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Living in the crowd: Influence of zoo visitors, social density and social interactions on cortisol levels in captive capuchin monkeys (*Sapajus libidinosus*)

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Dissertação apresentada à Universidade Federal do
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Programa de Pós-graduação em Psicobiologia, como
parte das exigências para a obtenção do título de
Mestre em Psicobiologia.

Orientadora: Prof. Dr. Renata Gonçalves Ferreira.

Co-orientadora: Prof. Dr. Hélderes Peregrino Alves da Silva.

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SUMÁRIO

LISTA DE TABELAS

CAPÍTULO 2

Supplemental materials

CAPÍTULO 3

LISTA DE FIGURAS

CAPÍTULO 2

RESUMO

Animais de cativeiro precisam enfrentar estressores crônicos em sua rotina, desde espaços reduzidos e alterados à perda de esconderijos. Macacos-prego (*Sapajus spp.*) são modelos úteis para estudar os efeitos da vida em cativeiro, não apenas por sua prevalência em centros de resgate e zoológicos brasileiros, mas também por sua flexibilidade comportamental, o que poderia influenciar sua habilidade de enfrentar o estresse. Este trabalho usa medidas comportamentais e fisiológicas (metabólicos fecais de glicocorticoides - MFG) em macacos-prego (*Sapajus libidinosus*) de diferentes ambientes de cativeiro (CETAS de Natal/RN e Cabedelo/PB, e zoológico de João Pessoa/PB) para testar as hipóteses de que a presença de visitantes, alta densidade populacional, e alta posição hierárquica são estressores, enquanto que catação social e comportamento sexual diminuem estresse. Na introdução geral, explicamos o conceito de estresse, carga alostática e como ela é afetada por fatores espaciais como densidade populacional e presença de visitantes, e por fatores sociais como posição hierárquica e expressão de comportamento social. Nos dois capítulos seguintes realizamos os testes de hipóteses. Selecionando modelos de regressão baseados em AIC (Akaike Information Criteria), corroboramos as hipóteses de que acesso a visitantes de zoológico é um estressor forte para macacos-prego como revelado pelo aumento em níveis de MFG médio, porém densidade populacional não foi um preditor forte de níveis de MFG. Tanto exposição a visitantes quanto densidade populacional afetam frequências comportamentais, com o primeiro aumentando observação ambiental e diminuindo alimentação, enquanto o segundo aumenta três tipos de comportamentos potencialmente indicativos de estresse enquanto diminui brincar sozinho e manipulação de ambiente. Aumento da densidade também prediz aumento do comportamento afastar-se, indicativo de uma estratégia de evitação social. Como esperado, indivíduos dominantes apresentaram maior nível de estresse do que subordinados de acordo com seus valores máximos de MFG. Contrário ao esperado, catação social não foi um preditor forte para menores níveis de MFG, mas comportamento sexual, afastar-se, proximidade e auto-catação se destacaram como correlacionados a baixos níveis de estresse. Comportamento sexual e proximidade atuaram como eficientes estratégias de suporte social que mitigam estresse, afastar-se aparece também como um comportamento que ajuda a evitar conflito no grupo cativo, e auto-catação como um comportamento auto-direcionado diminuidor de estresse.

Palavras-chave: cortisol, estresse, densidade populacional, efeito do visitante, rank, catação social

ABSTRACT

Captive animals must cope with chronic stressors in their lives, from reduced and altered space to lack of scape areas. Capuchin monkeys (*Sapajus spp.*) are useful models to study effects of life in captivity, not only due to their prevalence in Brazilian rescue centers and Zoos, but also because of their great behavioural flexibility, which may influence their ability to adapt to captivity. This thesis uses behavioural and physiological (fecal glucocorticoid metabolics - FMG) measures in capuchin monkeys (*Sapajus libidinosus*) from different captive environments (CETAS at Natal/RN and Cabedelo/PB, and zoo at João Pessoa/PB) to test the hypotheses that visitor presence, social crowding, and high hierarchical position are factors that increase stress levels, while social support behaviours like social grooming and sexual behaviour decreases stress levels. In the general introduction, we explain the concept of stress, allostatic load and how it is affected by spatial and social factors as social crowding, visitor effect, hierarchical position and social behaviour. At the two following chapters we present the hypotheses tests. Selecting regression models based on Akaike Information Criteria, our analyses corroborated the hypotheses that visitor exposure is a strong physiological stressor to capuchins, as revealed by increased mean FMG levels, while social crowding could not predict FMG levels. Both exposure to visitors and crowding significantly predicted increased frequency of three different behaviours potentially indicative of stress and decreased frequency of environmental manipulation and solitary playing. Increased social crowding also predicted higher retreat behaviour frequency, pointing towards a social avoidance strategy for crowding stress. As expected, dominant individuals were more stressed than subordinates according to maximum FMG values. Contrary to expected, social grooming was not a strong predictor of low cortisol levels, but sexual behaviour, retreat, proximity and self-grooming were. As a whole, these results indicate both social crowding and visitor presence as strong stressors to captive capuchin monkeys, proximity and sexual behavior as efficient social behaviours that mitigate stress, retreat behaviour as a good strategy to avoid conflict in captive groups, and self-grooming as a stress ameliorating self-directed behaviour.

Keywords: cortisol, stress, crowding, visitor effect, rank, social grooming

CAPÍTULO 1: Carga alostática: Uma introdução didática usando macacos-prego como modelos de estudo

Alostase: estabilidade pela mudança

Ao longo de suas vidas, animais selvagens experienciam mudanças de habitat que afetam sua aptidão, desde riscos estocásticos de predação e competição inter- e intragrupo, a flutuações cíclicas de estiagens e alagamentos, a mudanças ambientais rápidas induzidas por humanos (*human-induced rapid environmental changes* – HIREC) como desmatamento e urbanização (Mason *et al*., 2013; Sol *et al.*, 2013). Mudanças no comportamento e/ou fisiologia representam potenciais estratégias de ajuste ao desafio apresentado pelas mudanças ambientais.

A ideia de desafio fisiológico é estudada no conceito de alostase, o qual expande o conceito clássico de homeostase. Homeostase se refere à faixa ótima de variação de parâmetros funcionais do organismo, que deve ser preservada para manter a estabilidade interna dos mecanismos fisiológicos do corpo (Cannon, 1929). De acordo com o ambiente em que eles evoluíram, cada espécie é adaptada a certos parâmetros fisiológicos (ex. temperatura corporal ótima para metabolismo). Quando certas situações ou fatores ambientais (ex. calor excessivo), ou “estressores”, desafiam a homeostase ao mover o funcionamento fisiológico para fora dos parâmetros ótimos, mecanismos de retroalimentação negativa compondo a “resposta ao estresse” são ativados para enfrentar o estressor e trazer o corpo de volta para parâmetros ótimos. A resposta ao estresse pode ser comportamental (ex. mover-se para sombra) ou fisiológica (ex. começar a suar).

Baseados no conceito de homeostase, Sterling e Eyer (1988; p. 636) apresentam o conceito de alostase como “estabilidade por meio da mudança”. Isso inclui variações circadianas e anuais na ativação da resposta ao estresse, assim como resposta antecipatória a estressores, fatores que previamente não eram contemplados no conceito de homeostase. Alostase propõe que a faixa de funcionamento ótimo pode mudar ao longo da vida de um indivíduo, como uma resposta, por exemplo, à mudança de idade e à persistência de certos estressores crônicos (Sousa *et al*., 2015).

Fisiologicamente, a resposta ao estresse envolve a ativação do sistema nervoso simpático (resposta imediata) e do eixo hipotalâmico-pituitário-adrenal (HPA, representando uma resposta mais lenta), com a secreção final de catecolaminas e glicocorticoides pelos sistemas respectivos, havendo proeminente controle de retroalimentação negativa sobre o eixo neuroendócrino da resposta ao estresse (Allwin *et al.*, 2015). Catecolaminas (adrenalina e noradrenalina) são hormônios peptídicos responsáveis pelo aumento do fluxo sanguíneo e do ciclo respiratório durante resposta de fuga-ou-luta, com priorização de oxigênio para músculos esqueléticos ao invés de tecidos viscerais como o sistema gastrointestinal (Moyes & Schulte, 2010). Glicocorticoides (cortisol, cortisona e corticosterona) são hormônios esteroides relacionados principalmente ao metabolismo de energia, favorecendo reações catabólicas que liberam glicose de reservas de glicogênio para o sangue e promovem gliconeogênese a partir de reservas energéticas lipídicas e proteicas durante situações de maior demanda energética como fuga ou luta, consequentemente aumentando a glicemia (Sousa *et al.*, 2015; Phillips *et al.*, 2018).

Além de ser ativado após estímulo estressor, o eixo HPA varia durante ciclos sazonais e circadianos, causando maior liberação de glicocorticoides no começo do dia em espécies diurnas (pouco antes de acordar) e durante períodos de estiagem (Lynch *et al.*, 2002, *Sapajus nigritus*; Mendonça-Furtado *et al.*, 2017, *Sapajus libidinosus* e *S. nigritus*). A resposta fisiológica de estresse de curto tempo/agudo é benéfica para o animal, pois ela prepara seu corpo para uma possível resposta fuga-ou-luta. No entanto, uma resposta de longo tempo/crônica pode levar a desequilíbrios metabólicos, imunológicos e comprometimento na capacidade reprodutiva (Sapolsky *et al.*, 2000; Mason, 2010) estando associados também a quadros de transtornos comportamentais, sendo estes últimos muitas vezes acompanhados de distúrbios no mecanismo de retroalimentação negativa (Sapolsky *et al.*, 2000; Setchell *et al.*, 2008).

Estresse crônico com elevação continuada de cortisol também pode afetar o sistema imune por meio das citocinas, um grupo de peptídeos úteis para sinalização celular, sendo produzidas em resposta a estressores como feridas, inflamações e infecções (Pagliarone & Sforcin, 2009). Um estresse agudo envolveria inibição da resposta inflamatória enquanto focaliza linfócitos em áreas mais vulneráveis a infecções. No entanto, em uma resposta prolongada de estresse, a resposta anti-inflamatória aumenta em uma forma desregulada por causa do excesso de citocina pro-inflamatórias, uma característica envolvida no começo de algumas patologias como Depressão Maior e a imunodeficiência (Sousa *et al.*, 2015).

Brevemente revisando a literatura sobre estresse crônico e sua associação a quadros patológicos, Morgan e Tromborg (2007) listam como consequências de resposta ao estresse prolongada: capacidade reprodutiva suprimida; digestão suprimida; desequilíbrio hídrico, eletrolítico e glicêmico; dislipidemia; resposta imune suprimida; crescimento inibido por causa de menores níveis de hormônio de crescimento; peso corporal reduzido; maior agressão, o que pode levar a maiores chances de feridas; e aumento de comportamentos anormais, alguns dos quais podem causar ferimentos.

Por causa de seu papel na resposta ao estresse, níveis de cortisol são usados como um indicador fisiológico de estresse. Há diferentes meios biológicos usados para dosar cortisol de um animal, cada um com suas vantagens e desvantagens. A primeira matriz foi o sangue, o qual é mais preciso quando analisando níveis de cortisol, mas envolve um método invasivo e estressante de coleta de amostras. Em comparação, análise de metabólicos de glicocorticoides de amostras fecais oferecem um método não-invasivo e não estressante de coleta de amostras que melhor captura respostas crônicas ao estresse (Novak *et al.*, 2013; Sousa *et al.*, 2015).

Quando há um estressor crônico, o organismo pode passar por ajustes nos níveis basais homeostáticos para um parâmetro que melhor tolere o novo ambiente. Por exemplo, níveis basais de glicocorticoides podem permanecer altos, não retornando a níveis basais pré-estressor menores, mesmo após o estressor crônico ser removido (Sterling & Eyer, 1988). O custo fisiológico necessário para a ativação de processos alostáticos é nomeado carga alostática, e pode ser analisado pela quantificação dos níveis de glicocorticoides de um animal, por exemplo (Hodges *et al.*, 2010). Por exemplo, Moreira *et al.* (2016; *Sapajus nigritus*), analisando custos alostáticos ambientais em população livre de macacos-prego, utilizou níveis de glicocorticoides fecais como medidor de carga alostática.

Carga alostática: fatores individuais e sociais

Níveis de cortisol em primatas podem variar anualmente de acordo com variações sazonais em disponibilidade de alimento no ambiente, com cortisol circulante aumentando durante períodos com baixa disponibilidade de alimento (Cavigelli, 1999; Rangel-Negrín *et al.*, 2009). O cortisol livre também varia de acordo com mudanças no ciclo de vida do primata, como variações nos níveis de cortisol de acordo com o ciclo ovariano de fêmeas (Saltzman *et al.*, 1994), e aumentos durante períodos de acasalamento em machos (Lynch *et al.*, 2002; Strier *et al.*, 1999), gravidez (Smith *et al.*, 2012; Maestripieri & Georgiev, 2016) ou lactação (Maestripieri & Georgiev, 2016).

Já que as pressões evolutivas podem variar de acordo com o sexo, também verifica-se variação com relação aos níveis de glicocorticoides e estratégias comportamentais de enfrentamento de estresse entre machos e fêmeas. Com relação a níveis basais de cortisol, em espécies de primatas com grande dimorfismo sexual (ex. gorilas machos tem 60% mais massa corporal do que fêmeas coespecíficas), o custo energético necessário para manter um corpo maior demanda maior uso das reservas energéticas (Key & Ross, 1999), tornando os níveis basais de cortisol maiores em machos. Com relação a resposta ao estresse, bugios fêmeas na presença de machos invasores tendem a se comportar de forma passiva e não-agressiva com um simultâneo aumento em níveis de glicocorticoides, enquanto que machos do mesmo grupo respondendo ao mesmo estressor "macho invasor" tendem a se comportar de forma agressiva, mas não mostram um aumento significativo em níveis de glicocorticoides (Cristóbal-Azkarate *et al.*, 2007).

Idade e histórico de vida do indivíduo também afetam níveis de cortisol. Edes *et al.* (2016) encontraram uma correlação positiva entre idade (a partir dos seis anos) e níveis de glicocorticoides em gorilas cativos, assim como uma correlação positiva entre níveis de glicocorticoides e o número de eventos estressantes pelos quais eles passaram durante suas vidas (ex. anestesia, translocação, agressão). Entre primatas, também há uma tendência natural para níveis de cortisol serem maiores em filhotes, e então diminuírem ao longo do período juvenil inicial (Fourie & Bernstein, 2011; *P. hamadryas papio*).

Outro fator que pode influenciar carga alostática é a posição do indivíduo na hierarquia social. A hierarquia de dominância é uma característica filogeneticamente ancestral em espécies sociais (Pettinger *et al.*, 2011 – insetos; Simões *et al.*, 2015 – peixes; Sands & Creel, 2004 - mamíferos). Por definição, em uma hierarquia social, indivíduos dominantes tendem a ganhar mais conflitos intragrupo e tem maior acesso a recursos alimentares, suporte social e parceiros de acasalamento, enquanto indivíduos subordinados tendem a perder mais conflitos e, portanto, têm menor acesso a recursos preferidos e menos suporte social (Prall & Muehlenbein, 2017). A formação de uma hierarquia social é considerada uma estratégia evolutiva estável, porque isso permite que membros do grupo prevejam quem tem uma melhor chance de ganhar um conflito (quem é dominante sobre quem), resultando em menores quantidades de brigas intragrupo porque brigas em que eles perderiam seriam evitadas (Enquist & Leimar, 1990). Isso é útil para a vida em grupo, porque se posições hierárquicas tivessem de ser constantemente reafirmadas por meio de brigas ao invés de por meio de gestos de dominação e subordinação, seria difícil manter coesão grupal (de Waal, 1998).

Ambas as posições subordinado e dominante possuem certos desafios que requerem custo energético, aumentando carga alostática (Creel, 2001; Abbott *et al.*, 2003). Há o "estresse de subordinação", causado pelo limitado acesso de subordinados a recursos e sendo mais vulneráveis a agressão de dominantes. Também há "estresse de dominância", causado pelo custo energético necessário para conseguir e manter sua alta posição social (Ode *et al.*, 2015) e estressores psicológicos relacionados à possibilidade de perder seu alto *rank* (posto social hierárquico) para outro indivíduo (Higham *et al.*, 2013; Gesquiere *et al.*, 2011). Já que esses estressores relacionados ao *rank* variam junto com o tipo de organização social do grupo (despótico vs. igualitário), a forma de conseguir uma posição hierárquica alta (vencer conflitos ou herdar pela linhagem matrilinear) e a estabilidade da hierarquia (estável vs. instável), há estudos que corroboram ambas as hipóteses: alguns estudos reportam que subordinados são mais estressados que dominantes (ex. Virgin & Sapolsky, 1997; *Papio anubis*), outros corroboram a hipótese que dominantes são mais estressados que subordinados (ex. Coe *et al.*, 1979, *Saimiri sciureus*; Saltzman *et al.*, 1996, *Callithrix jacchus*), e há também estudos em que não foi achada qualquer correlação entre hierarquia de dominância e cortisol (ex. Sapolsky, 1983; *Papio anubis*).

Estresse relacionado a posição hierárquica pode ser afetado pela estabilidade social e o sexo de forma a inverter o relacionamento entre carga alostática e status social. Por exemplo, em babuínos machos em vida livre, subordinados apresentaram maiores níveis de cortisol do que dominantes durante períodos de estabilidade social, mas menores níveis cortisol durante períodos de instabilidade social (Sapolsky, 1992; *Papio anubis*). Saltzman *et al.* (1994) encontraram níveis mais altos de cortisol em saguis dominantes fêmeas do que em subordinadas. A revisão de Cavigelli e Caruso (2015) em diferentes espécies de primatas conclui que, durante períodos de instabilidade social, machos dominantes apresentam maior carga alostática que machos subordinados, enquanto nas fêmeas, durante períodos de instabilidade social, subordinadas apresentam maior carga alostática do que membros de grupo dominantes. Além disso, durante os períodos de estabilidade social, machos subordinados tendem a apresentar maior carga alostática do que machos dominantes, enquanto fêmeas dominantes tendem a apresentar níveis mais altos de glicocorticoide que subordinadas (Abbott *et al.*, 2003). O trabalho de Setchell *et al.* (2008; *Mandrillus sphinx*) oferece suporte a isso, mostrando que, nos machos, os níveis de cortisol são mais altos nos subordinados do que nos dominantes durante períodos de estabilidade social, enquanto o oposto acontece durante períodos de instabilidade social.

Também é possível haver uma relação não-linear entre os níveis de glicocorticoides e a posição hierárquica, como encontrado em Gesquiere *et al.* (2011; *Papio cynocephalus*), em que tanto os machos alfa quanto os machos subordinados tinham níveis glicocorticoides mais altos na hierarquia, embora provavelmente não para os mesmos estressores: os níveis de estresse dos machos alfa estão ligados a encontros agressivos e cópulas, enquanto machos subordinados devem ser estressados por causa do acesso limitado à comida. Indivíduos de nível intermediário na hierarquia, especialmente o macho beta, apresentaram os menores níveis de estresse.

