentável Uacari, rio Juruá, Amazonas. [master's thesis] [Manaus]: Instituto Nacional de Pesquisas da Amazônia.

Schenck C. 1999. Lobo de Río (*Pteronura brasiliensis*). Presencia, uso de hábitat y protección en el Perú. Agencia de Cooperación Técnica Alemana (GTZ), Sociedad Zoológica de Frankfurt, Lima, Peru.

Schweizer J. 1992. Ariranhas no Pantanal: Ecologia e Comportamento da *Pteronura brasiliensis*. Curitiba: Edibran-Editora Brasil Natureza Ltda.

Silva RE. 2010. Ecologia alimentar da ariranha (*Pteronura brasiliensis*) e da lontra neotropical (*Lontra longicaudis*) no Parque Nacional do Jaú, Amazonas, Brasil. [master's thesis] [Manaus]: Instituto Nacional de Pesquisas da Amazônia.

Stander PE. 1992. Cooperative hunting in lions: the role of the individual. Behav Ecol Sociobiol. 21(6):445–454.

Tomas WM, Caceres NC, Nunes AP, Fischer EA, Mourão G, Campos Z. 2011. Mammals in the Pantanal wetland, Brazil. In: Junk W, Silva CJ, Cunha CN, Wantzen KM, editors. The Pantanal: Ecology, biodiversity and sustainable management of a large neotropical seasonal wetland. Sofia: Pensoft Publishers. p. 563–595.

Uscamaita MR, Bodmer R. 2009. Recovery of the endangered giant otter *Pteronura brasiliensis* on the Yavarí-Mirin and Yavarí Rivers: a success story for CITES. Oryx. 44(1):83–88.

Van Damme P, Wallace R, Swaenepoel K, Painter L, Ten S, Taber A, Gonzalez J, Saravia I, Fraser A, Vargas J. 2002. Distribution and population status of the Giant Otter *Pteronura brasiliensis* in Bolivia. IUCN Otter Specialist Group Bulletin. 19(2):87–96.

Instituto Nacional de Pesquisas da Amazônia - INPA
Programa de Pós-graduação em Ecologia

Avaliação de tese de doutorado

Título: **Grau de parentesco e relações sociais em ariranha (*Pteronura brasiliensis*)**

Aluno: **CAROLINA RIBAS**

Orientador: **William E. Magnusson** Co-orientador: **Guilherme Mourão**

Avaliador: **Eduardo Eizirik**

Por favor, marque a alternativa que considerar mais apropriada para cada ítem abaixo, e marque seu parecer final no quadro abaixo

	Muito bom	Bom	Necessita revisão	Reprovado
Relevância do estudo	(X)	( )	( )	( )
Revisão bibliográfica	(X)	( )	( )	( )
Desenho amostral/experimental	(X)	( )	( )	( )
Metodologia	(X)	( )	( )	( )
Resultados	(X)	( )	( )	( )
Discussão e conclusões	(X)	( )	( )	( )
Formatação e estilo texto	(X)	( )	( )	( )
Potencial para publicação em periódico(s) indexado(s)	(X)	( )	( )	( )

PARECER FINAL

(X) **Aprovada** (indica que o avaliador aprova o trabalho sem correções ou com correções mínimas)

( ) **Aprovada com correções** (indica que o avaliador aprova o trabalho com correções extensas, mas que não precisa retornar ao avaliador para reavaliação)

( ) **Necessita revisão** (indica que há necessidade de reformulação do trabalho e que o avaliador quer reavaliar a nova versão antes de emitir uma decisão final)

( ) **Reprovada** (indica que o trabalho não é adequado, nem com modificações substanciais)

__Porto Alegre_____, __22.03.2012_____, _____
Local Data Assinatura

Comentários e sugestões podem ser enviados como uma continuação desta ficha, como arquivo separado ou como anotações no texto impresso ou digital da tese. Por favor, envie a ficha assinada, bem como a cópia anotada da tese e/ou arquivo de comentários por e-mail para pgecologia@gmail.com e claudiakeller23@gmail.com ou por correio ao endereço abaixo. O envio por e-mail é preferível ao envio por correio. Uma cópia digital de sua assinatura será válida.