Abbott *et al.* (2003) aponta que a força do estresse da subordinação sobre os indivíduos é maior quando eles não têm acesso a suporte social e são minimamente relacionados a outros membros do grupo social. Dunbar (1991) diz que cerca de um quinto do tempo de atividade de um primata é gasto fazendo catação social e argumenta que essa é uma quantidade excessiva de tempo se a única função da catação social fosse apenas higiênica. A catação social é amplamente aceita como importante para os primatas não-humanos, não só por razões de higiene, mas também porque ajuda a fortalecer laços sociais (Akinyi *et al.*, 2013). Esse maior apoio social (analisado por tempo em proximidade com coespecífico, tempo oferecendo catação a coespecífico e tempo recebendo catação de coespecífico) pode estar vinculado a uma maior chance de sobrevivência da prole (Silk *et al.*, 2003; *Papio cynocephalus*). O contato social através da catação entre primatas pode funcionar como um ansiolítico ou regulador contra estressores, ajudando a regular a ativação do eixo HPA (Grandi *et al.*, 2015; Crockford *et al.*, 2017). Ao mesmo tempo, receber catação de um parceiro próximo pode liberar ocitocina em primatas, um neuropeptídeo capaz de influenciar o eixo HPA, atenuando a liberação de glicocorticoides, ou diminuindo o tempo necessário para o feedback negativo do eixo HPA entrar em vigor e parar a resposta fisiológica ao estresse (Wittig *et al.,* 2016). Tem sido proposto que a afiliação social, como catação (ou mesmo a presença de um parceiro próximo no mesmo recinto), pode fazer um animal mudar sua percepção sobre o quão ameaçador um estressor psicológico é (Hostinar & Gunnar, 2015).

No entanto, a relação entre o comportamento de catação social e a carga alostática em primatas não-humanos é complexa, e ainda há dúvidas sobre como esse comportamento social afeta o enfrentamento ao estresse. Fontani *et al.* (2014) encontraram que tanto o recebimento quanto o oferecimento de catação social são fortemente e inversamente correlacionados com os níveis fecais de glicocorticoides em saguis cabeça-de-algodão (*Saguinus oedipus*). Gust *et al.* (1993), por outro lado, observaram uma correlação forte e negativa apenas entre catação recebida e os níveis de glicocorticoides em macacas rhesus (*Macaca mulatta*), enquanto Shutt *et al*. (2007; macacas-de-Gilbratar) e Wooddell *et al.* (2017; macacas rhesus) encontraram uma correlação negativa entre as concentrações metabólicas de glicocorticoides fecais e catação social oferecida. Essa controvérsia abre espaço para questões adicionais sobre se o ato do catar outro indivíduo também tem um efeito relaxante sobre o animal, ou se os indivíduos mais calmos do grupo são simplesmente mais propensos a oferecer catação do que indivíduos com maior estresse crônico.

Em conjunto ao tópico de catação social como tamponador de estresse, também há registros de relação entre auto-catação e carga alostática. Auto-catação é um comportamento auto-direcionado utilizado para limpeza pessoal, porém seu aumento de frequência durante momentos de estresse levanta a hipótese de que ele tenha a mesma capacidade apaziguadora da catação social. Tal hipótese foi corroborada em Galvão-Coelho *et al.* (2017; *Callithrix jacchus*), os quais encontraram uma relação negativa entre auto-catação e níveis de glicocorticoides fecais em saguis juvenis durante períodos de isolamento social). No entanto, Higham *et al.* (2009) e Ellis *et al.* (2011) não encontraram nenhuma relação entre auto-catação e níveis de glicocorticoides fecais em babuínos adultos (*Papio hamadryas anubis*), portanto ainda é interessante verificar se esse comportamento tem a habilidade de regular a ativação do eixo HPA.

Carga alostática e vida em cativeiro

Existe uma aparente contradição em relação aos animais que vivem em cativeiro: o ambiente cativo é um lugar sem predadores, com acesso mais fácil a alimentos e cuidados veterinários em caso de lesão ou doença em comparação com o ambiente natural (Mason, 2010). De fato, Smith *et al.* (2012) utilizando metabólitos fecais de corticosterona (MFC) em marmotas de barriga amarela para avaliar os níveis de estresse basal na espécie, encontraram menores níveis basais de MFC em cativeiro do que na natureza, um resultado que atribuem à maior previsibilidade de ambientes cativos. Ao mesmo tempo, recintos cativos são ambientes muito diferentes do ambiente ancestral das espécies e muitas espécies têm dificuldade em reproduzir ou sobreviver em cativeiro (Mason, 2010; Mason *et al.*, 2013). Isso revela o impacto negativo trazido por morar em recintos de espaço muito limitado com alta densidade populacional em um grupo escolhido não pelo animal, mas por seus cuidadores humanos. Além disso, a incapacidade de procurar por sua própria refeição (Ulyan *et al.*, 2006), a capacidade limitada de se distanciar de companheiros mais dominantes para evitar encontros agressivos (Sapolsky, 2005) ou simplesmente o tédio que pode vir de estar em um ambiente previsível com poucos estímulos (Mason, 2010; Pomerantz *et al.*, 2013) são estressores crônicos para animais em cativeiro.

A capacidade de adaptação do animal ao cativeiro é influenciada pela interação das características das espécies com os fatores sociais e físicos do ambiente cativo em que estão vivendo. Por exemplo, embora os primatas em geral pareçam ser influenciados pela presença de humanos desconhecidos em zoológicos, espécies de primatas de pequeno porte especificamente teriam maiores chances de ver esses humanos como ameaçadores, uma vez que humanos se assemelhariam a predadores para eles (Chamove *et al*., 1988).

Mason (2010) levantou a hipótese de que certas características dos animais podem ajudar ou dificultar de se adaptar ao cativeiro. Por exemplo, ser de uma espécie migratória pode dificultar sua adaptação, uma vez que o animal não será capaz de seguir seu comportamento migratório natural. Espécies que exploram grandes extensões de terra durante o dia e experimentam várias formas de estímulo mental enquanto buscam por alimentos podem ter dificuldade em se adaptar a um ambiente pequeno com poucos estímulos (Pomerantz *et al.*, 2013). Há também algumas dúvidas sobre como as espécies generalistas e onívoras lidariam com o cativeiro e se elas seriam mais propensas ao tédio ou mais fáceis de se adaptar (Mason *et al.*, 2013).

Vida em cativeiro (em oposição à vida livre ou semi-livre) restringe grandemente estratégias de mitigação de conflito focadas em se afastar dos indivíduos (Calhoun, 1962; Creel, 2001). A estratégia adotada para lidar com alta densidade populacional dentro do recinto, por exemplo, varia. Em chimpanzés de cativeiro, alguns grupos aumentaram os comportamentos socialmente positivos em resposta à alta densidade populacional, como forma de reduzir tensão intragrupo e evitar agonismo excessivo (Nieuwenhuijsen & de Waal, 1982; *Pan troglodytes*), enquanto outros grupos diminuíram todos os tipos de comportamento social e se afastaram uns dos outros como forma de evitar conflitos (Aureli & de Waal, 1997; *Pan troglodyte*). Existem assim com duas estratégias principais para esse estressor: redução de tensão e evitação social.

Como pode ser visto nos exemplos dados acima, diferentes grupos da mesma espécie não adotam necessariamente a mesma estratégia de enfrentamento. Parece que a estratégia de evitação é mais comum na alta densidade de curto prazo, enquanto a estratégia de redução de tensão ocorre mais durante situações com alta densidade a longo prazo (Crast *et al.*, 2015). É importante notar que, mesmo se o animal evitou agressão excessiva com sucesso, isso não significa automaticamente que ele está livre do estresse da alta densidade populacional (de Waal, 1989). Ademais, a estratégia de evitação de agressão intragrupo pode levar ao eventual colapso do grupo.

Efeito visitante, o possível efeito de exposição a visitantes humanos desconhecidos sobre animais de cativeiro (Hosey & Druck, 1987), tem sido estudado desde a década de 1970 (Hediger, 1970). Embora a maioria da literatura sobre efeito do visitante conclua que ele existe e tem consequências negativas sobre os animais cativos (Hosey, 2013), existem estudos que não encontraram mudanças comportamentais (Collins *et al.*, 2017; *Lemur catta*) ou encontraram um efeito positivo onde os humanos aparecem como uma forma de enriquecimento ambiental que estimula os animais (Fa, 1989; *Cercopithecus aethiops sabaeus*). Hosey *et al.* (2010) sugerem que o tipo de efeito que os visitantes têm sobre animais cativos (negativo, indiferente ou positivo) depende da forma como o animal interpreta os visitantes: uma fonte de enriquecimento ambiental (efeito positivo – Fa, 1989), um predador ameaçador ou potencial competidor (efeito negativo – Wells, 2005; *Gorilla gorilla*) ou simplesmente outra parte do ambiente (sem efeito – Collins *et al.*, 2017).

Teorizou-se inicialmente que espécies menores, como primatas arbóreos, são mais propensas a interpretar visitantes como predadores (Chamove *et al.*, 1988; Margulis *et al.*, 2003). No entanto, Queiroz e Young (2018) investigaram quais características da espécie prediziam uma maior influência dos visitantes sobre o repertório comportamental de mamíferos vivendo em zoológico, e encontraram que espécies arbóreas, que tinham acesso a árvores e esconderijos longe do olhar do público, foram menos afetadas do que espécies terrestres, que estavam mais expostas ao público. Peso corporal não foi capaz de predizer quais animais seriam mais afetados por visitantes, mas dieta, tipo de habitat natural e ciclo de atividade da espécie foram (Queiroz & Young, 2018): herbívoros e onívoros (espécies presas) são mais afetados que carnívoros (espécies predadoras); espécies de habitat fechado foram mais afetadas que espécies de habitat aberto; e espécies diurnas foram mais afetadas que espécies noturnas.

Entre primatas de zoológico, agressão intragrupo pode aumentar na presença de visitantes, enquanto afiliação social pode baixar (Glatston *et al.*, 1984; Mitchell *et al.*, 1991). Mallapur *et al.* (2005) encontraram 10 vezes mais níveis de agressão em macacos-de-cauda-de-leão (*Macaca silenus*) de zoológico do que em coespecíficos de vida livre, com a maioria desses comportamentos agressivos sendo direcionados aos visitantes do zoológico. Davis *et al.* (2005) viram que uma correlação positiva entre os níveis de glicocorticoides em macacos-aranha de zoológico com o número de visitantes. Macacos-aranha (*Ateles geoffroyi yucatanensis*) de vida livre vivendo em áreas conservadas apresentam níveis de cortisol mais baixos do que coespecíficos vivendo em áreas fragmentadas, e ambos apresentam níveis de cortisol mais baixos do que aqueles vivendo em zoológico (Rangel-Negrin *et al.*, 2009).

Ausência de mudança comportamental em um animal em cativeiro frente aos visitantes de zoológico não significa necessariamente que ele tenha deixado de ser afetado pelo estressor (Hosey, 2008), já que é possível animais continuarem ativando a resposta fisiológica ao estresse mesmo após parar a resposta comportamental (Line *et al.*, 1989; *Macaca mulatta*). Algumas espécies, especialmente as que são predadas em ambiente natural, costumam desenvolver uma expressão mais estoica a dor, de modo a não mostrar vulnerabilidade aos predadores (Jordan, 2005).

Morgan e Tromborg (2007) supõem que a maioria dos estressores em populações cativas são fatores que eles não podem controlar, nem escapar. Leopardos-nebulosos (*Neofelis nebulosa*) vivendo na área de exposição a visitantes apresentavam níveis de cortisol mais altos do que coespecíficos vivendo na área fora para exibição a visitantes, e esses níveis de glicocorticoides correlacionaram positivamente com comportamentos indicativos de estresse, como locomoção aberrante, se esconder e autoflagelação (Wielebnowski *et al*., 2002). A inclusão de esconderijos no recinto de leopardos-nebulosos reduziu os níveis de glicocorticoides (Shepherdson *et al.*, 2004), porque permitiu uma maior capacidade de escolha sobre quando os animais seriam vistos pelos visitantes. A capacidade de controlar quando será exposto a certos estressores atenua seu impacto sobre o animal.

Macacos-prego e a resposta ao estresse

O animal foco deste trabalho é o macaco-prego. Este é um primata neotropical que fazia parte do gênero *Cebus* junto com os macacos caiarara, mas recentemente foram separados de *Cebus* e colocados sozinhos no novo gênero *Sapajus*, agrupando oito espécies de macacos-prego robustos (Lynch-Alfaro *et al.*, 2012). Os membros do gênero *Sapajus* são assim conhecidos porque seu corpo é mais robusto, possui caninos mais longos que os ajudam durante a quebra de nozes e apresentam um estilo de forrageio durofágico quando comparados com os membros atuais do gênero *Cebus*, os macacos cairarara, que seriam chamados de macacos-prego gráceis. Devido ao seu alto nível de encefalização e habilidade de usar ferramentas (Fragaszy *et al.*, 2004) eles são usados como sujeitos experimentais para modelos comparativos e translacionais em estudos de evolução da cognição e psicopatologias.

Há variação sazonal nos níveis médios de glicocorticoides em macacos-prego em vida livre, embora seja inconclusivo se as maiores concentrações de cortisol são causadas pelo período de seca da região e/ou pelo início da temporada de acasalamento do grupo, os quais coincidiram (Lynch *et al.*, 2002; *Cebus apella nigritus*). Macacos-prego fêmeas de vida livre apresentam um aumento nos níveis de metabólicos fecais de glicocorticoides durante a gravidez, enquanto os machos do mesmo grupo apresentam um pico durante o início da estação reprodutiva (Moreira *et al.*, 2016; *Sapajus nigritus*).

A carga alostática dos juvenis de vida livre, medida por meio de metabólitos fecais de glicocorticoide, é influenciada principalmente pelo acesso a alimentos no ambiente (Moreira *et al.*, 2016). Carnegie *et al.* (2011) também encontram níveis mais elevados de cortisol em macacos-prego fêmeas (*Cebus capucinus*) de vida livre durante a gestação, lactação e temporada de estiagem.

Lynch *et al.* (2002; *Sapajus nigritus*) não encontraram diferenças significativas entre os níveis de glicocorticoides de machos subordinados e dominantes de vida livre. Mendonça-Furtado *et al.* (2014; *S. libidinosus*) por sua vez encontraram maiores níveis de glicocorticoides em machos dominantes do que em machos subordinados, independente da estabilidade social, o que é semelhante aos achados com macacos-prego caiarara (*Cebus capucinus*) machos, todos com maiores níveis de glicocorticoides em dominantes (Schoof *et al.*, 2012, 2014; Schoof & Jack, 2013). Estes diferentes resultados podem ser devido a variações na organização social, uma vez que os macacos-prego são primatas com uma hierarquia social menos despótica. Existe uma hierarquia entre as fêmeas (herdada através de vínculos familiares) e uma hierarquia entre os machos (vencida através de conflitos intragrupo). Durante períodos de instabilidade social na hierarquia dos machos, as fêmeas apresentam aumento nas concentrações de cortisol, independente do novo macho alfa estar relacionado a elas ou não (Carnegie *et al.*, 2011; *Cebus capucinus*).

Macacos-prego também parecem categorizar a presença de seus companheiros de recinto como uma necessidade básica. Em um experimento onde eles precisavam escolher entre comida e um companheiro social, cinco entre sete indivíduos escolheram o companheiro em condições basais (ausência do estressor fome), e um intervalo de 12-19 horas de privação de alimentos foi necessário para que seis de sete deles começassem a preferir alimentos ao invés de companheiro social (o sétimo sujeito continuou a preferir o companheiro social depois de 22 horas de privação de alimento, em que ponto o experimento precisou ser interrompido por razões de segurança de saúde) (Dettmer & Fragaszy, 2000; *Cebus apella*). De fato, comparando macacos-prego resgatados do comércio de animais de estimação que viviam isolados de coespecíficos com macacos-prego que viviam em um grupo social, aqueles que viviam em isolamento expressaram mais comportamentos anormais (ex.: auto-mutilação; balanço do corpo; giro de cabeça; morder a grade) e menores frequências de comportamento alimentar, sugerindo um prejuízo de longo prazo em seu comportamento devido ao isolamento social de coespecíficos (Bee, 2017; *Sapajus apella*).

Entre macacos-prego fêmeas, as dominantes oferecem mais catação social do que recebem, e oferecem a maior parte dessa catação a suas subordinadas (Parr *et al.*, 1997; O'Brien, 1993; Di Bitetti, 1997). Desta forma, esse comportamento de afiliação pode estar funcionando como um tipo de troca. Embora os macacos-prego geralmente não compartilhem alimentos diretamente, eles regularmente toleram a presença de colegas de grupo e filhotes quando estão comendo para fazer coletar restos (*scrounging),* que eles deixam vários pedaços de comida caírem no chão para que outros os comam, e até mesmo deixam filhotes "roubarem" parte da comida que ainda estão segurando. Tiddi *et al*. (2011; *Cebus apella nigritus*) argumentam a existência de um sistema de troca baseado em reciprocidade entre catação social e tolerância social durante o período de alimentação, sugerindo que podem existir outras formas de tolerância no grupo além de coalizões agressivas que podem ser trocadas por catação social. Por exemplo, há evidências de subordinadas solicitando catação a fêmeas dominantes, que então mostram mais tolerância e menos agressão à presença dessas subordinadas (Parr *et al.*, 1997; O'Brien, 1993).

O macaco-prego é o segundo gênero de primata mais comumente encontrado em zoológicos e centros de resgate no Brasil (Levacov *et al.*, 2011), logo é de grande importância prática estudar os padrões comportamentais e variações na carga alostática desses animais em ambiente de cativeiro. Tárano & López (2015) compararam o repertório comportamental e os orçamentos de tempo de macacos-prego (*Cebus olivaceus*) em zoológico e em semi-liberdade, descobrindo que os indivíduos cativos eram os únicos que expressavam comportamentos estereotipados. Macacos-prego cativos também gastaram uma maior parte do seu orçamento de tempo em forrageio e comportamento alimentar do que macacos-prego em ambiente aberto, o que pode ser um sinal de excesso de alimentação devido à falta de alternativas de atividades (Tárano & López, 2015). Em cativeiro, van Wolkenten *et al.* (2006; *Cebus apella*) encontraram que aumento agudo na densidade populacional dentro do recinto faz macacos-prego diminuírem todos os tipos de comportamento social independente da valência e manter distância dos companheiros de recinto, prevenindo assim um aumento em conflitos intragrupo. Outras situações comuns à vida em cativeiro que foram relacionadas ao estresse nesses primatas incluem realocação (Dufour *et al.*, 2011; *Cebus apella*), eventos de manejo imprevisíveis (Rimpley & Buchanan-Smith, 2013; *Sapajus apella*) e horários de alimentação imprevisíveis (Ulyan *et al.*, 2006; *Cebus apella*).

Ferreira *et al.* (2016, *Sapajus spp.*) encontraram cinco grandes componentes de estratégias de enfrentamento ao estresse em macacos-prego cativos, dois dos quais ("Restless" e "Self-protective") são similares às estratégias de enfrentamento proativa e reativa, respectivamente. Ferreira *et al.* (2018) encontraram que macacos-prego cativos diferem em seus padrões comportamentais ("Feeding", "Sociability", "Exploration" e "Activity"), cada um desses componentes correlacionando com diferentes estilos comportamentais de resposta ao estresse. À exceção de "Alimentação", os outros traços diferiram nos níveis de metabólicos fecais de glicocorticoides: maior “Sociabilidade” e “Exploração” reduziram os níveis médios, medianos e mínimos de glicocorticoides; e maior "Atividade" aumentou os níveis mínimos de glicocorticoides.