Endereço para envio de correspondência:

Claudia Keller
DCEC/CPEC/INPA
CP 478
69011-970 Manaus AM
Brazil

PG·ECO·INPA
PÓS-GRADUAÇÃO EM ECOLOGIA

Instituto Nacional de Pesquisas da Amazônia - INPA
Programa de Pós-graduação em Ecologia

INPA
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

Avaliação de tese de doutorado

Título: **Grau de parentesco e relações sociais em ariranha (*Pteronura brasiliensis*)**

Aluno: **CAROLINA RIBAS**

Orientador: **William E. Magnusson** Co-orientador: **Guilherme Mourão**

Avaliador: **Fernando C. C. Azevedo (UFSJ)**

Por favor, marque a alternativa que considerar mais apropriada para cada ítem abaixo, e marque seu parecer final no quadro abaixo

	Muito bom	Bom	Necessita revisão	Reprovado
Relevância do estudo	(X)	( )	( )	( )
Revisão bibliográfica	( )	(X)	( )	( )
Desenho amostral/experimental	( )	(X)	( )	( )
Metodologia	(X)	( )	( )	( )
Resultados	(X)	( )	( )	( )
Discussão e conclusões	( )	(X)	( )	( )
Formatação e estilo texto	(X)	( )	( )	( )
Potencial para publicação em periódico(s) indexado(s)	(X)	( )	( )	( )

PARECER FINAL

(X) **Aprovada** (indica que o avaliador aprova o trabalho sem correções ou com correções mínimas)

( ) **Aprovada com correções** (indica que o avaliador aprova o trabalho com correções extensas, mas que não precisa retornar ao avaliador para reavaliação)

( ) **Necessita revisão** (indica que há necessidade de reformulação do trabalho e que o avaliador quer reavaliar a nova versão antes de emitir uma decisão final)

( ) **Reprovada** (indica que o trabalho não é adequado, nem com modificações substanciais)

São João del Rei, 23/02/2012, _____

Local **Data** **Assinatura**

Comentários e sugestões podem ser enviados como uma continuação desta ficha, como arquivo separado ou como anotações no texto impresso ou digital da tese. Por favor, envie a ficha assinada, bem como a cópia anotada da tese e/ou arquivo de comentários por e-mail para pgecologia@gmail.com e claudiakeller23@gmail.com ou por correio ao endereço abaixo. O envio por e-mail é preferível ao envio por correio. Uma cópia digital de sua assinatura será válida.

Endereço para envio de correspondência:

Claudia Keller
DCEC/CPEC/INPA
CP 478
69011-970 Manaus AM
Brazil

Instituto Nacional de Pesquisas da Amazônia - INPA
Programa de Pós-graduação em Ecologia

Avaliação de tese de doutorado

Título: **Grau de parentesco e relações sociais em ariranha (*Pteronura brasiliensis*)**

Aluno: **CAROLINA RIBAS**

Orientador: **William E. Magnusson** Co-orientador: **Guilherme Mourão**

Avaliador:

Por favor, marque a alternativa que considerar mais apropriada para cada ítem abaixo, e marque seu parecer final no quadro abaixo

	Muito bom	Bom	Necessita revisão	Reprovado
Relevância do estudo	(X)	( )	( )	( )
Revisão bibliográfica	(X)	( )	( )	( )
Desenho amostral/experimental	(X)	( )	( )	( )
Metodologia	( )	(X)	( )	( )
Resultados	(X)	( )	( )	( )
Discussão e conclusões	( )	(X)	( )	( )
Formatação e estilo texto	( )	(X)	( )	( )
Potencial para publicação em periódico(s) indexado(s)	(X)	( )	( )	( )