Quanto ao estudo do efeito do visitante, Sherwen *et al.* (2015; *Cebus apella*) encontraram que o número de agressões intragrupos, comportamentos anormais (ex. coçar) e níveis de glicocorticoides diminuíram em macacos-prego de zoológico após pôr uma barreira visual no recinto que permitia os visitantes verem aos macacos sem serem vistos de volta, demonstrando que os visitantes eram interpretados comos um estressor com efeito negativo sobre os macacos-prego. Rodrigues e Azevedo (2016; *Sapajus xanthosternos*) mostraram aumento em catação social, auto-catação e agressão intragrupo quando visitante estavam presentes no zoológico, com os dois primeiros comportamentos possivelmente sendo usados para aliviar o estresse do efeito do visitante. Quadros *et al.* (2014; *Sapajus xanthosternos*) constataram que o número de visitantes estava correlacionado positivamente com vigilância (algo que sugeriria maior estresse) e negativamente com comportamento anormal (o que sugeriria menor estresse), o que corrobora a existência do efeito visitante, mas não a valência do efeito.

Objetivos gerais

Este estudo analisou o efeito de estressores ambientais e das relações sociais intragrupo sobre os níveis de estresse comportamental e fisiológico em macacos-prego cativos. O segundo capítulo objetiva verificar a capacidade preditiva de densidade populacional dentro do recinto e exposição aos visitantes de zoológico sobre a carga alostática dos indivíduos cativos (hipóteses 1 e 2). O terceiro capítulo objetiva verificar a capacidade preditiva de posição hierárquica e comportamentos afiliativos sobre a carga alostática dos indivíduos cativos (hipóteses 3 e 4).

Hipóteses e predições

H1. Carga alostática aumenta com maior densidade populacional dentro do recinto;

P1. Aumento da densidade populacional dentro do recinto irá predizer aumento nos níveis de MFG e aumento nas frequências de comportamentos potencialmente indicativos de estresse.

H2. Carga alostática aumenta na presença de visitantes;

P2. Níveis de MFG e frequências de comportamentos potencialmente indicativos de estresse são maiores em indivíduos vivendo em zoológico do que indivíduos vivendo em centros de resgate.

H3. Carga alostática é maior em dominantes do que em subordinados;

P3. Níveis de MFG são maiores em indivíduos com maior posição hierárquica.

H4. Suporte social diminui carga alostática.

P4. Aumento dos comportamentos sociais positivos catação social e comportamento sexual diminuem níveis de MFG.

P5. Aumento dos comportamentos sociais negativos de afastar-se e receber agressão aumentam níveis de MFG.

P6. Aumento dos comportamentos sociais negativos de afastar alguém e fazer agressão diminuem níveis de MFG.

CAPÍTULO 2: Trouble with crowds: density and visitor effect on behaviour and glucocorticoid levels in captive capuchin (*Sapajus libidinosus*)

Abstract

The captive environment offers various types of stressors to animals that trigger their allostatic systems, including activation of the hypothalamic-pituitary-adrenal axis. These stressors include limited space in the enclosure and, in the case of zoo animals, exposure to visitors. We analyzed fecal metabolite glucocorticoid (FMG) levels in captive capuchins (*Sapajus libidinosus*) living in zoos and rescue centers to study the effect of chronic crowding and visitor access on their allostatic load and behavioural frequencies. We collected fecal samples and observed the behaviour of 30 adult capuchins and used regression model selection by Akaike Information Criteria to find if sex, cage density and/or visitor access could predict allostatic load by means of mean, median, maximum and minimum FMG levels, and if any of them could predict any behavioural frequency. We found that visitor access is a stressor which increases allostatic load in captive capuchins by increasing mean FMG levels, and visitor access also increases vigilance while decreasing eating frequency. Crowding does not affect FMG, although it does predict higher frequency of three behaviours potentially indicative of stress while lowering frequencies of solitary play and environment manipulation. Higher crowding also predicts higher retreat behaviour without affecting frequencies of allogrooming or aggression, suggesting our captive capuchins are adopting a social avoidance coping strategy. These findings highlight the negative effect of visitor exposure on zoo animals despite years of exposure and the need to design ways to limit visitor effect over zoo capuchins.

Keywords: allostatic load, animal welfare, capuchin monkey, cortisol, social crowing, visitor effect

Introduction

Allostasis, or keeping stability through change, refers to variations on individuals´ physiological and behavioural parameters to adjust to predictable (eg.: seasonal variations, reproductive cycle) or unpredictable changes (eg. predation attempts) in the environment (Sterling & Eyer 1988; McEwen & Wingfield 2003, 2010). Allostatic load refers to the energy to maintain the activity of allostatic systems, in order to keep the organism's physiology within the range of optimal parameters needed to survive and reproduce (Maestripieri & Hoffman 2011).

The allostatic system encompasses behavioural, physiological, cognitive and social strategies. For example, wild white-faced capuchins rest more, travel less and expose the tongue (and yawn) more during dryer periods, to keep body temperature below 35ºC (Campos & Fedigan 2009). Using cortisol levels as an index of allostatic load, Creels *et al* (2013) showed that presence of predator and high population density are two ecological sources of stress in a variety of taxa of wild vertebrates (fish, reptiles, birds and mammals). Achieving alpha status increases cortisol and testosterone secretion in wild capuchin monkeys (*Cebus capucinus* - Schoof *et al* 2011; *Sapajus libidinosus* - Mendonça-Furtado *et al* 2014), and wild mantled howlers (*Allouatta paliatta* – Cristobal-Azkarate *et al* 2007). However, when this allostatic load is chronically kept high it usually leads to depression of the immune system and reduced reproduction (Wingfield 2005), as well as damaging the hypothalamic-pituitary-adrenal (HPA) axis itself in a way that might make it unable to restore basal cortisol levels after the stressor is gone, remaining higher than it was before (Sterling & Eyer 1988).

Animals kept in zoos, aquariums and research labs live in environments radically different from that experienced by their wild conspecifics, presenting further challenges to their allostatic systems. However, so many parameters differ from captive to wild, and among *ex situ* settings themselves (ranging from temperature and background noise to constant offer of food and health care), that scientific evidence show controversial patterns on the impacts of captivity for different species (Mason 2010; Pomerantz *et* *al* 2013; Kroshko *et al* 2016). Among those, crowding and visitor effects are of most interest to zoos and aquariums management practices.

Visitor effect, or the potential effect that human strangers such as zoo visitors might have on captive animals, has been investigated since the 1970's (Hediger 1970; Thompson 1976; Oswald & Kuyk 1977). Some studies report animals being positively affected by visitor presence (Fa 1989), particularly if a connection between "zoo visitor" and "food source" has been made. Margulis *et al* (2003), on the other hand, analyzed the behaviour of six species of zoo-housed felids (lion, Amur leopard, Amur tiger, snow leopard, clouded leopard and fishing cat) during and without visitor presence and found no visitor influence on activity of animals. Behaviour pattern and cortisol levels also did not vary between three cotton-top tamarin groups kept under different exposition to humans and in areas of different dimensions (Fontani *et al* 2014; *Saguinus oedipus*). However, most studies on the subject conclude that visitors have a negative impact on captive animals (Wells 2005), arguing that visitors mimic predators or are themselves a stressor the captive animals that have limited possibility to escape (Fernandez *et al* 2009). Birke (2002) found that zoo-housed orangutans spent more time hiding their faces in browse sacs in the presence of large human crowds, and that noisy visitor crowds had greater impact than quiet visitor crowds. Increases in intra-group aggression and decreases in affiliation have been reported for captive primates (Glatston *et al* 1984; Mitchell *et al* 1991) during presence of zoo visitors. Mallapur *et al* (2005) describe a 10-fold increase in aggressive display (directed at humans) in lion-tailed macaques (*Macaca silenus)* kept in zoos relative to their wild conspecifics.

Further studies on HPA activity in zoo animals show a possible increase in allostatic load related to the visitor effect. Davis *et al* (2005) found a positive correlation between visitor number and urinary cortisol in zoo-housed spider monkeys (*Ateles geoffroyii rufiventris*). Carlstead and Brown (2005) registered higher mean corticoid concentrations in zoo-housed black rhinos (*Diceros bicornis*) when they were kept in enclosures with greater access to public view. Rajagopal *et al* (2011) found a significantly higher mean fecal cortisol in zoo-housed Indian Blackbucks (*Antelope cervicapra*) when they were in high visitor density levels, than when visitor density levels were low or zero. They also expressed more intragroup aggression and rested less during high and extremely high visitor density. Pifarré *et al* (2012) showed that Mexican wolves (*Canis lupus baileyi*) living in zoos excrete more cortisol metabolites and spend more time lying down where the public could not see them on Sundays, when there are more visitors. Increases in cortisol concentrations, more hiding and less foraging behaviour were registered in pied tamarins (*Saguinus bicolor bicolor)*, a highly endangered new world monkey, when housed on-audience than when housed off-audience (Armstrong & Santymire 2013).

For social species, as well as for solitary but territorial species, the forced coexistence with conspecifics and lack of space to escape is a second source of environmental disruption that imposes physiological costs during captive living. This subject has been studied since the 1960’s (Christian 1961), with the original understanding that cage crowding would result in lowered reproductive capacity and increased frequency of aggression and abnormal behaviours (Calhoun 1962; Russell & Russell 1968). Like visitor effect, research on the effect of cage size on animal stress has produced varying results, with cage size and density being found at times to lower and at others to be of no consequence to stress (Morgan & Tromborg 2007). In a review on primate coping mechanisms for crowded conditions, Judge (2000) found that, from 29 published studies on cage density and aggression, 15 found a positive correlation between aggression and cage crowding, while six found a negative correlation, and the remaining eight report no connection. This doesn't necessarily mean nonhuman primates have habituated to crowded conditions, but it does mean they are adaptable enough to change their behaviour and use coping strategies to diminish risks of increased injuries or fighting in the group (de Waal 1989). De Waal *et al* (2000) found no differences in self-scratching rates or fecal glucocorticoid levels between captive chimpanzees (*Pan troglodytes*) housed in small and large enclosures until they were seeing aggressive displays from conspecifics, at which point those in smaller enclosures increased stress levels while also being less likely to respond aggressively than those in larger enclosures, illustrating how complicated the relationship between stress and aggression can be in primates.

Hosey (2008) argues that the absence of behavioural changes in captive animals does not indicates lack of stress. For example, Line *et al* (1989) found increased heart rate in laboratory rhesus macaques in the presence of human observers even after two years as study subjects, at which point all behavioural responses to the humans had stopped. For baboons (*Papio hamadryas*) Pearson *et al* (2015) found higher salivary cortisol levels during periods of chronic and acute crowding than during periods pre- and post-crowding, although stress related behaviours (such as scratch, self-groom, shake and self-touch) did not elevate during crowding events. Different species may also respond differently to crowding. Captive Japanese macaques (*Macaca fuscata*) decrease affiliation and do not exhibit significant plasma cortisol elevation, while captive rhesus macaques (*Macaca mulatta*) do not show behavioural differences to higher social crowding but do present higher plasma cortisol levels in response to higher crowding (Lee *et al* 2018).

Rangel-Negrín *et al* (2009) used fecal glucocorticoid levels to show that for spider monkeys (*Ateles geoffroyi yucatanensis),* allostatic load is higher in crowded populations living in fragmented environments than in more conserved environments, and higher than both of these conditions when animals are inside captive zoo environments. Gabriel *et al* (2017) found that lemurs (*Lemur catta)* living in high-density forest fragment had higher allostatic load than the low-density population. Dantzer *et al* (2013) showed that female red squirrels (*Tamiasciurus hudsonicus*) present 49% higher fecal cortisol levels in response to 75% higher population density. Dettmer *et al* (2014) saw an increase in hair cortisol and severe fight wounds due to high density housing condition in rhesus monkeys (*Macaca mulatta*).

Robust capuchin monkeys (*Sapajus spp*) are omnivorous, medium sized (3-4.5kg) neotropical primates with a wide distribution in South America (Lynch-Alfaro *et al* 2012). Presenting high neocortical ratio and exhibiting complex behavioural patterns (e.g. use of instruments, coalitions, behavioural traditions) these monkeys are focus of many studies in the areas of Cognition, Psychology and Comparative Evolutionary Anthropology (Fragaszy *et al* 2004). *Sapajus spp* is the second most present genus in rescue centers in Brazil (Levacov *et al* 2011), and colonies of endangered (and non-endangered) species are kept in diverse Latin American zoos (Ferreira *et al* in press). The socio-ecological characteristics of the clade, such as day range of 2.5km and large home range (up to 900ha - Izar *et al* 2012), manipulative foraging (Lynch-Alfaro *et al* 2012), and large and complex social groups (Fragaszy *et al* 2004), make these animals prone to increased stress when in captivity (Pomerantz *et al* 2013; Mason 2010).

In a study regarding their potential reaction to human presence while living in forest fragment, Izar *et al* (2012) show that two groups of free-living capuchin monkeys (one composed by *Sapajus libidinosus* and the other by *S. nigritus*) differed in their monthly rate of alarm calls, with the group of *S. libidinosus* vocalizing more alarm calls. Upon further inspection, about 38% of the *S. lididinosus* group’s alarm calls were in response to human disturbance (Izar *et al* 2012). This suggests that humans can be a source of threat and stress to capuchin monkeys, as was predicted by Chamove *et al* (1988) for zoo animals of small size compared to humans.

Previous studies show that capuchin monkeys exhibit varied levels of behaviours indicative of stress, ranging from 54% of activity budget in single housed animals, to 10%-17% in group housed animals, with minor sex differences reported (Rimpley & Buchanan-Smith 2013; Pomerantz *et al* 2013; Sorrentino *et al* 2012; Ferreira *et al* 2016). The way they respond to tension inducing situations is influenced by the predictability of these stressful situations, such as husbandry practices (Rimpley & Buchanan-Smith 2013; *Sapajus apella*) and feeding times (Ulyan *et al* 2006; *Cebus apella*), with animals showing less anxious behaviour (such as self-scratching) when these situations are made easier to predict. During acute crowding conditions, captive capuchin monkeys (*Cebus apella*) adopt a social avoidance strategy wherein they decrease affiliative and aggressive social behaviours (Van Wolkenten *et al* 2006). Black-capped capuchin monkeys show lower intragroup aggression and FMG levels when a one-way vision screen is put in their zoo enclosures (Sherwen *et al* 2015), thus lowering their stress by giving them a bigger ability to hide from zoo visitors. Sherwen *et al* (2015) found that visitor number (but not visitors' noise levels near the enclosure) is stressful for capuchin monkeys.

In this manuscript, we describe analyses of behavioural patterns and fecal glucocorticoid concentration in a sample of seven groups of captive brown capuchin monkeys (*Sapajus libidinosus*), kept under two different conditions: one group in a zoo living in large area with access to visitor, two groups in a zoo living in a quarantine enclosure, with less space but with access to visitors, and four groups kept in rescue centers, with less space available and no access to visitors (except the care takers and researchers).

We tested the hypothesis that both social crowding and visitors will impose allostatic costs to animals, shown as increases in stress related behaviour frequencies and in cortisol concentrations. As groups were under different conditions, by selecting models of multiple linear regression analyses we aim to distinguish the effect of each factor separately: sex, density and visitor effect. Analyses including social organization parameters and individual differences in coping strategies are treated elsewhere (Chagas *et al.* in prep.; Haeberlin *et al.* in prep).

Materials and methods

Experimental subjects and captive enclosures

We collected data on 18 male and 12 female capuchin monkeys (*Sapajus libidinosus*), distributed across seven groups allocated on CETAS of Natal (RN) and João Pessoa (PB), as well as enclosures of different sizes in zoo Parque Zoobotânico Arruda Câmara (PB). Data were collected between 2014 to 2016 (for the specific data collection periods for each group, see Table 1). All enclosures had their density and number of animals noted for statistical analysis (Table 1).

Table 1 – capuchin monkeys used in this study, divided by: their group (cage), number of adult females and adult males, total number of individuals in their group (including infants and juveniles), cage volume (m³), cage density and access to visitors (0 = no access; 1 = access).

Group	# adult females	# adult males	Total # animals	Cage volume	Cage density	Visitor access	# fecal samples	Sample collection period	Location	Observation hours per animal
1	2	2	4	50	0.08	0	115	Sep/2013 to Nov/2013	CETAS (RN)	6
2	1	1	5	45	0.11	0	50	Sep/2013 to Nov/2013	CETAS (RN)	6
3	3	6	9	45	0.2	0	701	Nov/2015 to Jan/2016	CETAS (PB)	4.2
4	2	2	6	45	0.1333	0	407	Nov/2015 to Jan/2016	CETAS (PB)	4.2

5**	4	2	8	112	0.0714	1	134	Oct/2015 to Jan/2016	Zoo (PB)	15.8
6	1*	2	3	4	0.75	1	36	Aug/2015 to Sep/2015	Zoo (PB)	15.8
7	0	3	3	4	0.75	1	73	Aug/2015 to Sep/2015	Zoo (PB)	15.8

* = This female's samples were not collected because she was pregnant, thus being deemed an outlier.

** = This enclosure did not have a ceiling, although it had a tree in the center the animals could climb on, so we used area (m²) instead of volume (m³) in this case.

All groups had a stable composition during the period of data collection. Fecal samples were collected throughout the day, from 07:30 in the morning to 17:50 in the afternoon, while the subjects were observed for behavioural data collection.

Behavioural sampling

Each individual was observed in their captive enclosure for 10 consecutive minutes each day by focal-scan method, with behaviour state (i.e. behaviours lasting more than 3 seconds) registered at every 30 seconds intervals, totaling 317.56 hours of behavioural observation.

We recorded all occurrences of behavioural events (i.e. behaviours lasting less than 3 seconds). For example, if a monkey makes head twirl three times successively, we note three separate head twirls. Ethogram is based in Ferreira *et al* (2016, 2018) and can be found in Table 1 of Supplemental materials.

Physiological analysis

Fecal samples (N= 675) were collected in eppendorf anytime an animal defecated (excluding times fecal samples were eaten before researcher could collect them), identified with name do the animal, hour and day of defecation, then stored in -5ºC on freezer under 2 hours after defecation. They were taken to the Hormonal Measures Laboratory (LMH) at UFRN for cortisol extraction based on Mendonça-Furtado *et al* (2017). Steroid metabolites were extracted by mixing 0.1 g of feces with 1 mL of methanol 80% (v/v), kept in the vortex for 30 minutes, followed by 10 minutes of centrifugation at 122633 g. Afterwards, 500uL of supernatant was collected in eppendorf and stored in the freezer (-20 ºC) until further analysis.

Samples were dosed in a competitive ELISA FMG assay that had been previously validated in Chagas (2016). We used the polyclonal anti-cortisol antibody (R4866; 1:15000) obtained from T. Ziegler at University of Wisconsin (EUA), which cross-reactivities can be found listed in Ziegler *et al* (1995). The horseradish peroxidase conjugate used (1:37500) was obtained from C. J. Munro (University of California, USA). Assay sensitivity was 27.63 ng/g of fecal sample. 96-well microtiter plates were coated with 100 µL antibody solution and incubated at 20ºC for 6 hours, followed by 18 hours of incubation at 8 ºC. Afterwards, plates were washed three times with wash solution (0.15 M NaCl, 0.05% Tween 20, pH 7.0), then each well was given 150 µl of EIA-buffer (0.1 M NaPO4, 0.149 M NaCl, 0.1% bovine serum albumin, pH 7.0). Plates were stored in freezer (-20 ºC) overnight.

Extracted samples were dosed by leaving the plates incubating on room temperature for 2 hours, then adding 25 µL of diluted samples (1:40 in EIA-buffer) with 275 µL of HRP-F in the aforementioned concentration. Plates were then incubated for 2 hours before being washed twice and filled with 100 µL of substract solution per well. After 40 minutes of final incubation, plates were read at 405 nm in a microplate reader (Bio-Tek®, Epoch). Inter CVs were 18% and 19% for high and low pools, and intra CV was < 10%.

Statistical analysis

We calculated the daily percentage of behavioural states and daily rates per hour of behavioural events. Behaviours that were calculated as daily rates (states) and number of events (event) had separate columns and were tested as different behavioural measures. We calculated mean, median, maximal and minimum cortisol level for each individual. In order to attend for normality and homocedasticity requirements, all cortisol levels were log-transformed. In order to standardize parameters, behavioural data, FMG levels (log) and cage density were transformed in their zscores before analysis. All values with $Z \geq \pm 3$ were excluded from analyses.

We used SPSS 21 program for a linear regression model selection, calculating models for FMG levels (mean, median, maximum and minimum levels) using visitor access (“access to visitors/closed to visitors”), sex (“male/female”) and/or population density inside the captive environment as predictor variables, while all behavioural measures and FMG measures were dependent variables. Best model for each FMG level and behavioural measure was decided according to lowest Akaike’s Information Criteria (AIC) value. In case two or more models had the same AIC value, the model with the least number of variables was considered the best model. All predictor variables from best model had to have p-value < 0.07 to be considered a significant model.

Results

FMG mean, median, maximum and minimum levels in nanograms per gram of fecal sample according to sex are arranged on Table 2. Two-tailed T-test shows there is no significant difference between female and male mean (t= -1.085; P=0.287), median (t= -1.428; P=0.164), maximum (t= -0.650; p=0.521) or minimum (t= -0.912; P=0.369) FMG levels. Another two-tailed T-test, this time between FMG levels for animals with and without visitor access, shows a significant difference regarding mean FMG levels (t= -2.557; p=0.016), with FMG levels being higher for animals with visitor access than for animals without visitor access. There was no significant difference for median (t= -1.696; p=0.101), maximum (t= -1.775; p=0.087) and minimum (t= -0.685; p=0.499) FMG values between animals with and without visitor access.

Table 2 – Mean, median, maximum and minimum FMG levels (in ng/g of fecal sample) for all individuals, female subjects and male subjects.

	Mean FMG	**Median FMG**	**Maximum FMG**	**Minimum FMG**
Total (n = 30)	7 067.651	2 697.49200	40 804.0333	542.3567
Male (n = 18)	8 962.830	2 962.315	53 777.231	608.874
Female (n = 12)	4 224.883	2 300.257	21 344.237	442.581

Best model to predict each FMG level can be found on Table 3. Cage density was the only predictor for median FMG values, wherein median FMG increases when cage density increases; however, p-value was higher than 0.08, so the model was considered weak and discarded.

Visitor access is the only variable capable of predicting mean and maximum FMG levels, but it’s p-value is only significant for mean FMG. Therefore, the regression model for maximum FMG was considered weak and discarded, while the best model for mean FMG was considered strong and kept (Fig. 1). In both cases, FMG values decrease when animals are not exposed to zoo visitors.

Sex could not predict any FMG variable, and no variable used could predict minimum FMG levels.

Table 3 – Akaike’s Information Criteria (AIC) per regression model. FMG is the dependent variable. Density (population density in the captive environment), Access (to visitors) and Sex are predictors. Best models for each dependent variable are in bold letters.

	AIC

FMG	**Mean**	**Median**	**Maximum**	**Minimum**
Sex	-//-	-//-	-//-	-//-
Access	**-2.868** Bno-access= -0.888 *P=0.016*	-//-	**0.230** Bno-access= -0.649 P=0.087	-//-
Density	-//-	**-3.137** B=0.795 P=0.084	-//-	-//-
Sex + Access	-2.868	-//-	0.230	-//-
Sex + Density	-//-	-3.137	-//-	-//-
Access + Density	-//-	-3.137	-//-	-//-
Sex + Access + Density	-//-	3.137	-//-	-//-

*"-//-" means none of the variables here tested was significant enough to appear on any of the regression models. All models only gave Beta0 values.

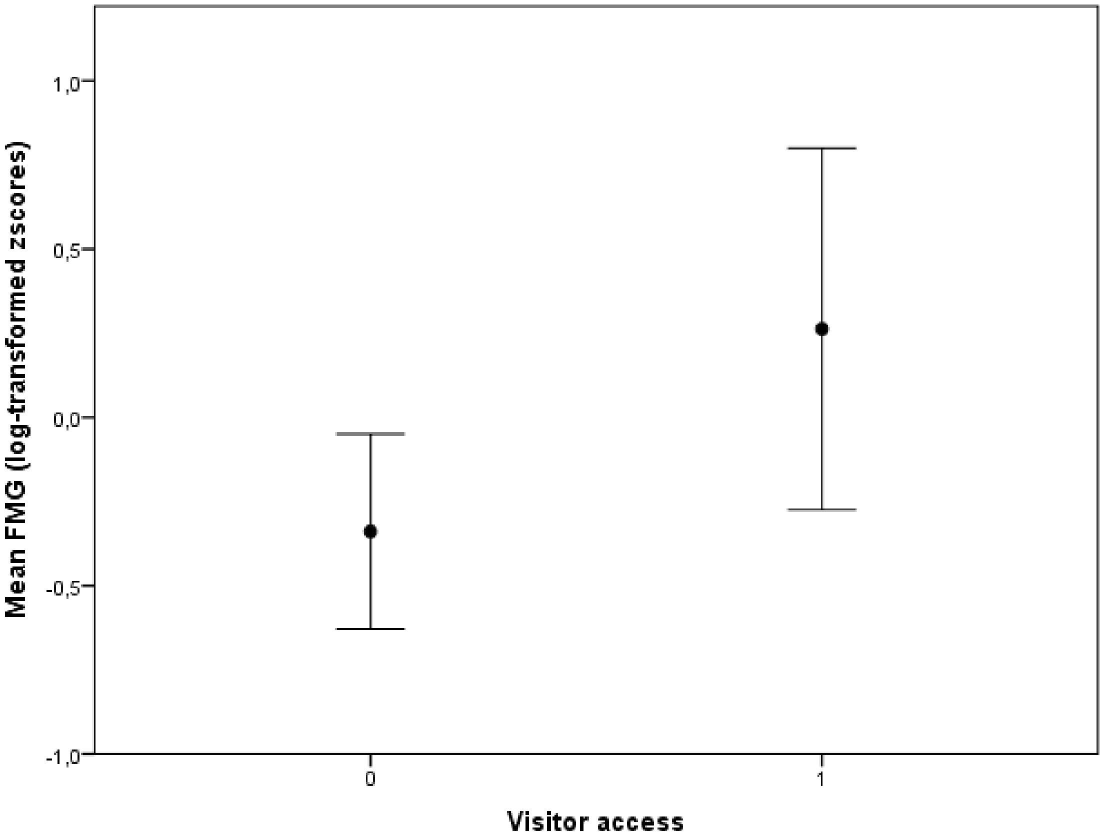

Figure 1 - Mean FMG values (log-transformed z scores) by access to visitors. Group 0 represents no access to visitors; group 1 represents access to visitors.

Out of the 32 behaviour variables, three could be predicted by Sex, eight could be predicted by Visitor access and 12 could be predicted by Cage density (all AICs presented in Table 2 of Supplemental materials). After comparing AIC values for regression models of each behavioural variable and discarding models wherein no p-values were significant or had a tendency towards statistical significance (P<0.07), we selected best models to predict each behavioural variable (Table 4).

Table 4 – Akaike's Information Criteria (AIC) per regression model. Each behaviour frequency works as a dependent variable in each model. Density (population density in the captive environment), Access (to visitors) and Sex are predictors. Best models for each dependent variable are in bold letters. Bna is Beta for no access to visitors; Bd is Beta for density.

	AIC						
Predictors / Behaviours	**Sex**	**Access**	**Density**	**Sex + Access**	**Sex + Density**	**Access + Density**	**Sex + Access + Density**
Eat	-//-	**-10.534** Bna=1.245 *P<0.000*	-3.860	-10.534	-3.860	-3.860	-3.860
Environment manipulation	-//-	-//-	**-25.110** Bd= -0.683 *P=0.015*	-//-	-25.110	-25.110	-25.110
Vigilance	-//-	**-32.167** Bna= -1.701 *P<0.000*	-20.951	-32;167	-20.951	-20.951	-20.951
Play alone	-//-	-//-	**-17.536** Bd= -0.821 *P=0.012*	-//-	-17.536	-17.536	-17.536
Scrounging	-//-	**-18.639** Bna= -0.514 P=0.064	-//-	-18.639	-//-	-//-	-//-
Sexual behaviour	-//-	**-19.791** Bna=0.515 P=0.059	-11.915	-19.791	-11.915	-11.915	-11.915
Retreat	-//-	**-//-**	**-24.007** Bd=0.702	-//-	-24.007	-24.007	-24.007

			P=0.012				
Self-scratching (event)	-1.054	-//-	**-11.017** Bd=1.325 *P=0.002*	-1.054	-11.017	-11.017	-11.017
Self-masturbation (event)	-//-	-//-	**-37.450** Bd=0.331 P=0.069	-//-	-37.450	-37.450	-37.450
Urine/fecces/sperm manipulation (event)	-//-	-//-	**-11.658** Bd=0.862 *P=0.022*	-//-	-11.658	-11.658	-11.658

* “-//-“ means none of the variables here tested was significant enough to appear on any of the regression models. All models only gave Beta0 values.

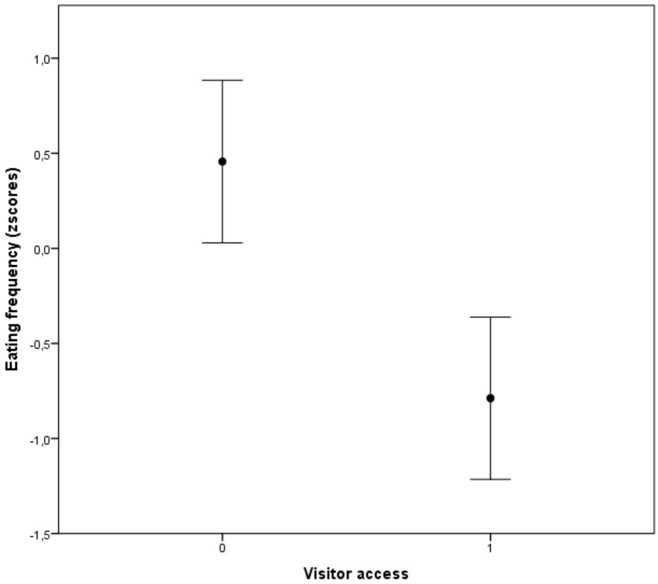

Figure 2 - Eating frequency (z scores) by access to visitors. Group 0 represents no access to visitors; group 1 represents access to visitors.

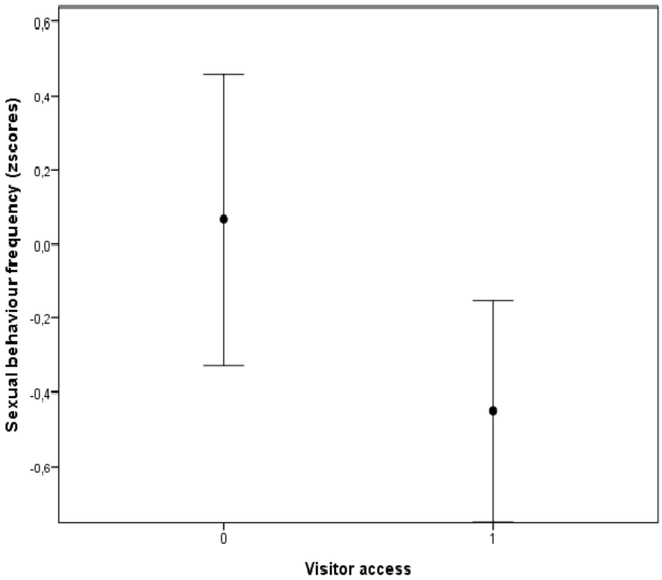

Figure 3 - Sexual behaviour frequency (z scores) by access to visitors. Group 0 represents no access to visitors; group 1 represents access to visitors.

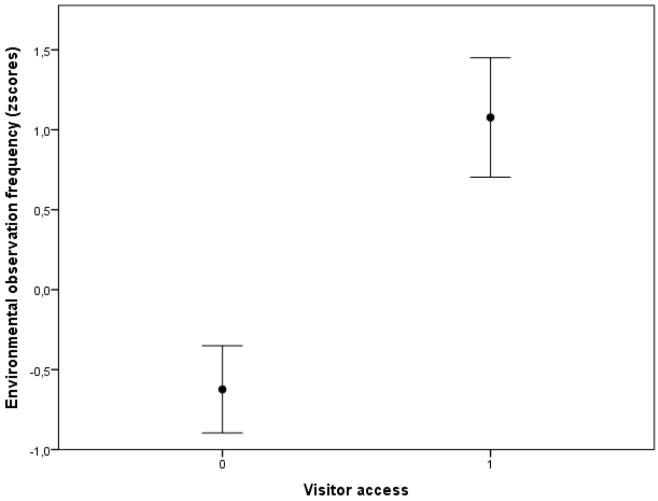

Figure 4 - Environment observation frequency (z scores) by access to visitors. Group 0 represents no access to visitors; group 1 represents access to visitors.

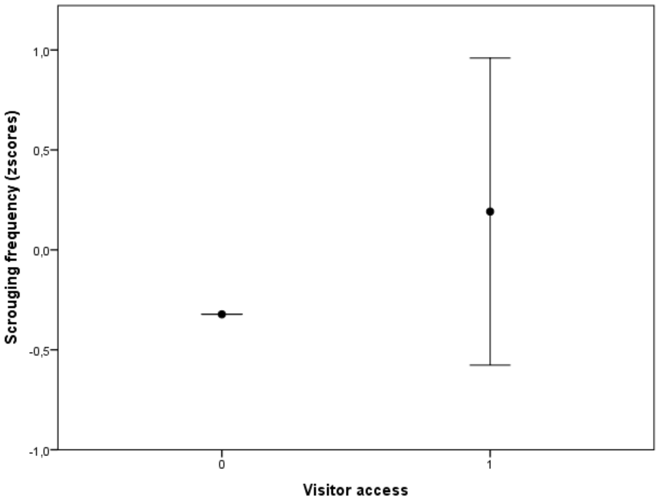

Figure 5 - Scrounging frequency (z scores) by access to visitors. Group 0 represents no access to visitors; group 1 represents access to visitors.

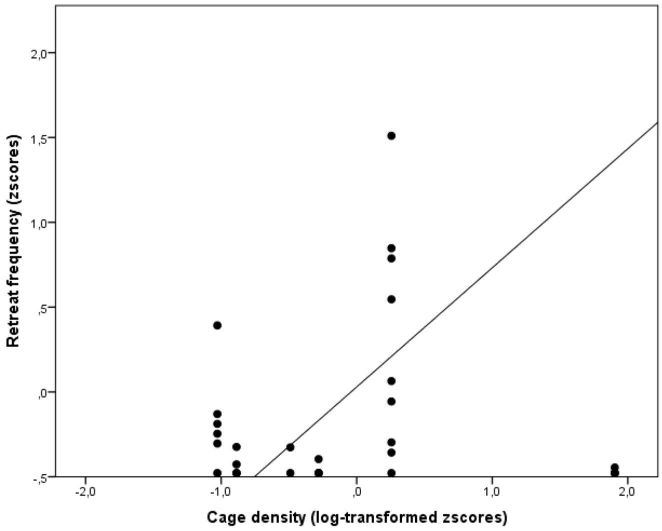

Figure 6 - Dispersion graph where cage density (log-transformed z scores) predicts retreat frequency (z scores).

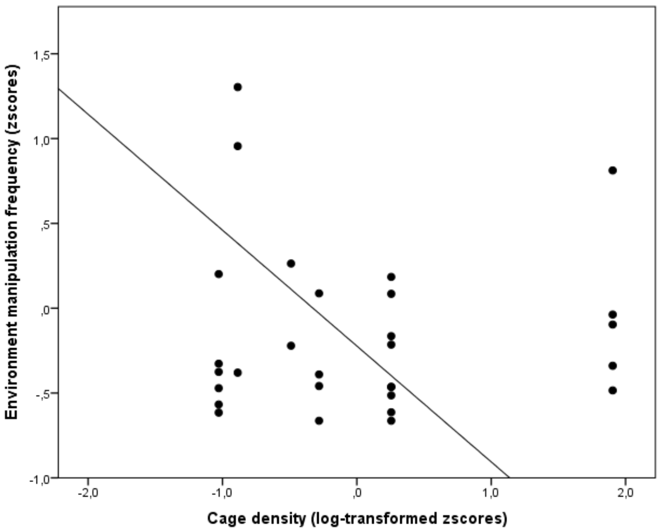

Figure 7 - Dispersion graph where cage density (log-transformed z scores) predicts environment manipulation frequency (z scores).

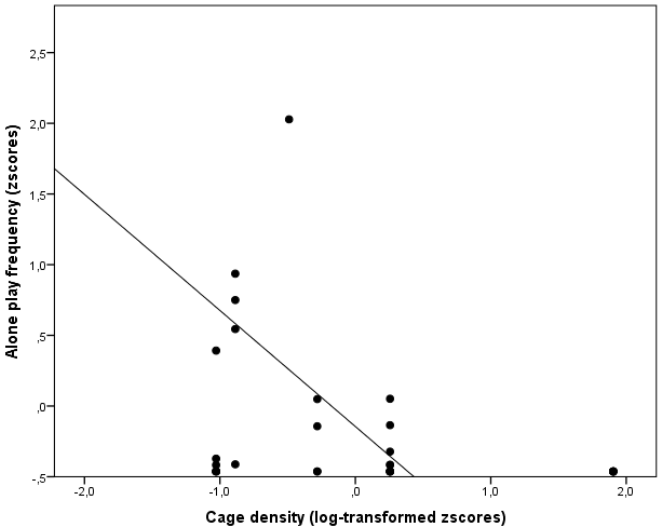

Figure 8 - Dispersion graph where cage density (log-transformed z scores) predicts alone playing frequency (z scores).