PARECER FINAL

(X) **Aprovada** (indica que o avaliador aprova o trabalho sem correções ou com correções mínimas)

( ) **Aprovada com correções** (indica que o avaliador aprova o trabalho com correções extensas, mas que não precisa retornar ao avaliador para reavaliação)

( ) **Necessita revisão** (indica que há necessidade de reformulação do trabalho e que o avaliador quer reavaliar a nova versão antes de emitir uma decisão final)

( ) **Reprovada** (indica que o trabalho não é adequado, nem com modificações substanciais)

Obs: A avaliação segue no corpo da tese.

Izeni Pires Farias
Profa Dra. Izeni Pires Farias
Lab de Evolução e Genética Animal LEGAL
Departamento de Biologia ICB
Universidade Federal do Amazonas - UFAM

Manaus, Local **Manaus, 15/03/2012, Data** **Izeni Pires Farias Assinatura**

Comentários e sugestões podem ser enviados como uma continuação desta ficha, como arquivo separado ou como anotações no texto impresso ou digital da tese. Por favor, envie a ficha assinada, bem como a cópia anotada da tese e/ou arquivo de comentários por e-mail para pgecologia@gmail.com e claudiakeller23@gmail.com ou por correio ao endereço abaixo. O envio por e-mail é preferível ao envio por correio. Uma cópia digital de sua assinatura será válida.

Endereço para envio de correspondência:

Claudia Keller
DCEC/CPEC/INPA
CP 478
69011-970 Manaus AM
Brazil

Instituto Nacional de Pesquisas da Amazônia - INPA
Graduate Program in Ecology

Referee evaluation sheet for PhD thesis

Title: **Relatedness and social relation of giant otters (*Pteronura brasiliensis*)**

Candidate: **CAROLINA RIBAS**

Supervisor: **William E. Magnusson** Co-supervisor: **Guilherme Mourão**

Examiner: **Matthew Gompper**

Please check one alternative for each of the following evaluation items, and check one alternative in the box below as your final evaluation decision.

	Excellent	Good	Satisfactory	Needs improvement	Not acceptable
Relevance of the study	(x)	( )	( )	( )	( )
Literature review	(x)	( )	( )	( )	( )
Sampling design	(x)	( )	( )	( )	( )
Methods/procedures	(x)	( )	( )	( )	( )
Results	(x)	( )	( )	( )	( )
Discussion/conclusions	(x)	( )	( )	( )	( )
Writing style and composition	( )	(x)	( )	( )	( )
Potential for publication in peer reviewed journal(s)	(x)	( )	( )	( )	( )

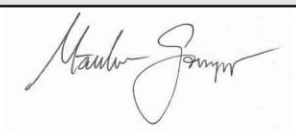
FINAL EVALUATION

( ) **Approved without or minimal changes**

(x) **Approved with changes** (no need for re-evaluation by this reviewer)

( ) **Potentially acceptable, conditional upon review of a corrected version** (The candidate must submit a new version of the thesis, taking into account the corrections asked for by the reviewer. This new version will be sent to the reviewer for a new evaluation only as acceptable or not acceptable)

( ) **Not acceptable** (This product is incompatible with the minimum requirements for this academic level)



Columbia, Missouri, USA **22 February 2012**

Place **Date** **Signature**

Additional comments and suggestions can be sent as an appendix to this sheet, as a separate file, and/or as comments added to the text of the thesis. Please, send the signed evaluation sheet, as well as the annotated thesis and/or separate comments by e-mail to pgecologia@gmail.com and claudiakeller23@gmail.com or by mail to the address below. E-mail is preferred. A scanned copy of your signature is acceptable.