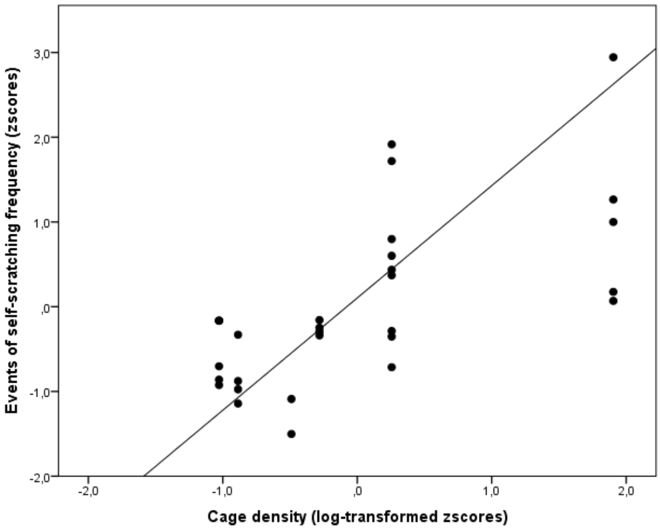

Figure 9 - Dispersion graph where cage density (log-transformed z scores) predicts events of self-scratching frequency (z scores).

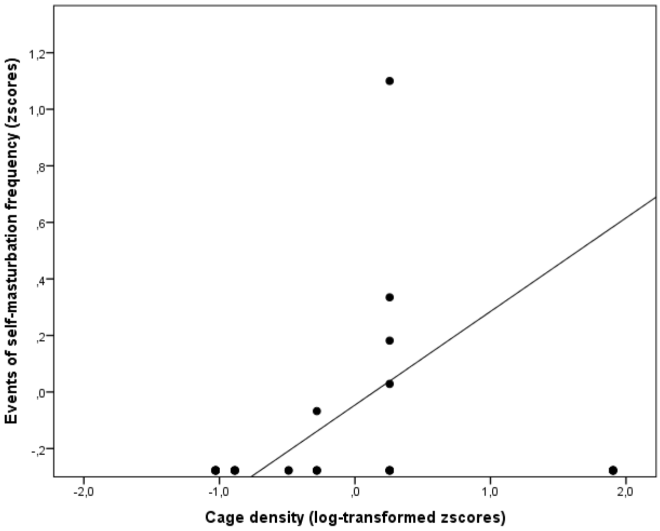

Figure 10 - Dispersion graph where cage density (log-transformed z scores) predicts events of self-masturbation frequency (z scores).

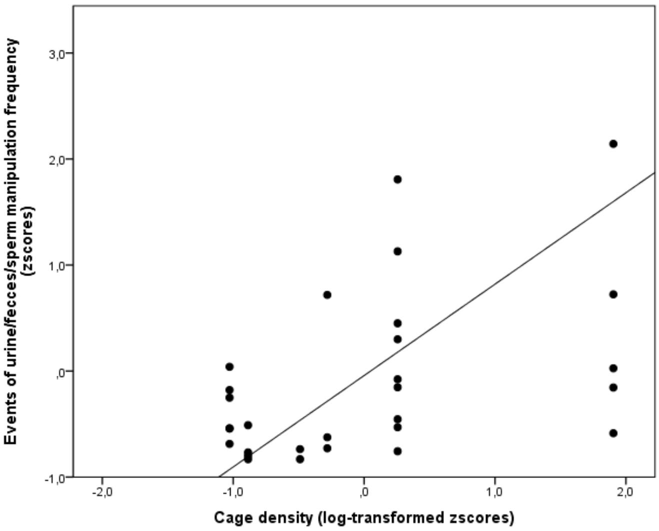

Figure 11 - Dispersion graph where cage density (log-transformed z scores) predicts events of urine/fecces/sperm manipulation frequency (z scores).

Absence of visitor access predicts more frequency of eating (Fig. 2) and sexual behaviour (Fig. 3), while also predicting less vigilance (Fig. 4) and scrounging (Fig. 5). Higher cage density predicts higher frequency of retreat behaviour (Fig. 6) and more events of self-scratching (Fig. 9), self-masturbation (Fig. 10) and urine/fecces/sperm manipulation (Fig. 11), while also predicting lower frequencies of environment manipulation (Fig. 7) and solitary play (Fig. 8).

Discussion

Using fecal metabolites of glucocorticoid levels as indicators of allostatic load, our data shows that visitor access was the best predictor for physiological stress levels, influencing mean concentrations of FMG. Regarding how that translated into individual behaviour, animals exposed to zoo visitors ate less, tended to express less sexual behaviour, tented to do more scrounging, and observed the environment more.

This pattern of more vigilance indicates a condition of alert and escape, since humans can be categorized as a random sources of stress that have to be constantly monitored, as suggested by Hosey (2000). Arboreal, small-sized primates like capuchins are predicted to be more prone to interpret humans as predators (Chamove *et al* 1988), a pattern corroborated by the rates of increased vigilance detected in this study. This increased vigilance could be compromising feeding behaviour by using the time to eat to be more vigilant. In his review, Hosey (2005) cites examples in which concealment screens and other types of barriers around the enclosure allowed the animals some control over whether they could or not be seem by the audience. The presence of these barriers diminished stress in captive capuchin. Sherwen *et al* (2015) also finds lowered glucocorticoid levels among captive capuchin monkeys after their enclosure receives a concealment barrier on one part of it.

Crowding did not significantly affect FMG values, however their effects on behavioural measures indicate a persistent behavioural stress response. Despite increased social partners, animals in high density presented more self-directed behaviours (scratching and masturbation) and more manipulation of urine/feces/sperm, while also interacting less with the environment and playing alone less. Self-scratching is a behaviour indicative of stress (Sorrentino *et al* 2012; *Cebus apella nigritus*), as well as urine/fecces/sperm manipulation (Prates & Bicca-Marques 2005; *Cebus apella*). Masturbation, reported as “extremely rare in captive capuchins housed in mixed-sex social groups” (Fragaszy *et al* 2004; p.240), can be considered a behaviour indicative of stress and has been used as such previously (e.g. Boinski *et al* 1999). Therefore, our subjects’ increase in events of self-scratching, urine/fecces/sperm manipulation and self-masturbation could mean the chronic activation of behavioural allostatic systems even as physiological ones stop responding to higher social crowding. It is interesting to note that, although we tested these behaviours both as events and as states, only events were predicted by social crowding.

The severity of crowding stress may be reduced by increased grooming exchange and social ties (Judge & de Waal 1997; *Macaca mulatta*) or avoidance strategies where intragroup aggression is prevented by avoiding any behaviour involving social contact, including positive ones (van Wolkenten *et al* 2006; *Cebus apella*). Out of the two main coping styles for social crowding, avoidance strategy is more common for short-term situations and tension reduction is more common for long-term situations (Crast *et al* 2015). Indeed, in another study on capuchin monkeys (*Cebus apella*) during short-term crowding, capuchins decreased both affiliation and aggression, adopting the conflict avoidance strategy (Van Wolkenten *et al* 2006). Since retreat increased with higher social crowding, while allogrooming and aggression (done and received) could not be predicted by cage density, our capuchin monkeys seem to have adopted a social avoidance strategy to cope with long-term crowding.

Sex of animals was not a strong predictor of allostatic load. However, there were two models in which sex was a significant predictor of behaviour: males tended to do more crouching (AIC= -47.020; b=0.306; p=0.068) and did more events of self-scratching (AIC= -1.054; b=0.748; p=0.043) than female conspecifics, both of which are behaviours indicative of stress (Sorrentino *et al* 2012 – scratching; *Cebus apella nigritus*) and fear (Blanchard & Blanchard 1969 - crouching; rats). However, none of them were best model for these behaviours (Table 5). It appears that in captivity, there is a tendency for any sort of sex difference in natural selection pressure to disappear while both sexes converge into the same type of coping behaviour (Uher 2008).

Allostatic overload type 2 occurs when animals live in environments with easier access to food and water in comparison to free environments, but with high frequency of random factors, such as predator risk (Wingfield 2005). In such cases there is a chronic elevation of glucocorticoid secretion that does not exceed the amount of energy available in the environment, however, it remains chronically high without triggering the emergency life-history stage required for the individual to lower allostatic load. Allostatic overload will only stop through environmental change or learning (Wingfield 2005). Our results indicate that captive capuchins have not gone through habituation. Instead, the physiological and behavioural stress response has persisted (both of them in the case of visitor exposure stress, and only behavioural stress response when it comes to crowding stress), a pattern expected for animals in the temporary permanence at rescue centers, but not so much for animals living for years in zoos.

Disruptions or distortions on behavioural and homeostatic parameters resulting from chronically high allostatic load jeopardizes the proposed functions of zoos of scientific education and reproduction of endangered species (and for laboratory centers, the function of generating reliable data). As a major concern of zoos, aquarium and research facilities is to keep the allostatic load of the captive animals at minimum, they must have in mind the capacity of human exposure, number, noise and behaviour to affect the behaviour and welfare of its captive species (Sherwen *et al* 2015; Quadros *et al* 2014). Identifying and disentangling the effects of different stress sources on captive animals is therefore a major quest for that species management and study practices.

Conclusion

In summary, our study shows that zoo visitors are strong stressors for capuchin monkeys, affecting their physiology and behaviour by resulting in increased vigilance and stereotypic behaviours while inhibiting eating and sexual behavior. These robust capuchins use avoidance strategies to cope with stress of crowded conditions; crowding is a further challenge that primates like *Sapajus libidinosus* must cope with. These allostatic pressures might be mitigated by changes in the captive environment, such as putting screens on zoo enclosures that make it impossible for the monkeys to see the visitors or making it so capuchins can stay above ground and looking down on visitors, making them seem less threatening.

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Supplemental materials

Table 1 - Ethogram used; based on Ferreira *et al*. (2016):

Genus normative behaviours	
Behaviours	**Definition**
Eat	Individual puts food inside its mouth and ingests
Food manipulation	Individual handles food without putting inside mouth during the interval
Forage	Individual moves around the cage searching for food without manipulating or eating it during the interval
Environment manipulation	Individual touches, moves, licks, bites or rubs objects or part of the environment
Vigilance	Individual rotates its head while looking at two or more spatial positions without doing any other behaviour, individual is alert and not in a resting position or hanging from a wire mesh of cages

Locomotion	Individual moves vertically or horizontally around the cage without manipulating environment or foraging; there is an end point to the movement and the path is not repeated right after
Allogrooming	Individual slowly manipulates the fur of another one with hands or mouth
Play alone	Individual is pushing or hanging on a rope, twig, branch or wires
Social play	Two or more individuals hold, grasp, chase and/or bite each other without aggression
Scrounging	Individual approaches another one that its eating and feeds from the food scraps that fall from their mouth or hands
Inactivity	Individual remains static in a relaxed position, may be lying, sitting or hanging in a mesh, eyes may be open or closed
Sexual behaviour	Individual crosses arms around chest and opens eyes wide while swaying from side to side in the direction of another individual; individual rides another individual, with possible genital contact
Proximity	Individual stands close to another individual
Retreat	Individual moves away from another individual to avoid being threatened by way of lunging, screaming or showing teeth
Displace	Individual moves in the direction of another individual, making them move from the place they were occupying
Receive aggression	Individual is hit, bitten or attacked by another individual
Do aggression	Individual hits, bites or attacks another individual
Behaviours potentially indicative of stress	
Behaviours	**Definition**
Aberrant locomotion (state)	Individual moves repeatedly across the same circuit inside the cage without an obvious goal
Pendulous movement (state)	Individual remains sitting while it sways body back and forth or side to side repeatedly for more than 3 seconds
Crouching (state)	Individual holds itself in a tense position with arms, legs and tail, eyes open while not moving body or observing the environment.
Self-masturbation (state)	Individual stimulates its own genitals for more than 3 seconds
Urine/fecces/ sperm manipulation (state)	Individual handles urine, fecces and/or sperm for more than 3 seconds
Head twirl (event)	Individual turns its own head while looking sideways or upwards repeatedly, The subject turns his head looking sideways and upwards, may occur while moving or sitting
Self-scratching (event)	Individual rubs or pulls fur or skin in its own body
Pirouette (event)	Individual rotates around itself in a complete 360◦ rotation, may occur while moving or sitting
Pendulous movement (event)	Individual remains sitting while it sways body back and forth or side to side repeatedly for less than 3 seconds
Self-masturbation (event)	Individual stimulates its own genitals for less than 3 seconds
Urine/fecces/sperm manipulation (event)	Individual handles urine, fecces and/or sperm for less than 3 seconds
Sexual display to humans (event)	Individual crosses arms around chest and opens eyes wide while swaying from side to side in the direction of a human
Total stereotypy (event)	Sum of the frequency of all event-type stereopypic behaviours described above
Total stereotypy (state)	Sum of the frequency of all state-type stereopypic behaviours described above

Table 2 – Akaike's Information Criteria (AIC) per regression model. Each behaviour frequency works as a dependent variable in each model. Density (population density in the captive environment), Access (to visitors) and Sex are predictors. Best models for each dependent variable are in bold letters. Bna is Beta for no access to visitors; Bd is Beta for density.

	AIC						
Predictors / Behaviours	**Sex**	**Access**	**Density**	**Sex + Access**	**Sex + Density**	**Access + Density**	**Sex + Access + Density**
Eat	1.126*	**-10.534** Bna=1.245 *P<0.000*	-3.860	-10.534	-3.860	-3.860	-3.860
Food manipulation	1.126*	**0.100**	3.495*	0.100	3.495*	3.495*	3.495*
Forage**	-11.810*	-11.810*	-6.041*	-11.810*	-6.041*	-6.041*	-6.041*
Environment manipulation	-39.905*	-39.905*	**-25.110** Bd= -0.683 *P=0.015*	-39.905*	-25.110	-25.110	-25.110
Vigilance	1.126*	**-32.167** Bna= -1.701 *P<0.000*	-20.951	-32;167	-20.951	-20.951	-20.951
Locomotion	1.126*	**0.045**	-10.566*	0.045	-10.566*	-10.566*	-10.566*
Allogrooming	1.126*	**0.058**	4.949	0.058	4.949	4.949	4.949
Play alone	-30.776*	-30.776*	**-17.536** Bd= -0.821 *P=0.012*	-30.776*	-17.536	-17.536	-17.536
Social play**	-203.408*	-203.408*	-117.149*	-203.408*	-117.149*	-117.149*	-117.149*
Scrounging	-17.205*	**-18.639** Bna= -0.514 P=0.064	-//-	-18.639	-//-	-//-	-//-
Inactivity**	-23.096*	-23.096*	-12.046*	-23.096*	-12.046*	-12.046*	-12.046*
Sexual behaviour	-18.196*	**-19.791** Bna=0.515 P=0.059	-11.915	-19.791	-11.915	-11.915	-11.915
Proximity**	1.160*	1.160*	2.867*	1.160*	2.867*	2.867*	2.867*
Retreat	-38.954*	-38.954*	**-24.007** Bd=0.702 *P=0.012*	-38.954*	-24.007	-24.007	-24.007

Displace	**-20.108**	-19.425*	-5.023*	-20.108	-5.023*	-5.023*	-5.023*
Receive aggression	1.160*	**0.186**	7.707*	0.186	7.707*	7.707*	7.707*
Do aggression	-29.290*	-29.290*	-10.532*	-29.290*	-10.532*	-10.532*	-10.532*
Aberrant locomotion**	1.126*	1.126*	5.550*	1.126*	5.550*	5.550*	5.550*
Pendulous movement**	-128.572*	-128.572*	-70.699*	-128.572*	-70.699*	-70.699*	-70.699*
Crouching	-47.020	-45.692*	**-57.810**	-47.020	-57.810	-57.810	-57.810
Self-masturbation**	-71.327*	-71.327*	-35.462*	-71.327*	-35.462*	-35.462*	-35.462*
Urine/fecces/sperm manipulation**	-20.157*	-20.157*	-18.353*	-20.157*	-18.353*	-18.353*	-18.353*
Head twirl (event)**	-10.466*	-10.466*	-3.180*	-10.466*	-3.180*	-3.180*	-3.180*
Self-scratching (event)	-1.054	1.126*	**-11.017** Bd=1.325 *P=0.002*	-1.054	-11.017	-11.017	-11.017
Pirouette (event)**	1.126*	1.126*	-0.740*	1.126*	-0.740*	-0.740*	-0.740*
Pendulous movement (event)**	-147.545*	-147.545*	-88.587*	-147.545*	-88.587*	-88.587*	-88.587*
Self-masturbation (event)	-71.135*	-71.135*	**-37.450** Bd=0.331 P=0.069	-71.135*	-37.450	-37.450	-37.450
Urine/fecces/sperm manipulation (event)	-13.324*	-13.324*	**-11.658** Bd=0.862 *P=0.022*	-13.324*	-11.658	-11.658	-11.658
Sexual display to humans (event)**	-63.483*	-63.483*	-29.834*	-63.483*	-29.834*	-29.834*	-29.834*
Total stereotypy (event)**	-10.671*	-10.671*	-3.822*	-10.671*	-3.822*	-3.822*	-3.822*
Total stereotypy (state)**	1.126*	1.126*	3.793*	1.126*	3.793*	3.793*	3.793*

* None of the variables here tested was significant enough to appear on any of the regression models. All models only gave Beta0 values.

** This behavioural measure could not be predicted by any variable tested here.

CAPÍTULO 3: Retreat, have sex and stay in proximity: social dynamics and physiological stress in captive capuchin monkeys (*Sapajus libidinosus*)

Abstract

Group life can involve tension due to fight for resources and partners, which need to be regulated by social support to keep the group stable. Establishing a dominance hierarchy can help control intragroup aggression in general, but it also aggregates different stressors for dominants and for subordinates. A variety of sociosexual behaviours such as social grooming, sexual behaviour, social play and proximity have been shown to reduce social tension and possibly ameliorate stress by regulating the physiological stress response. The present study tests physiological stress levels in captive capuchin monkeys (*Sapajus libidinosus*) to verify two hypothesis: dominant capuchins are more stressed than subordinate conspecifics; and positive social behaviours such as receiving or offering grooming and sexual behaviour would decrease stress. We collected behavioural and fecal samples from 45 subjects living in different enclosures around Rio Grande do Norte (Brazil) and Paraíba (Brazil). Using ELISA method on fecal samples, we calculated mean, median, maximum and minimum fecal glucocorticoid metabolite (FMG) levels from each individual. We calculated behavioural frequency of 10 social behaviours (positive and negative ones) and two self-directed behaviours (self-grooming and masturbation), as well as the rank position of 24 individuals. Selecting the best linear regression model for each FMG measure according to their Akaike Information Criteria, we found that: 1) rank could predict maximum FMG levels, being higher in dominants than in subordinates, which corroborates our first hypothesis that dominants are more stressed in this socially tolerant species; 2)from the twelve behaviours tested, only sexual behaviour, retreat, proximity and self-grooming were capable of predict any of the FMG measures, all of them decreasing stress with higher behavioural frequencies. Social grooming was not included in any of the best regression models. The mixture of submissive approaches such as retreat behaviour with socially positive behaviours such as sexual behaviour and proximity shows the complexity of the strategies captive capuchins use to reduce social tension.

Keywords: social stress, capuchin monkey, dominance hierarchy, social support.

1. Introduction

In a social group individuals must constantly balance two forces, aggregating pressures (e.g. increased efficiency in group defense of feeding sites, dilution effects) and pressures that disperse individuals (e.g. increased competition for food or mates), (Giraldeau and Caraco, 2000). These opposite pressures can lead to conflicts between individuals within a group. The establishment of dominance hierarchies is considered an evolutionary stable strategy that diminishes such costs, since future conflicts can be avoided based on results of previous conflicts (Majolo and Huang, 2017; Enquist and Leimar, 1990).

By definition, in a dominance hierarchy higher-ranking individuals have privileged access to resources such as food and breeding partners, while the lower-ranking members (subordinates) have fewer access and might regularly face aggression, or the threat of aggression from dominants (Albeck *et al.*, 1997; Prall and Muehlenbein, 2017). However, as dominance ranks are constantly being contested, studies differ over who is more stressed in the hierarchy (Creel, 2001). Some studies find higher stress levels (as measured by cortisol levels and behaviours potentially indicative of stress) down the hierarchy (Virgin and Sapolsky, 1997; *Papio anubis;* Sapolsky *et al.*, 1997*; Papio anubis*), others find that higher ranking individuals are more stressed (Coe *et al.*, 1979; spider monkeys; Muller and Wrangham, 2004; *Pan troglodytes*), while others can not find significant difference over stress levels along the hierarchy (Burgess, 2015; *Lemur catta*; Sapolsky, 1983; *Papio Anubis*; Brent *et al.*, 2011; *Macaca mulatta*).

Social hierarchies vary on how an individual achieves dominance (physical competition *versus* matrilineal inheritance *versus* by chance), on stability (stable *versus* unstable) and linearity (despotic *versus* egalitarian). Each of these factors affect what exactly constitutes dominance in a specific species or group and, consequently, how much stress dominants and subordinates are going through. This complexity is well demonstrated by Gesquiere *et al.* (2011), who analyzed cortisol levels as a way to quantify physiological stress in wild male baboons (*Papio cynocephalus*). The author found a non-linear relationship between stress and rank position, with middle ranking individuals showing less stress levels, and both the alpha male and the lowest ranking members had similarly high stress levels, albeit the reasons for higher cortisol levels were different between them. The alpha males were probably more stressed because of the higher agonism, the psychological stress of losing their high rank to a competitor and because of the mating opportunities, while the low-ranking subordinates' high stress levels were likely linked to less access food sources and mating opportunities (Gesquiere *et al.*, 2011). In stable social groups, dominant females and subordinate males are usually the ones to experience higher stress (Abbott *et al.*, 2003). Accordingly, Cavigelli and Caruso (2015)'s review shows that in unstable social groups, dominants males and subordinate females tended to show higher stress levels.

Another important aspect of group life and conflict avoidance is the maintenance of social bonds. Abbott *et al.* (2003) verified that subordinates are less stressed in a group when they have more access to sources of social support. This social support can occur by way of allogrooming, proximity and agonistic support (Majolo and Huang, 2017). In the case of allogrooming, primates can spend about 20% of their activity budget participating in grooming (Dunbar, 1991), which suggests this is an activity used not just to clean each other's fur out of ectoparasites and debris, but also to establish social bonds (Akinyi *et al.*, 2013). It has been shown that more social support can bring long-term benefits with heightened fitness levels, as measured by higher infant survival rate in *Papio cynocephalus* (Silk *et al.*, 2003). Social support also confers short-term benefits by ameliorating the effect of stress, since grooming can lower the activation of physiological responses responsible for cortisol release (Grandi *et al.*, 2015; Crockford *et al.*, 2017; Wittig *et al.*, 2016). Lower stress levels are detected when the animal is receiving grooming (Gust *et al.*, 1993; rhesus macaques), when it is grooming others (Shutt *et al.*, 2007; Barbary macaques; Wooddell *et al.*, 2017; rhesus macaques) and during both situations (Fontani *et al.*, 2014; cotton-top tamarins).

Social grooming is possibly the most studied positive social behaviour (Schino, 2001), but it is not the only one. Non-reproductive sexual behaviours like genital rubbing are intensified in captive bonobos (*Pan paniscus*), which suggests both social grooming and sexual behaviours might work as stress reducers and/or as conflict avoidance methods (Paoli *et al.*, 2007). Bonobos perform non-conceptive forms of sexual behaviour possibly as a means of tension regulation, specifically same-sex sexual behaviours among females can occur in socially tense periods of food competition (Manson *et al*., 1997). However, rates of genital contact do not correlate with salivary cortisol levels during periods of possible food competition in bonobos (Hohmann *et al*., 2009; *Pan paniscus*), so their actual effect for stress regulation rather than just conflict avoidance is still debatable.

A third positive social behaviour is social play. It has been theorized that playful activities in general are behaviours that only happen when primates are healthy, have access to enough nutrients and are free from stress, being a potential signal of positive welfare (Oliveira *et al.*, 2010). However, Held and Spinka (2011) argue the current literature shows conflicting evidence, indicating play behaviour has more complex functions than that. For example, social play also increases before co-feeding in bonobos, a socially tense situation, which suggests social play could regulate social stress (Palagi *et al.*, 2006).

A special case of stress reducing strategies runs in the opposite direction and regards self-directed behaviours and withdrawn from social interactions. Stereotypic behaviours in general and self-directed behaviours in particular have been used as a signal of poor welfare, possibly indicating the animal is doing it because they are unable to carry out species-normative behaviours (Pomerantz *et al.*, 2013). For example, Pizzutto *et al*. (2015) found a positive correlation between cortisol levels and rates of alopecia due to self-mutilation in captive chimpanzees. Maestripieri *et al.* (1992) theorizes self-directed behaviours like self-grooming and self-scratching could be signals of anxiety in primates, although they do not necessarily equal anxiety to stress. Mason and Latham (2004) suggested some forms of stereotypic behaviours might work as a type of “do-it-yourself” environmental enrichment in captive animals, in which case some of them might be successful forms of stress relievers that lower cortisol levels. Galvão-Coelho *et al.* (2017; *Callithrix jacchus*) for example found a negative correlation between self-grooming and fecal glucocorticoid levels in social isolation. Self-grooming also can be found in free-living primates (eg.: Fuente *et al.*, 2014; *Callithrix jacchus*), being a type of species normative behaviour instead of a stereotypy. However, for female and male olive baboons, Higham *et al.* (2009) and Ellis *et al.* (2011) did not find a correlation between fecal glucocorticoid levels and rates of the self-directed behaviours self-grooming, self-scratching, yawing and body shaking, nor between glucocorticoid levels and total rates of self-directed behaviour, which suggests the relationship between self-directed behaviours and the physiological stress response might not be as simple as expected.

Capuchin monkeys (*Sapajus spp.*) are medium sized, omnivorous neotropical primates considered detached theoretical models for evolutionary studies due to their high degree of encephalization and their ability to use tools both in captivity and in the wild (Fragaszy *et al.,* 2004). Capuchin monkeys tend to form matrilineal-tolerant, less linear social hierarchy (Izar *et al.,* 2006; 2012). They show great capacity for behavioural flexibility according to their environment (Izar and Ferreira, 2007; *Cebus apella* and *C. Nigritus; Lins and Ferreira, 2019; Sapajus flavius*). Males are usually dominant over females, except for the alpha female, who can be dominant over some adult and subadult males (O'Brien, 1991).

Lynch *et al.* (2002) did not find significant difference in cortisol levels between subordinate and dominant males in black horned capuchin monkeys (*Sapajus nigritus*) living in Atlantic forest, while Mendonça-Furtado *et al.* (2014) found that dominant males in bearded capuchin monkeys (*S. libidinosus*) living in Caatinga had higher levels cortisol levels than subordinates, both during unstable and stable periods in the hierarchy. In the phylogenetically close genus *Cebus*, composed of gracile capuchin monkeys, male dominants seem to have higher cortisol levels than subordinates (Schoof *et al.,* 2012; *Cebus capucinus;* Schoof *et al.,* 2014; *Cebus capucinus;* Schoof and Jack, 2013; *Cebus capucinus*), suggesting that among capuchin, social stress tends to go up the hierarchy. The dominant position can bring advantages; for example, it can affect infant survival (Janson, 1984; *Cebus apella*) and access to feeding sites (Izawa, 1992), with higher-ranking females having higher infant survival rates and access to food resources than female subordinates. However, the dominant position may also involve social stress, such as intervention in intragroup fights, which the alpha male does more than any other individual in the group (Ferreira *et al.,* 2008). It can be that, at least in the case of male capuchin hierarchy, being the alpha male comes with many energetically costly activities, which is why their stress levels tend to be higher.

Female capuchins do more grooming than male conspecifics, showing a preference for grooming other females or immatures in the group (O'Brien, 1991; di Bitetti, 1997). According to Tiddi *et al.* (2012) grooming behaviours in capuchins has two functions. Long lasting grooming bouts are indicative of affiliation between partners, with subordinates offering long bouts of grooming to dominant individuals. Fast grooming bouts seems to work as a signal of tolerance, and they are requested by subordinates and offered by dominants, mainly near feeding sites. As most bouts of grooming are of short duration, grooming runs down the hierarchy, a pattern contrary to observed in old world monkeys (Seyfarth, 1977; O´Brien, 1993, Parr *et al*., 1997). This bargain seems to be influenced by the sex of the participants, as dominant individuals of either sex showed more tolerance for subadults and juvenile males than for females (Tiddi *et al*., 2011; *Sapajus nigritus*). Receiving grooming also seems to have a calming effect on capuchin monkeys, as shown in Schino *et al.* (2016; *Sapajus spp*.), wherein receiving larger quantities of grooming increased "optimism" in their performance during cognitive bias tests.

Capuchin monkeys' sexual behaviour is slightly different from other primates' in that females during periovulatory phase are the ones to court their chosen male (usually, the alpha male), who initially ignores them instead of immediately reciprocating their proceptive behaviour (Falótico and Ottoni, 2013. After copulating with the alpha male, female capuchins start showing proceptive behaviour towards subordinate males in the group, copulating with several of them on the same day (Carosi *et al.,* 2005), a pattern interpreted as an attempt at paternity confusion to avoid male aggression against their infants. Sexual behaviour in capuchin monkeys also occurs outside of the female's ovulating phase, during socially tense situations such as post-fight reconciliation and during play or affiliation, indicating it might be important for social functions other than as a breeding behaviour (Fragaszy *et al.,* 2004). Same-sex sexual behaviour has also been reported in *Cebus apella* (Fragaszy *et al.,* 2004) and *Cebus capucinus* (Manson *et al.,* 1997), especially among male capuchins post-fights and during coalition formation (Manson *et al.,* 1997).

Sapajus spp. is the second most common primate genus found in Brazilian rescue centers and zoos (Levacov *et al.,* 2011). They spend more time foraging and eating in captivity than in semi-free condition, which might suggest captive capuchins are doing hyperphagia (Tárano and López, 2015; Ferreira *et al.,* 2018). Crowding and visitor exposure are factors that have been shown to cause stress in these captive animals, with the latter increasing fecal glucocorticoid levels and vigilance frequency while the former increases events of self-scratching, self-masturbation and urine/fecces/sperm manipulation (Chagas in prep). Self-directed behaviours (such as self-grooming and masturbation) are used as a stress coping mechanism and as a stress indicator, respectively (Quadros *et al.,* 2014; Boinski *et al.,* 1999). In captive environments, and particularly for rescue centers, group composition changes frequently. Therefore, the study of how animals minimize their social stress is of paramount importance for management practices.

In this study we analyzed if rank, social grooming and sexual behaviours are predictors of physiological stress in captive capuchin monkeys (*Sapajus libidinosus*), as quantified through their fecal metabolite glucocorticoid (FMG) levels. Unlike previous studies on the subject, verifying dominance hierarchy in male capuchins and social grooming in female capuchins, we will analyze both dominance and grooming in males and females together. We predicted that higher ranking individuals will be more stressed than subordinates, and that increased engagement in socio-sexual behaviours such as social grooming and sexual behaviour is related to decreased stress levels.

2. Material and methods

2.1 Experimental subjects

We collected data from 45 adult capuchin monkeys (*Sapajus libidinosus*), 25 males and 20 females, all of them located on CETAS of Natal (RN), CETAS of João Pessoa (PB) or at Parque Zoobotânico Arruda Câmara (PB) (for each group's specific sample collection period, see Table 1).

Table 1 – capuchin monkeys used in this study, divided by location, number of adult females and adult males, total number of samples collected and observation hours.

Location	# of adult females	# of adult males	# of fecal samples	Sample collection period	Hours of observation
CETAS (RN)	8	7	158	Oct/2016 to Nov/2016 and Feb/2017 to Sep/2017	3.0h/individual
CETAS (PB)	8	11	449	Sep/2013 to Nov/2013 and Nov/2015 to Jan/2016	4.2h/individual
Zoo (PB)	4	7	226	Aug/2015 to Jan/2016	15.8h/individual

Fecal samples were collected from 07:30 in the morning to 17:50 in the afternoon, while the subjects were observed for behavioural data collection. In total, we collected 362.64 hours of behavioural data and 833 fecal samples.

2.2 Physiological analysis

Fecal samples were collected in eppendorfs, identified with name do the animal, day and hour of defecation, before being stored in -20 ºC before a maximum of 2 hours post-defecation, until time for ELISA procedure. They were taken to the Hormonal Measures Laboratory (LMH) at UFRN for cortisol extraction. Based on Mendonça-Furtado *et al.* (2017): glucocorticoid metabolites were extracted by mixing 0.1g of feces with 1mL of methanol 80% (v/v), mixed in the vortex for 30 minutes, then centrifugated for 10 minutes at 122633 g. Afterwards, 500uL of supernatant were collected in eppendorf and stored at -20 ºC.

Samples were dosed using competitive ELISA assay for fecal metabolites of glucocorticoid (FMG), previously validated in Chagas (2016) for *S. libidinosus*. We used the polyclonal anti-cortisol antibody (R4866; 1:15000) from T. Ziegler (University of Wisconsin, EUA), which cross-reactivities can be found listed in Ziegler *et al.* (1995). The horseradish peroxidase conjugate used (1:37500) was obtained from C. J. Munro (University of California, USA). 96-well microtiter plates were coated with 100 µL antibody solution and incubated at 20ºC for 6h, followed by 18 hours of incubation at 8 ºC. Plates were then washed three times with wash solution (0.15 M NaCl, 0.05% Tween 20, pH 7.0) before each well was filled with 150 µl of EIA-buffer (0.1 M Na-PO4, 0.149 M NaCl, 0.1% bovine serum albumin, pH 7.0). Plates were stored at -20 ºC at least overnight before further analysis.

Samples were dosed by plate incubating on room temperature for 2 hours, then adding 25 µL of diluted samples (1:40 in EIA-buffer) and 275µL of HRP-F. Each sample was duplicated in their plate. Plate was incubated for 2 hours before being washed twice in the same wash solution prepared for sensibilization, then filled with 100µL of substract solution per well. Plate passed through a final incubation period 40 minutes long, before being read at 405 nm in a microplate reader (BioTek®, Epoch). Inter CVs were 18% and 19% for high and low pools, and intra CVs < 10%.

2.3 Behavioural analysis

Subjects were observed from the year 2013 to 2017 (Table 1). Each individual was observed in their captive enclosure for 10 consecutive minutes by focal-scan method, with behaviours lasting longer than 3 seconds (states) registered at every 30 seconds intervals. Behaviours that were too fast (lasted 3 seconds or less to complete) were categorized as events and were recorded at an all occurrence basis. For events, rates per hour were calculated per day of observation, and daily percentages of scans in behavioural states records were calculated for each animal. Both event- and state-type behavioural variables resulted in individual relative frequencies for each behaviour. The ethogram used for data collection is based on Ferreira *et al.* (2016; 2018) and can be seen in Table 2.

Table 2 – ethogram used in this study.

Behaviour	**Description**
Offers grooming (state)	Slowly manipulates the fur of another individual with hands or mouth
Receives grooming (state)	Has their fur slowly manipulated by another individual with hands or mouth
Sexual behaviour (state)	Opens eyes wide and arms folded across chest while doing writhing and swaying body movements towards another individual; ride on top of another individual, with possible genital contact
Scrounging (state)	Approaches another individual eating and feeds from the food that falls from their mouth or hands on the ground
Social play (state)	Two or more individuals hold, grasp, chase or bite each other without aggression
Retreat (event)	Moves away from another individual to avoid or after being threatened by way of lunging, screaming or showing teeth
Displace (event)	Moves in the direction of another individual, making them move from the place they were occupying
Does aggression (event)	Hit, bite or attack another individual
Receives aggression (event)	Is hit, bitten or attacked by another individual
Proximity (state)	Stands close to another individual
Masturbation (state)	Stimulation or manipulation of their own genitals for more than 3 consecutive seconds
Masturbation (event)	Stimulation or manipulation of their own genitals for less than 3 consecutive seconds
Self-grooming (state)	Repetitive slow manipulation of their own fur with hand or mouth

Dominance rank was calculated by modified David's score on SOCPROG 2.8 (Whitehead, 2016), based on a dominance matrix for each group, which was done based on the result of agonistic and approach-avoidance interactions. Ranks were then manually scored on excel ranging from one to nine, with the number one position being the highest modified David's score value in the group, and thus the most dominant position in the group (the alpha position). Out of the 45 animals from 14 groups analyzed, only

30 subjects from 11 groups were ranked. Each of these individual's position can be found on Table 3.

Table 3 – Individual hierarchical position by captive group. Rank 1 is the alpha position, while rank 9 is the most subordinate position. There are gaps in groups 7 to 11 because not all individuals had fecal samples collected.

Group	1	2	3	4	5	6	7	8	9
1	Hulk	Lula	Ester	Soleil	-//-	-//-	-//-	-//-	-//-
2	Golias	Queen	-//-	-//-	-//-	-//-	-//-	-//-	-//-
3	Buraco	Maguila	Júnior	Sandy	Sanfona	Cláudio	Libi	Inês	Gal
4	Catra	Jony	Tufo	Ramos	-//-	-//-	-//-	-//-	-//-
5	Mi	Romário	-//-	-//-	-//-	-//-	-//-	-//-	-//-
6	Sivuca	Acordeon	Cabelinho	-//-	-//-	-//-	-//-	-//-	-//-
7	Wildão	0000	0000	0000	-//-	-//-	-//-	-//-	-//-
8	0000	Big	Dedinho	0000	-//-	-//-	-//-	-//-	-//-
9	0000	Lola	0000	0000	-//-	-//-	-//-	-//-	-//-
10	Peruca	0000	0000	0000	-//-	-//-	-//-	-//-	-//-
11	0000	0000	Juan	0000	0000	-//-	-//-	-//-	-//-

- "-//-" means there are no animals with this rank in this group.

** "0000" means the animal in this rank could not have it's fecal samples analyzed.

2.4 Statistical analysis

Data collected on each subject's FMG level was log transformed in order to attend normality and homocedasticy requirements. Behavioural frequencies and FMG levels were Z-transformed before analysis. All samples with $Z > \pm 3$ were considered outliers and excluded from analyses.