Mailing address:

Claudia Keller
DCEC/CPEC/INPA
CP 478
69011-970 Manaus AM
Brazil

Instituto Nacional de Pesquisas da Amazônia - INPA
Programa de Pós-graduação em Ecologia

Avaliação de tese de doutorado

Título: **Grau de parentesco e relações sociais em ariranha (*Pteronura brasiliensis*)**

Aluno: **CAROLINA RIBAS**

Orientador: **William E. Magnusson** Co-orientador: **Guilherme Mourão**

Avaliador: **Thomas E. Lacher, jr.**

Por favor, marque a alternativa que considerar mais apropriada para cada ítem abaixo, e marque seu parecer final no quadro abaixo

	Muito bom	Bom	Necessita revisão	Reprovado
Relevância do estudo	(X)	( )	( )	( )
Revisão bibliográfica	(X)	( )	( )	( )
Desenho amostral/experimental	( )	(X)	( )	( )
Metodologia	( )	(X)	( )	( )
Resultados	( )	(X)	( )	( )
Discussão e conclusões	(X)	( )	( )	( )
Formatação e estilo texto	( )	(X)	( )	( )
Potencial para publicação em periódico(s) indexado(s)	( )	( )	(X)	( )

PARECER FINAL

( ) **Aprovada** (indica que o avaliador aprova o trabalho sem correções ou com correções mínimas)

(X) **Aprovada com correções** (indica que o avaliador aprova o trabalho com correções extensas, mas que não precisa retornar ao avaliador para reavaliação)

( ) **Necessita revisão** (indica que há necessidade de reformulação do trabalho e que o avaliador quer reavaliar a nova versão antes de emitir uma decisão final)

( ) **Reprovada** (indica que o trabalho não é adequado, nem com modificações substanciais)

__College Station, TX, _1 março de 2012___, _____
Local Data Assinatura

Comentários e sugestões podem ser enviados como uma continuação desta ficha, como arquivo separado ou como anotações no texto impresso ou digital da tese. Por favor, envie a ficha assinada, bem como a cópia anotada da tese e/ou arquivo de comentários por e-mail para pgecologia@gmail.com e claudiakeller23@gmail.com ou por correio ao endereço abaixo. O envio por e-mail é preferível ao envio por correio. Uma cópia digital de sua assinatura será válida.

Endereço para envio de correspondência:

Claudia Keller
DCEC/CPEC/INPA
CP 478
69011-970 Manaus AM
Brazil

INPA | DIVISÃO DOS CURSOS DE PÓS-GRADUAÇÃO | PIPG BTRN | Ministério da Ciência e Tecnologia | BRASIL

AULA DE QUALIFICAÇÃO
PARECER

Aluno(a): CAROLINA RIBAS PEREIRA
Curso: ECOLOGIA
Nível: DOUTORADO
Orientador(a): WILLIAM ERNEST MAGNUSSON

Título:

GRAU DE PARENTESCO E RELAÇÕES SOCIAIS EM GRUPOS DE ARIRANHAS (PTERONURA BRASILIENSIS) NO PANTANAL BRASILEIRO

BANCA JULGADORA:

TITULARES:
Jansen Zuanon (INPA)
Tânia Sanaiotti (INPA)
José Alves Gomes (INPA)
Marcelo Menin (UFAM)
Gislene Carvalho-Zilse (INPA)

SUPLENTES:
Mario Cohn-Haft (INPA)
Claudia Keller (INPA)

EXAMINADORES	PARECER		ASSINATURA
Jansen Zuanon (INPA)	(X) Aprovado	( ) Reprovado	
Tânia Sanaiotti (INPA)	(X) Aprovado	( ) Reprovado	
José Alves Gomes (INPA)	(X) Aprovado	( ) Reprovado	
Marcelo Menin (~~INPA~~ UFAM)	(X) Aprovado	( ) Reprovado	
Gislene Carvalho-Zilse (INPA)	(X) Aprovado	( ) Reprovado	
Mario Cohn-Haft (INPA)	( ) Aprovado	( ) Reprovado	
Claudia Keller(INPA)	( ) Aprovado	( ) Reprovado	

Manaus(AM), 11 de dezembro de 2007

OBS: A banca recomenda um aprofundamento teórico nos gestores relacionados às análises genéticas e suas aplicações no trabalho, incluindo a possibilidade de uso de um marcador mitocondrial para traçar as linhagens maternas

INPA | DIVISÃO DOS CURSOS DE PÓS-GRADUAÇÃO

PIPG BTRN

Ministério da Ciência e Tecnologia | BRASIL UM PAÍS DE TODOS

AULA DE QUALIFICAÇÃO

Aluno(a): CAROLINA RIBAS PEREIRA
Curso: ECOLOGIA Nível: ( ) Mestrado (X) Doutorado
Dia: 11 / 12 / 2007
Hora: 15:00 hs
Local: AUDITÓRIO DO BADPI
Orientador(a): WILLIAM ERNEST MAGNUSSON

LISTA DE PRESENÇA

Nº	NOME DO ALUNO	CURSO	ASSINATURA
01	Andreia Cavalcante Peixoto	BADPI	
02	José Carlos de Almeida	BADPI	
03	Maíra Benchimol de Souza	CPEC	
04	Dani Teles Vinhas Santos	CPEC	
05	Fernando C. Pereira	BADPI	Fernando C. Pereira
06	Maria Muniz da Costa	[illegible]	
07	Carolina Jorge dos Santos	ECOLOGIA	
08	Gaia Alves de Matos	—	
09	Dayse Campista	—	Dayse Campista
10	José Antônio Lopes Neto	—	
11	Fabrícia Oliveira Macedo	BADPI	
12	Patrícia Farias Rosas Ribeiro	CPEC	Patrícia Farias R. Ribeiro
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			
32			
33			
34			
35			

FAVOR DEVOLVER ESTA LISTA À SECRETARIA DE PÓS-GRADUAÇÃO

PG·ECO·INPA
PÓS-GRADUAÇÃO EM ECOLOGIA

INPA
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

COCP
COORDENAÇÃO DE CAPACITAÇÃO

Ministério da Ciência ,Tecnologia e Inovação

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 10 dias do mês de maio do ano de 2012, às 09:00 horas, na Sala de Aula do PPG-ECO, Campus III, INPA/V8, reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a). Dr(a). **Vera Maria Ferreira da Silva**, do Instituto Nacional de Pesquisas da Amazônia o(a) Prof(a). Dr(a). **Fernando César Weber Rosas**, do Instituto Nacional de Pesquisa da Amazônia e o(a) Prof(a). Dr(a). **Izeni Pires Farias**, da Universidade Federal do Amazonas, tendo como suplentes o(a) Prof(a). Dr(a). Richard Carl Vogt do Instituto Nacional de Pesquisas da Amazônia e o(a) Prof(a). Dr(a). Gislene Carvalho Zilse do Instituto Nacional de Pesquisa da Amazônia, sob a presidência do(a) primeiro(a), a fim de proceder a argüição pública da **TESE DE DOUTORADO** de **CAROLINA RIBAS PEREIRA,** intitulada "Grau de parentesco e relações sociais em ariranhas (Pteronura brasiliensis)", orientada pelo(a) Prof(a). Dr(a). William Ernest Magnusson, do Instituto Nacional de Pesquisa da Amazônia e co-orientada pelo(a) Prof(a). Dr(a) Guilherme de Miranda Mourão, da Empresa Brasileira de Pesquisa Agropecuária.

Após a exposição, o(a) discente foi argüido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

☒ APROVADO(A)	☐ REPROVADO(A)
☒ POR UNANIMIDADE	☐ POR MAIORIA

Nada mais havendo, foi lavrada a presente ata que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a). Dr(a). Vera Maria Ferreira da Silva

Prof(a). Dr(a). Fernando César Weber Rosas

Prof(a). Dr(a). Izeni Pires Farias

Coordenação PPG-ECO/ INPA