We used SPSS 21 program for an exploratory independent two-tailed T-test between behavioural frequencies and FMG values of male and female subjects ($p<0.05$), before doing a linear multiple regression model selection, calculating models for FMG levels (mean, median, maximum and minimum levels) using 10 social behaviour categories related with allogrooming, intragroup aggression and proximity (Table 2), three self-directed behaviours (masturbation as a state, masturbation as an event, and self-grooming as a state), rank (1 to 9) and sex ("male/female") as predictor variables. After finding which of the 15 individual variables were strong enough to be capable of forming a model, we built models using combinations of variables, to test if the joint effect is greater or lower than the individual effect.

Best model for each FMG level was decided according to lowest Akaike's Information Criteria (AIC) value. Additionally, all variables in the best model must be either significant (p=0.05) or have a tendency towards significance (p<0.07); in case one variable does not have it, the model is discarded for the next best model according to AIC values. In case two or more models had the same AIC value, the one with the fewest variables was considered best model.

3. Results

According to exploratory t-test, only Displace was significantly different between males and females (t= -2.171; p=0.048), with males doing displacement more frequently than female conspecifics. All other behaviours did not offer a p-value lower than 0.05 (view Supplementary data, Table 1). However, neither Sex nor Displacement were significant predictors of any physiological variables (mean FMG, median FMG, maximum FMG and minimum FMG) during exploratory regression model selection.

Out of the 13 behaviours (Table 2), 10 were individually capable of predicting at least one of the physiological measures, with seven of them presenting a significant p-value (p=0.05) and two a tendency towards significance (p<0.07) in some of the models (Table 4). Retreat, Receive grooming and Masturbation (state) could form at least one regression model individually, although none of these models was statistically significant or had a tendency towards significance (see Table 2 of Supplementary data for all AIC values of regression models with one predictor), so they were kept for further multi-variable model selection. Sex alone could not predict any of the physiological variables, but dominance rank alone could predict maximum FMG values, with a tendency towards significance.

Table 4 – Regression model according to FMG measure and predictor behaviour. FMG measures are in log-transformed zscores. Behaviour frequencies are in zscores. Each model displays, in order: AIC value, beta and p-value. Models with significant p-value are in italics.

Predictors	**Mean FMG**	**Median FMG**	**Maximum FMG**	**Minimum FMG**
Does aggression	-//-	AIC= -6.071; B= -1.667; *P=0.003*	-//-	AIC= -0.091; B= -1.033; P=0.075
Receives aggression	-//-	-//-	AIC= -1.270; B= -1.124; *P=0.038*	-//-

Offers grooming	AIC= -12.436; B= -1.022; *P=0.025*	AIC= -0.746; B= -1.001; P=0.051	AIC= -0.075; B= -0.917; P=0.075	-//-
Self-grooming	AIC= -13.264; B= -1.729; *P=0.016*	AIC= -0.989; B= -1.640; *P=0.045*	AIC=0.881; B= -1.259; P=0.127	-//-
Sexual Behaviour	AIC= -14.589; B= -1.071; *P=0.008*	AIC= -14.873; B= -1.772; *P<0.000*	-//-	AIC= -0.941; B= -0.920; *P=0.046*
Social Play	AIC= -10.168; B= -0.696; P=0.090	AIC= -5.193; B= -1.268; *P=0.005*	-//-	-//-
Proximity	AIC= -16.485; B= -0.432; *P=0.003*	AIC= -7.690; B= -0.520; *P=0.001*	-//-	AIC= -11.915; B= -0.599; *P<0.000*
Rank	-//-	-//-	AIC= -0.353; B= -0.160; P=0.064	-//-

- -//- means this predictor variable was not capable of forming a model.

We combined all variables capable of individually predicting each of the same FMG measure, building models with two or more predictors. There were in total 91 models with more than one predictor for mean FMG, 50 models for median FMG, 25 models for maximum FMG and 11 models for minimum FMG (for AIC values of all models tested with more than one predictor, see Tables 3 to 6 of Supplementary data). Putting these additional models' results into consideration, best model for Mean FMG levels was: Retreat + Proximity + Self-grooming (AIC= -28.729); for Median FMG levels: Sexual Behaviour + Proximity (AIC= -25.333); for Maximum FMG levels: Rank + Self-grooming (AIC= -1.999); and for Minimum FMG: Sexual Behaviour + Proximity (AIC= -13.523). All predictors mentioned above predicted decreased cortisol. These AIC values are lower than those using variables individually, indicating the combined influence of behaviours on FMG levels (See tables 5 to 8).

Table 5 – Predictor variables for best regression model for mean FMG. Significant p-values are in italic.

Predictor	**Beta**	**p-value**	**R**
Retreat	-0.688	*0.020*	0.162
Proximity	-0.456	*<0.000*	0.412
Self-grooming	-2.268	*<0.000*	0.426

Table 6 – Predictor variables for best regression model for median FMG. Significant p-values are in italic.

Predictor	**Beta**	**p-value**	**R**

Sexual Behaviour	-1.639	*<0.000*	0.630
Proximity	-0.457	*0.001*	0.370

Table 7 – Predictor variables for best regression model for maximum FMG. Significant p-values are in italic.

Predictor	**Beta**	**p-value**	**R**
Rank	-0.186	*0.030*	0.567
Self-grooming	-1.538	0.056	0.433

Table 8 – Predictor variables for best regression model for minimum FMG. Significant p-values are in italic.

Predictor	**Beta**	**p-value**	**R**
Sexual Behaviour	-0.754	0.058	0.188
Proximity	-0.570	*<0.000*	0.812

4. Discussion

Individual sex could not predict cortisol levels, which indicates small sexual dimorphism in capuchin monkeys' stress response in captivity. As the alpha position received the number one (1) in hierarchy, the decrease of cortisol found when rank number increases means the higher-ranking dominance position correlates to maximum FMG levels. This means our study subjects show dominant members as the most stressed ones in the hierarchy, which corroborates results from Mendonça-Furtado *et al.* (2014; *Sapajus libidinosus*), Schoof *et al.* (2012, 2014; *Cebus capucinus*) and Schoof and Jack (2013; *Cebus capucinus*), who also found higher stress levels up the hierarchy in wild male capuchins. Rank was retained as a significant predictor in multivariable model for maximum FMG, which might mean rank has a particular effect on peak stress levels among capuchin monkeys. The increased stress in higher ranking individuals in stable social groups is typical of a more tolerant egalitarian society (Abbott *et al.*, 2003; de Vries *et al*., 2003). In the case of capuchin monkeys, where social hierarchy is well determined and there is low intragroup aggression (Izawa, 1980; *Sapajus spp*. – formerly *Cebus apella*), the alpha position can aggregate social stress because of responsibilities such as breaking down fights between group members (Ferreira *et al.*, 2008)..

Bergman *et al.* (2005) found no correlation between fecal glucocorticoid levels and rates of aggression done or received in wild male chacma baboons. In our study, doing aggression predicted lower cortisol levels (specifically, median and minimum

FMG), and previous literature shows displacement aggression can be used, primarily by dominants, to ameliorate stress (Virgin and Sapolsky, 1997; *Papio anubis*). However, it was a weak predictor which was not retained in any of the four best models. Curiously enough, receiving aggression could predict lower maximum cortisol levels, going against our predictions; but, like doing aggression, receiving aggression did not appear in any of the four best models. On the other hand, retreat behaviour was included in multi-variable models predicting mean FMG. Higher frequencies of retreat lowered cortisol instead of increasing it, indicating capuchin monkeys, especially subordinate capuchins, may be using a conflict avoidance strategy as a way to avoid social stress (Majolo and Huang, 2017; Enquist and Leimar, 1990).

Offering and receiving grooming ameliorates stress levels, with higher frequency of grooming predicting lowered mean, median and maximum FMG levels (Shutt *et al.*, 2007; Wooddell *et al.*, 2017; Fontani *et al.*, 2014) and receiving grooming predicting lower minimum FMG levels, corroborating previous studies (Shutt *et al.*, 2007; Wooddell *et al.*, 2017; Fontani *et al.*, 2014; Gust *et al.*, 1993). However, social grooming's effect was not as strong as previously expected, since neither offered nor received grooming were retained in any of the best regression models for cortisol. Since in capuchin monkeys grooming runs down the hierarchy (Tiddi *et al.*, 2012), the effect of social grooming may be derived from the rank effects. Social grooming’s stress reducing effects might also have been lost in this study because we only took long-term grooming bouts, losing short-term grooming bouts related with social tolerance (Tiddi *et al.*, 2012). In comparison, self-grooming was a stronger predictor of cortisol, being able to predict lower mean, median and maximum stress levels, and it was retained in the best models for mean and maximum FMG levels. Self-grooming is a self-directed behaviour expressed by some mammals when they are experiencing negative emotions (Maestripieri *et al.*, 1992). It also has a buffering effect on HPA axis activation, as seem in captive juvenile common marmosets (*Callithrix jacchus*) during periods of social isolation, where self-grooming rates correlated negatively with fecal glucocorticoid levels (Galvão-Coelho *et al.*, 2017). Their stronger effect over maximum FMG levels in this study could mean self-grooming has a blunting effect over HPA axis reactivity, lowering peak values.

Social play was another behaviour which lowered cortisol levels when alone (mean and median FMG) but, like social grooming, it did not have a strong enough effect to appear in any of the four best multi-variable regression models. Social play has been shown to increase in primates before stressful events as a coping strategy that prevents stress from escalating into intragroup competition, working as a potential short-term stress regulator (Palagi *et al.*, 2006; *Pan paniscus*) and as an indicator to researchers of positive primate welfare (Norscia and Palagi, 2011; *Callithrix jacchus*; Yamanashi *et al*., 2018; *Pan troglodytes*). However, it is possible that the energy required to perform social play masks their calming effect on the HPA axis, although that does not necessarily mean it is not useful as a conflict avoidance strategy.

Sexual behaviour has been hypothesized to be useful not only for breeding, but also for tension regulation, coalition formation and reconciliation (Vasey, 1995; reviewing same-sex sexual behaviour specifically). In a 10-year longitudinal study, higher frequency of reported orgasms was related to lower risk of mortality in men, especially deaths from coronary heart disease (Smith *et al.*, 1997). Conspicuous sexual behaviours such as consortship and copulation was related to higher levels of cortisol in male subordinate olive baboons (Virgin and Sapolsky, 1997), but that was probably because such behaviour can lead to punishment in subordinates, as seem by the fact that covert copulations did not influence cortisol levels (Virgin and Sapolsky, 1997) and that male dominants' cortisol levels are not affected by consortship behaviour (Sapolsky and Ray, 1989). In the present study, sexual behaviour frequency could predict mean, median and minimum FMG levels, and it was kept in multivariable best models for median and minimum FMG levels. Therefore, higher frequency of sexual behaviour lowered chronic and peak stress levels. In contrast, another sex-related behaviour, masturbation as a state, could weakly predict lower mean FMG levels individually but was not retained in any of the four best models, and masturbation as an event could not even built a regression model.

Proximity was a variable expected to predict stress levels, but it had a stronger effect than previously anticipated: proximity could individually predict mean, median and minimum cortisol, all of them strongly enough to appear in their best regression models. According to Silk (2002), proximity is one of the most common behaviour measures used to analyze the quality of primate social relationships, but it is questionable how effective it is as a signal of relationship quality (because two individuals might stay in proximity to each other because they are attracted to the same third individual, and because being in proximity to other individual does not require active engagement). For example, Manson and Perry (2000) found rates of self-directed behaviours (scratching and self-grooming) increased in wild female white-faced capuchins when they were in proximity to other conspecifics, regardless of dominance hierarchy and especially right before they started social grooming. The authors hypothesized whether this means self-directed behaviours are a signal of anxiety in platyrrhine primates, or if the moment before grooming bouts is a socially tense situations where female capuchins use grooming to establish coalitions. Ferreira *et al.* (2008; *Sapajus spp.* – formerly *Cebus apella*) found that capuchin monkeys seemed to form coalitions based on proximity rather than social grooming, with grooming facilitating proximity tolerance between individuals, which could lead to stronger social bonds. For female rhesus macaques, Brent *et al.* (2011) found that proximity reach (the amount of proximity partners an individual has, and how many proximity partners these proximity partners have) was positively correlated with glucocorticoid levels in dominants, who worked better in more focused network groups. However, our study found the opposite pattern, with higher proximity frequency predicting lower cortisol levels, ameliorating both basal and chronic stress, and doing so much more effectively than social grooming frequencies.

5. Conclusion

Using fecal glucocorticoid metabolics as a measure of physiological stress response, we found that dominant capuchin monkeys are more stressed than subordinates in captivity. We also found that capuchin monkeys use a mixture of avoidance strategy (as seen by retreat behaviours), proximity to others and self-grooming behaviours as behavioural strategies to cope with life in captivity, decreasing mean cortisol levels. Chronic stress as measured by median cortisol is best predicted by Proximity and Sexual behaviour, all of which help decrease it; peak stress as seen by maximum cortisol levels is best predicted by Rank and Self-grooming; and basal stress as seen by minimum cortisol levels is best predicted by Sexual behaviour and Proximity. In summary, retreating, having sex, self-grooming and staying in proximity are all behaviours which help decrease stress in captive capuchin monkeys.

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Supplementary data

Table 1 - T-test comparing behavioural frequencies and FMG levels in females and males (significant results in italics):

Variable	F	t	p-value	95% confidence interval	
				Inferior	Superior
Offers grooming	0.545	0.413	0.682	-0.24330197	0.36839297
Receives grooming	0.428	-0.167	0.869	-0.63720477	0.54044668
Sexual behaviour	8.078	1.530	0.139	-0.07626687	0.51648541
Scrounging	1.054	0.569	0.572	-0.31466497	0.56160225
Social play	0.442	0.311	0.757	-0.29672910	0.40503518
Retreats	0.081	0.251	0.803	-0.27507443	0.35317342
Displaces	6.617	-2.171	*0.048*	-0.90424717	-0.00433258
Does aggression	0.162	-0.248	0.805	-0.18643311	0.14559918
Receives aggression	6.904	-1.363	0.192	-0.72546715	0.15866856
Proximity	0.745	-0.576	0.568	-0.84767393	0.47259425
Mean FMG	0.091	0.407	0.686	-0.43751571	0.65882639
Median FMG	0.427	-0.026	0.980	-0.61973730	0.60425784
Maximum FMG	1.125	-0.153	0.879	-0.65821277	0.56545919
Minimum FMG	0.140	-0.346	0.731	-0.71588031	0.50642792
Self-masturbation (state)	5.213	-1.337	0.194	-0.25534912	0.05459219
Self-masturbation (event)	9.369	-1.973	0.065	-0.34078132	0.01142059
Self-grooming	0.969	-0.187	0.852	-0.14254404	0.11830984
Rank	0.753	1.513	0.142	-0.425092	2.827006

Table 2 – AIC values of all linear regression models tested with only one predictor (only variables which were capable of forming at least one model):

Predictors	Mean FMG	Median FMG	Maximum FMG	Minimum FMG
Offers grooming	AIC= -12.436; B= -1.022; *P=0.025*	AIC= -0.746; B= -1.001; P=0.051	AIC= -0.075; B= -0.917; P=0.075	-//-
Receives grooming	-//-	-//-	-//-	AIC=0.254; B= -0.370; P=0.091
Sexual Behaviour	AIC= -14.589; B= -1.071; *P=0.008*	AIC= -14.873; B= -1.772; *P<0.000*	-//-	AIC= -0.941; B= -0.920; *P=0.046*
Social Play	AIC= -10.168; B= -0.696; P=0.090	AIC= -5.193; B= -1.268; *P=0.005*	-//-	-//-
Proximity	AIC= -16.485; B= -0.432; *P=0.003*	AIC= -7.690; B= -0.520; *P=0.001*	-//-	AIC= -11.915; B= -0.599; *P<0.000*
Retreats	AIC= -10.366; B= -0.628; P=0.081	-//-	AIC=0.224; B= -0.682; P=0.090	-//-
Does aggression	-//-	AIC= -6.071; B= -1.667; *P=0.003*	-//-	AIC= -0.091; B= -1.033; P=0.075
Receives aggression	-//-	-//-	AIC= -1.270; B= -1.124; *P=0.038*	-//-
Masturbation (state)	AIC= -10.057; B0=0.073; P0=0.907 if zscore= -0.200067;	-//-	-//-	-//-

	B1=2.040; P1=0.059 if zscore= -0.164349			
Self-grooming	AIC= -13.264; B= -1.729; *P=0.016*	AIC= -0.989; B= -1.640; *P=0.045*	AIC=0.881; B= -1.259; P=0.127	-//-
Rank	-//-	-//-	AIC= -0.353; B= -0.160; P=0.064	-//-

*-//- means predictor variable was not capable of forming a regression model.

Table 3 - AIC values of all linear regression models tested for Mean FMG with more than one predictor:

Predictors	**AIC**
Offers grooming, Sexual behaviour	-14.649
Offers grooming, Social play	-12.436
Offers grooming, Retreats	-12.806
Offers grooming, Proximity	-17.876
Offers grooming, Self-masturbation	-12.436
Offers grooming, Self-grooming	-14.596
Sexual behaviour, Social play	-14.589
Sexual behaviour, Retreats	-17.313
Sexual behaviour, Proximity	-21.271
Sexual behaviour, Self-masturbation	-16.233
Sexual behaviour, Self-grooming	-14.589
Social play, Retreats	-12.732
Social play, Proximity	-18.587
Social play, Self-masturbation	-10.905
Social play, Self-grooming	-13.264
Retreats, Proximity	-16.683
Retreats, Self-masturbation	-10.633
Retreats, Self-grooming	-17.093
Proximity, Self-masturbation	-16.485
Proximity, Self-grooming	-25.084
Self-masturbation, Self-grooming	-15.207
Offers grooming, Sexual behaviour, Social play	-14.649
Offers grooming, Sexual behaviour, Retreats	-17.313
Offers grooming, Sexual behaviour, Proximity	-21.271
Offers grooming, Sexual behaviour, Self-masturbation	-16.233
Offers grooming, Sexual behaviour, Self-grooming	-14.649
Offers grooming, Social play, Retreats	-12.806
Offers grooming, Social play, Proximity	-18.587
Offers grooming, Social play, Self-masturbation	-12.436
Offers grooming, Social play, Self-grooming	-14.596
Offers grooming, Retreats, Proximity	-17.876
Offers grooming, Retreats, Self-masturbation	-12.806
Offers grooming, Retreats, Self-grooming	-17.329
Offers grooming, Proximity, Self-masturbation	-17.876
Offers grooming, Proximity, Self-grooming	-25.084
Offers grooming, Self-masturbation, Self-grooming	-15.328
Sexual behaviour, Social play, Retreats	-17.313
Sexual behaviour, Social play, Proximity	-21.271
Sexual behaviour, Social play, Self-masturbation	-16.233
Sexual behaviour, Social play, Self-grooming	-14.589
Sexual behaviour, Retreats, Proximity	-22.997
Sexual behaviour, Retreats, Self-masturbation	-18.320
Sexual behaviour, Retreats, Self-grooming	-18.313
Sexual behaviour, Proximity, Self-masturbation	-21.271

Sexual behaviour, Proximity, Self-grooming	-25.084
Sexual behaviour, Self-masturbation, Self-grooming	-16.238
Social play, Retreats, Proximity	-20.398
Social play, Retreatst, Self-masturbation	-12.866
Social play, Retreatst, Self-grooming	-17.093
Retreats, Proximity, Self-masturbation	-16.683
Retreats, Proximity, Self-grooming	-28.729
Retreats, Self-masturbation, Self-grooming	-18.454
Proximity, Self-masturbation, Self-grooming	-25.285
Offers grooming, Sexual behaviour, Social play, Retreats	-17.313
Offers grooming, Sexual behaviour, Social play, Proximity	-21.271
Offers grooming, Sexual behaviour, Social play, Self-masturbation	-16.233
Offers grooming, Sexual behaviour, Social play, Self-grooming	-14.649
Offers grooming, Social play, Retreats, Proximity	-20.398
Offers grooming, Social play, Retreats, Self-masturbation	-12.806
Offers grooming, Social play, Retreats, Self-grooming	-17.329
Offers grooming, Retreats, Proximity, Self-masturbation	-17.876
Offers grooming, Retreats, Proximity, Self-grooming	-28.729
Offers grooming, Proximity, Self-masturbation, Self-grooming	-25.285
Sexual behaviour, Social play, Retreats, Proximity	-22.997
Sexual behaviour, Social play, Retreats, Self-masturbation	-18.320
Sexual behaviour, Social play, Retreats, Self-grooming	-18.313
Sexual behaviour, Retreats, Proximity, Self-masturbation	-23.151
Sexual behaviour, Retreats, Proximity, Self-grooming	-28.729
Sexual behaviour, Proximity, Self-masturbation, Self-grooming	-25.285
Social play, Retreats, Proximity, Self-masturbation	-20.398
Social play, Retreats, Proximity, Self-grooming	-28.729
Social play, Proximity, Self-masturbation, Self-grooming	-25.285
Retreats, Proximity, Self-masturbation, Self-grooming	-29.611
Offers grooming, Sexual behaviour, Social play, Retreats, Proximity	-22.997
Offers grooming, Sexual behaviour, Social play, Retreats, Self-masturbation	-18.320
Offers grooming, Sexual behaviour, Social play, Retreats, Self-grooming	-18.313
Offers grooming, Social play, Retreats, Proximity, Self-masturbation	-20.398
Offers grooming, Social play, Retreats, Proximity, Self-grooming	-28.729
Offers grooming, Social play, Proximity, Self-masturbation, Self-grooming	-25.285
Offers grooming, Retreats, Proximity, Self-masturbation, Self-grooming	-29.611
Sexual behaviour, Social play, Retreats, Proximity, Self-masturbation	-23.151
Sexual behaviour, Social play, Retreats, Proximity, Self-grooming	-28.729
Sexual behaviour, Social play, Proximity, Self-masturbation, Self-grooming	-25.285
Sexual behaviour, Retreats, Proximity, Self-masturbation, Self-grooming	-29.611
Social play, Retreats, Proximity, Self-masturbation, Self-grooming	-29.611
Offers grooming, Sexual behaviour, Social play, Retreats, Proximity, Self-masturbation	-23.151
Offers grooming, Sexual behaviour, Social play, Retreats, Proximity, Self-grooming	-28.729
Offers grooming, Sexual behaviour, Retreats, Proximity, Self-masturbation, Self-grooming	-29.611
Offers grooming, Social play, Retreats, Proximity, Self-masturbation, Self-grooming	-29.611
Sexual behaviour, Social play, Retreats, Proximity, Self-masturbation, Self-grooming	-29.611
Offers grooming, Sexual behaviour, Social play, Retreats, Proximity, Self-masturbation, Self-grooming	-29.611

Table 4 - AIC values of all linear regression models tested for Median FMG with more than one predictor:

Predictors	**AIC**
Offers grooming, Sexual behaviour	-14.873
Offers grooming, Social play	-5.193
Offers grooming, Does aggression	-6.214
Offers grooming, Proximity	-8.010

Offers grooming, Self-grooming	-1.429
Sexual behaviour, Social play	-14.873
Sexual behaviour, Does aggression	-14.873
Sexual behaviour, Proximity	-25.333
Sexual behaviour, Self-grooming	-14.873
Social play, Does aggression	-9.293
Social play, Proximity	-17.963
Social play, Self-grooming	-5.193
Does aggression, Proximity	-13.202
Does aggression, Self-grooming	-6.431
Proximity, Self-grooming	-13.685
Offers grooming, Sexual behaviour, Social play	-14.873
Offers grooming, Sexual behaviour, Does aggression	-14.873
Offers grooming, Sexual behaviour, Proximity	-25.333
Offers grooming, Sexual behaviour, Self-grooming	-14.873
Offers grooming, Social play, Does aggression	-9.293
Offers grooming, Social play, Proximity	-17.963
Offers grooming, Social play, Self-grooming	-5.193
Offers grooming, Does aggression, Proximity	-13.202
Offers grooming, Does aggression, Self-grooming	-6.431
Offers grooming, Proximity, Self-grooming	-13.685
Sexual behaviour, Social play, Does aggression	-14.873
Sexual behaviour, Social play, Proximity	-25.333
Sexual behaviour, Social play, Self-grooming	-14.873
Sexual behaviour, Does aggression, Proximity	-25.333
Sexual behaviour, Does aggression, Self-grooming	-14.873
Sexual behaviour, Proximity, Self-grooming	-25.333
Social play, Does aggression, Proximity	-20.008
Social play, Does aggression, Self-grooming	-9.293
Social play, Proximity, Self-grooming	-18.593
Does aggression, Proximity, Self-grooming	-16.841
Offers grooming, Sexual behaviour, Social play, Does aggression	-14.873
Offers grooming, Sexual behaviour, Social play, Proximity	-25.333
Offers grooming, Sexual behaviour, Social play, Self-grooming	-14.873
Offers grooming, Social play, Does aggression, Proximity	-20.008
Offers grooming, Social play, Does aggression, Self-grooming	-9.293
Offers grooming, Does aggression, Proximity, Self-grooming	-16.841
Sexual behaviour, Social play, Does aggression, Proximity	-25.333
Sexual behaviour, Social play, Does aggression, Self-grooming	-14.873
Sexual behaviour, Does aggression, Proximity, Self-grooming	-25.333
Social play, Does aggression, Proximity, Self-grooming	-20.008
Offers grooming, Sexual behaviour, Social play, Does aggression, Proximity	-25.333
Offers grooming, Sexual behaviour, Social play, Does aggression, Self-grooming	-14.873
Offers grooming, Social play, Does aggression, Proximity, Self-grooming	-20.008
Sexual behaviour, Social play, Does aggression, Proximity, Self-grooming	-25.333
Offers grooming, Sexual behaviour, Social play, Does aggression, Proximity, Self-grooming	-25.333

Table 5 - AIC values of all linear regression models tested for Maximum FMG with more than one predictor:

Predictors	**AIC**
Offers grooming, Retreats	-0.253
Offers grooming, Receives aggression	-1.310
Offers grooming, Self-grooming	-0.075
Offers grooming, Rank	-0.381
Retreats, Receives aggression	-1.509

Retreats, Self-grooming	-1.465
Retreats, Rank	-0.353
Receives aggression, Self-grooming	-1.270
Receives aggression, Rank	-1.275
Self-grooming, Rank	-1.999
Offers grooming, Retreats, Receives aggression	-1.509
Offers grooming, Retreats, Self-grooming	-1.465
Offers grooming, Retreats, Rank	-0.381
Offers grooming, Receives aggression, Self-grooming	-1.310
Offers grooming, Receives aggression, Rank	-1.310
Offers grooming, Self-grooming, Rank	-1.999
Retreats, Receives aggression, Self-grooming	-2.668
Retreats, Receives aggression, Rank	-1.509
Retreats, Self-grooming, Rank	-1.999
Receives aggression, Self-grooming, Rank	-2.285
Offers grooming, Retreats, Receives aggression, Self-grooming	-2.668
Offers grooming, Retreats, Receives aggression, Rank	-1.509
Offers grooming, Receives aggression, Self-grooming, Rank	-1.310
Retreats, Receives aggression, Self-grooming, Rank	-2.668
Offers grooming, Retreats, Receives aggression, Self-grooming, Rank	-2.668

Table 6 - AIC values of all linear regression models tested for Minimum FMG with more than one predictor:

Predictors	**AIC**
Receives grooming, Sexual behaviour	-0.941
Receives grooming, Does aggression	-0.091
Receives grooming, Proximity	-11.915
Sexual behaviour, Does aggression	-0.941
Sexual behaviour, Proximity	-13.523
Does aggression, Proximity	-11.915
Receives grooming, Sexual behaviour, Does aggression	-0.941
Receives grooming, Sexual behaviour, Proximity	-13.523
Receives grooming, Does aggression, Proximity	-11.915
Sexual behaviour, Does aggression, Proximity	-13.523
Receives grooming, Sexual behaviour, Does aggression, Proximity	-13.523

Considerações finais gerais

Animais que vivem em cativeiro enfrentam diferentes tipos de estressores relacionados ao espaço limitado em comparação com o ambiente natural (Pomerantz *et al.*, 2013), ou agravados pelo cativeiro, como a densidade populacional dentro do recinto (Creel, 2001) e a incapacidade de se fugir de indivíduos dominantes que possam agredi-los (Sapolsky, 2005). A maioria desses estressores estão relacionados à falta de controle que os animais possuem sobre certas características de sua vida (Morgan & Tromborg, 2007), o que confere imprevisibilidade a esses momentos. Todos esses fatores desafiam a homeostasia dos animais de cativeiro, consequentemente ativando sistemas alostáticos como o eixo hipotalâmico-pituitário-adrenal (HPA) para enfrentar os estressores (Sousa *et al.*, 2015).

Ativação do sistema alostático a fim de enfrentar estressores crônicos requer gasto energético chamado de carga alostática, aqui calculado como níveis de metabólitos fecais de glicocorticoides. O uso crônico de sistemas alostáticos pode fazer a carga alostática aumentar até o ponto em que o animal alcança sobrecarga alostática, ponto em que há maior risco de desenvolvimento de patologias (Sousa *et al*., 2015). Em especial, sobrecarga alostática do tipo 2, que ocorre quando o animal se mantém com aumento crônico de níveis de cortisol sem nunca exceder a quantidade de energia disponível no ambiente, já que nesse ambiente há maior disponibilidade de comida e água (Wingfield, 2005), é interessante de se ter em mente no estudo de animais de cativeiro, onde há mais assegurado acesso a comida do que no ambiente natural. Caso o animal não se habitue ao estressor, a sobrecarga eventualmente prejudica a capacidade reprodutiva, resposta imunológica (Morgan & Tromborg, 2007) e o próprio feedback negativo do eixo HPA (Sterling & Eyer, 1988).

Animais com alta flexibilidade comportamental como o macaco-prego são um modelo interessante para analisar como animais selvagens reagem ao estresse de cativeiro e se eles serão capazes de enfrentar os estressores. Ou seja, até que ponto essa flexibilidade possibilita que eles modifiquem seus parâmetros comportamentais e fisiológicos a fim de se adaptar ao cativeiro (Mason *et al*., 2013)? Mesmo na ocasião que ele pareça ter ajustado seu comportamento com sucesso, também é interessante verificar se essa mudança ocasionou em um aumento de carga alostática, caso em que ele ainda está estressado mesmo sem sinais evidentes de estresse (de Waal, 1989), ou se a resposta fisiológica ao estresse também foi atenuada e carga alostática baixou. Neste trabalho, testamos as hipóteses de que alta densidade populacional, acesso aos visitantes de zoológico e posição hierárquica alta seriam estressores em macacos-prego (*Sapajus libidinosus*) cativos que aumentariam carga alostática, enquanto suporte social atenuaria carga alostática. No total, nossas predições eram, em ordem de hipóteses sendo testadas: aumento de densidade populacional aumentariam níveis de glicocorticoides fecais e frequências de comportamentos potencialmente indicativos de estresse; níveis de glicocorticoides fecais e comportamentos indicativos de estresse são maiores em animais expostos a visitantes de zoológico do que em animais não expostos a visitantes; posição hierárquica alta prediz níveis altos de glicocorticoides fecais; comportamentos afiliativos positivos como catação social e comportamento sexual predizem menores níveis de glicocorticoides fecais, enquanto que receber agressão aumentaria níveis de glicocorticoides fecais e fazer agressão em alguém prediz menores níveis de glicocorticoides fecais.

Os resultados aqui encontrados corroboram com a ideia que alta densidade populacional é um estressor que afeta o comportamento do animal (van Wolkenten *et al.*, 2006; *Cebus apella*), aumentando frequência de comportamentos potencialmente indicativos de estresse e de afastar-se, mas não influenciando a carga alostática do animal. O fato de que afastar-se foi influenciado pela alta densidade, mas catação social e agressão intragrupo não foram, sugere que eles estão adotando uma estratégia de evitação social para controlar as tensões dentro do grupo (de Waal, 1989). Acesso aos visitantes, por sua vez, mostrou um aumento de vigilância, comportamento potencialmente indicativo de maior estresse em recinto com acesso a visitantes de zoológico (Sherwen *et al.*, 2015; *Cebus apella*), assim como aumentou carga alostático como demonstrado pelos maiores níveis médios de glicocorticoides em sujeitos expostos a visitantes de zoológico.

Como visto em outros estudos em macacos-prego, a posição dominante agrega maior carga alostática do que a posição subordinado, especificamente no quesito de níveis de pico de glicocorticoide, o que sugere que em macacos-prego os níveis de agressão que subordinados sofrem de dominantes é baixo (Abbott *et al.*, 2003), o que pode estar relacionado ao fato dos macacos-prego serem primatas socialmente tolerantes (Izar *et al.*, 2012).

Os comportamentos sociais capazes de predizer níveis de glicocorticoides, todos eles baixando carga alostática, foram: afastar-se; comportamento sexual; e proximidade. Novamente, o afastar-se indica que esses animais estão utilizando uma estratégia de evitação ao conflito como forma de lidar com tensões no cativeiro (Judge & de Waal, 1993; *Macaca mulatta* fêmeas). A proximidade e comportamento sexual são ambos comportamentos sociais positivos e, portanto, não é incomum que eles baixem níveis de glicocorticoides. Entretanto, foi inesperado que nem catação social recebida ou oferecida estejam inclusos na lista, visto que esse é considerado um indicador clássico de suporte social e que trabalhos anteriores em outras espécies de primatas tenham achado uma relação entre maiores níveis de catação e menores níveis de glicocorticoides (Fontani *et al.*, 2014; *Saguinus oedipus*; Gust *et al.*, 1993; *Macaca mulatta*; Shutt *et al.*, 2007; *Macaca sylvanus*; Wooddell *et al.*, 2017*; Macaca mulatta*). Por outro lado, a auto-catação também reduz os níveis de glicocorticoides fecais, reforçando esse comportamento como um bom indicador de enfrentamento ao estresse em macacos-prego cativos.

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MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE
COMISSÃO DE ÉTICA NO USO DE ANIMAIS – CEUA
Av. Salgado Filho, S/N – CEP: 59072-970 – Natal / RN
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CERTIFICADO

Natal (RN), 10 de outubro de 2017.

Certificamos que a proposta intitulada "**Diferenças individuais em ambientação experimental de macacos-pregos (*Sapajus libidinosus*) em area de caatinga RN**", **PARECER nº 053.063/2017**, sob a responsabilidade de **Renata Gonçalves Ferreira**, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica encontra-se de acordo com os preceitos da Lei n.º 11.794, de 8 de outubro de 2008, do Decreto n.º 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS da Universidade Federal do Rio Grande do Norte – CEUA/UFRN.

Vigência do Projeto	**ABRIL 2019**
RELATÓRIO	**MAIO 2019**
Espécie/Linhagem	**Macacos-pregos**
Número de Animais	**38**
Idade/Peso	**Infant-Adulto / 0,5-3,8kg**
Sexo	**20 machos + 18 fêmeas**
Origem	**CETAS – IBAMA SUPES RN**
Manutenção	**CETAS Natal e recinto na mineradora SUSA – Cruzeta RN**

Informamos ainda que, segundo o Cap. 2, Art. 13, do Regimento Interno desta CEUA, é função do professor/pesquisador responsável pelo projeto a **elaboração de relatório** de acompanhamento que deverá ser entregue tão logo a pesquisa seja concluída. **O descumprimento desta norma inviabilizará a submissão de projetos futuros.**

José de Castro Souza Neto Júnior
Coordenador da CEUA-UFRN

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PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA

1) PROTOCOLO Nº : 063-2017

2) TÍTULO DO PROJETO:

Diferenças individuais em ambientação experimental de macacos-pregos (*Sapajus libidinosus*) em area de caatinga RN

3) PESQUISADOR RESPONSÁVEL:

Renata Gonçalves Ferreira

4) DATA DO PARECER:

10 outubro 2017

5) RESUMO DO PROJETO:

6) OBJETIVOS DO PROJETO:

Geral: Avaliar as diferenças individuais em mudança no padrão comportamental e níveis de cortisol fecal em macacos-prego alojados: ex-situ (CETAS RN e PB) e in-situ (fase de ambientação em Cruzeta- RN).

Específicos: 1- Determinar o perfil comportamental de macacos-prego mantidos nos recintos dos CETAS RN e CETAS PB; 2- averiguar diferenças nas estratégias de enfrentamento ao estresse de macacos-prego mantidos nos recintos dos CETAS RN e CETAS PB; 3- Adquirir indicadores hormonais de estresse (i.e. Cortisol fecal) dos indivíduos em cativeiro; 4- Comparar a variância inter- e intra-individual do padrão comportamental dos animais no recinto do CETAS e no recinto de ambientação; 5- Comparar a variância inter- e intra-individual do perfil hormonal dos animais no recinto do CETAS e no recinto de ambientação.

7) FINALIDADE DO PROJETO: ☐ Ensino ☐ Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS:

Título	☒ Adequado	☐ Comentários
Objetivos	☒ Adequados	☐ Comentários
Introdução e Justificativa	☒ Adequadas	☐ Comentários
Cronograma para execução da pesquisa	☒ Adequado	☐ Comentários
Orçamento e fonte financiadora	☒ Adequados	☐ Comentários
Referências Bibliográficas	☒ Adequadas	☐ Comentários

9) O PROJETO DESTA PESQUISA ESTA ADEQUADO À LEGISLAÇÃO VIGENTE: ☐ Adequado ☐ Comentários

10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de severidade: ☒ Brando ☐ Médio ☐ Elevado

Espécie: Macacos prego **Número Amostral:** 38

Redução Amostral: ☐ Sim ☒ Não

Substituição de Metodologia: ☐ Sim ☒ Não
Aprimoramento da Metodologia: ☐ Sim ☒ Não
Acomodação e manutenção dos animais: ☒ Adequado ☐ Inadequado
Manipulação dos animais: ☒ Adequado ☐ Inadequado

Analgesia dos animais (se aplicável): ☐ Adequado ☐ Inadequado
Justificativa:
Não se aplica

Anestesia dos animais (se aplicável): ☐ Adequado ☐ Inadequado
Justificativa:
Não se aplica

Eutanásia dos animais (se aplicável): ☐ Adequado ☐ Inadequado
Justificativa:
Não se aplica

11) PARECER:

☒ **Aprovado**

☐ Recomendações

☐ Pendência

☐ Não aprovado

Comentários gerais sobre o projeto: