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AVALIAÇÃO E IMPLICAÇÕES DO ESTRESSE DO TURISMO DE INTERAÇÃO E INTERVALO DE REFERÊNCIA BIOQUÍMICA DO SANGUE DE *MELANOSUCHUS NIGER* E *CAIMAN CROCODILUS* NO PARQUE NACIONAL DE ANAVILHANAS, AMAZÔNIA CENTRAL BRASILEIRA

WASHINGTON CARLOS DA SILVA MENDONÇA

Manaus - AM

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Tese apresentada ao Programa de Pós-Graduação em Zoologia da Universidade Federal do Amazonas/ Instituto Nacional de Pesquisas da Amazônia, como parte dos requesitos para obtenção do título de Doutor em Zoologia.

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Resumo

Crocodilianos são indivíduos de biologia complexa e críptica, predadores de topo, são de grande relevância econômica e ecológica nos sistemas naturais onde ocorrem ou quando são introduzidos. A região equatorial é onde ocorrem as maiores diversidade de crocodilianos no mundo. O Brasil é um dos países com maior diversidade de crocodilianos, ocorrendo cinco espécies com ampla distribuição natural, que são popularmente chamados de jacarés, quatro destas espécies ocorrem na região amazônica brasileira, sendo que duas delas o jacaré-açu (*Melanosuchus niger*) e o jacaré-tinga (*Caiman crocodilus*) são as mais abundantes e mais estudas até os dias de hoje. Historicamente são importantes na cadeia produtiva, pois há mais de meio século estão submetidas ao aproveitamento econômico legal/ilegal no estado do Amazonas, porém de forma destrutivas para aproveitamento dos seus subprodutos. Atualmente o turismo de iteração com fauna, uma alternativa de aproveitamento econômico não destrutivo aliado à conservação. Neste sentido, a crescente procura pelo turismo de interação os jacarés amazônicos alertou a preocupação quanto aos efeitos do turismo de interação sobre os jacarés. Contudo é difícil ponderar tais efeitos, devido métodos apropriados e aplicados, além disso, por conta da falta de valores de referência de parâmetros bioquímicos do sangue para *M. niger* e *C. crocodilus*. Estes parâmetros são de suma relevância para avaliar efeitos diretos não somente do turismo ou outras intervenções humanas, mas também nas análises na cliníca veterinária assim como na interpretação do estado biológico animal. Neste contexto, a presente tese investigou o efeito do turismo de interação com jacarés e registrou intervalo de referência para 11 parâmetros bioquímicos do sangue das populações de *M. niger* e *C. crocodilus* no Parque Nacional de Anavilhanas, no baixo rio Negro. O **primeiro capítulo** apresenta uma avaliação do estresse fisiológico direto do turismo de interação com *M. niger* e *C. crocodilus*. No **segundo capítulo** estabelece intervalos de referência de 11 parâmetros sanguíneos para *M. niger* e *C. crocodilus*. Por fim, os resultados encontrados nesta tese poderão embasar decisões que podem direcionar o rumo do manejo sustentável não destrutivo de jacarés, o turismo de interação com jacarés, também auxiliarão veterinários e biólogos da vida silvestre nos diagnósticos clínicos e de avaliação da condição física de jacarés. Espero que esta tese incentive outras pesquisas como desdobramentos dos resultados aqui apresentados.

Abstract

Crocodilians are individuals with complex and cryptic biology, top predators, and are of great economic and ecological importance in the natural systems where they occur or when they are introduced. The equatorial region is home to the greatest diversity of crocodilians in the world. Brazil is one of the countries with the greatest diversity of crocodilians. There are five species with a wide natural distribution, which are popularly called caimans. Four of these species occur in the Brazilian Amazon region, two of which, the Black caiman (*Melanosuchus niger*) and the Spectacled caiman (*Caiman crocodilus*), are the most abundant and most studied to date. Historically, they are important in the production chain, as they have been subject to legal/illegal economic exploitation in the state of Amazonas for more than half a century, albeit in a destructive way in order to make use of their by-products. Today, wildlife tourism is an alternative for non-destructive economic exploitation combined with conservation. In this sense, the growing demand for interaction tourism with Amazonian caimans has raised concerns about the effects of interaction tourism on caimans. However, it is difficult to assess these effects due to the lack of appropriate and applied methods, as well as due to the lack of reference intervals for blood biochemical parameters for *M. niger* and *C. crocodilus*, which are of parameters importance when it comes to assessing the direct effects not only of tourism or other human interventions, but also in veterinary clinical analyses as well as in the interpretation of animal biological status. In this context, the present thesis investigated the effect of tourism on caiman interaction and recorded reference intervals for 11 blood biochemical parameters of *M. niger* and *C. crocodilus* populations in the Anavilhanas National Park, on the lower Negro River. The first chapter presents an assessment of the direct physiological stress of tourist interaction with *M. niger* and *C. crocodilus*. The second chapter establishes reference intervals for 11 blood parameters for *M. niger* and *C. crocodilus*. Finally, the results found in this thesis can support decisions that can direct the course of sustainable, non-destructive caiman management, caiman interaction tourism, as well as assist veterinarians and wildlife biologists in clinical diagnoses and assessment of the physical condition of caimans. I hope that this thesis will encourage further research as a result of the findings presented here.

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INTRODUÇÃO GERAL

Crocodylia é um grupo monofilético de amniótas com biologia e ecologia peculiares, e juntamente com as aves representam os Archosauria viventes (Green et al. 2014). No geral, a maioria das 25 espécies atuais habita principalmente ambientes tropicais no globo terrestre (Lourenço-de-Moraes et al. 2023), sendo vertebrados de médio a grande porte, de vida longa, com crescimento lento e maturidade sexual tardia (Da Silveira et al. 2013). Também apresentam comportamento complexo e críptico, atuando como predadores generalistas e oportunistas, com acentuado cuidado parental materno (Marioni et al. 2008; Campos et al. 2012; Villamarín-Jurado et al. 2007).

Algumas das espécies atuais geram subprodutos (pele, carne) com valor comercial, sendo que, no geral depende de ações de conservação devido a fatores socioambientais como manejo econômico inadequado e desinteresse de manutenção das populações naturais pelo medo que causam às pessoas, agravado por casos de ataques freqüentes (Bergamasco et al. 2018) que podem levar a óbito em humanos (Fukuda et al. 2014, Brackhane et al. 2018, Bitencourt & Maschio 2023) e suas criações domésticas (Pierre et al. 2021, Pooley et al. 2021).

O Brasil é o país com maior diversidade de crocodilianos do mundo, ocorrem seis espécies de crocodilianos que pertencem à família Alligatoridae, sendo popularmente e tecnicamente chamados de jacarés (Roberto et al. 2021). Cinco dessas espécies ocupam a região Amazônica brasileira, quatro delas apresentam distribuição ampla e ocupam basicamente todos os tipos de corpos hídricos em ambientes de terra Firme, várzeas e igapós no estado do Amazonas (Marioni et al. 2021).

O jacaré-coroa (*Paleosuchus trigonatus* Schneider, 1801) pode atingir 2,2 m de comprimento total (CT) e habita primariamente os riachos de terra firme (igarapés) na floresta densa (Magnusson, 1992b). O jacaré-paguá (*Paleosuchus palpebrosus* Cuvier,

1807) pode atingir até 2,1 m de CT, e sua ocorrência inclui florestas alagadas perto dos principais rios e lagos (Magnusson, 1992a), além de corpos hídricos artificiais formados ao longo de rodovias amazônicas, como a BR319 (Botero, 2007). Pesquisas em andamento indicaram que esta espécie é mais generalista no uso dos habitats do que anteriormente se pensava, não ocupando somente os igarapés de ordens inferiores (Marioni et al. 2013).

Ambas as espécies de *Paleosuchus* não possuem valor comercial para a produção de peles por serem muito ossificadas por osteodermos e por serem de pequeno porte para a produção de carne (ver Da Silveira, 2002 para uma exceção). São amplamente distribuídas no norte da América do sul, mas apresentam densidade e abundância relativamente baixa (Magnusson, 1992).

As espécies mais abundantes e com mais informações biológicas são jacaré-açu (*Melanosuchus niger* Spix, 1825) e jacaré-tinga (*Caiman crocodilus* Linnaeus, 1758) (Maioni et al. 2021). Estas espécies já tiverem suas populações largamente exploradas para abastecer o comércio de peles que quase levou a extinções locais de jacaré-açu (Smith 1981, Medem 1983, Plotkin et al. 1983, Rebêlo & Magnusson 1983, Antunes et al. 2016), após a queda do comércio de peles de jacarés amazônicos o mercado focou no comércio ilegal de carne (Da Silveira e Thorbjarnarson, 1999; Da Silveira, 2001; Mendonça et al. 2016). A caça para obtenção de iscas para pesca de piracatinga (*Colophysus macropterus*) também remonta uma prática de exploração de jacarés insustentável e ilegal (Da Silveira e Viana, 2003; Brum e Silva, 2017). Na verdade, historicamente os crocodilianos amazônicos na maioria das vezes sempre foram submetidos ao mau manejo caracterizado pela retirada de indivíduos de suas populações naturais de forma destrutiva, para aproveitamento de seus subprodutos.

O turismo de interação com jacarés no estado do Amazonas (D'Cruze et al. 2017) tem se apresentado como uma alternativa não "destrutiva" de uso sustentável de jacarés. Do ponto de vista biológico, social, econômico e governamental faz-se necessário avaliar os impactos do turismo, investindo em métodos científicos adequados em diferentes especificidades, e que possam ser replicados em espaço ou tempo, além de que previsões de impactos são raramente quantificados evidenciando a falta de ciência aplicada para avaliar tais impactos como ferramenta para tomada de decisão.

Considerando que problemas ambientais, sociais e físicos causam perturbações que podem comprometer não só a sua sobrevivência, mas outros fatores importantes do histórico de vida dos répteis, como a reprodução (Jessop et al. 2003), as interações com humanos devem acentuar diferentes respostas aos estímulos submetidos, podendo estimular a glândula adrenal a produzir glicocorticoides (Guillette et al. 1995).

Num cenário de estresse causado pelo turismo de interação com os crocodilianos amazônicos, a captura, a luminosidade excessiva e o manuseio do animal, bem como as inúmeras percepções sensoriais que o animal poderá sofrer resultam numa via clássica de resposta ao estresse por meio da ativação do eixo hipotálamo-hipófise-adrenal (HPA), uma modelar via de resposta ao estresse (Sheriff et al. 2011, Dantzer et al. 2014)

As diversas zonas hipotalâmicas regulam o comportamento de defesa, reprodução, respostas motoras somáticas e controle endócrino. No suposto cenário de estresse agudo citado anteriormente para os crocodilianos, os neurônios hipotalâmicos especializados devem aumentar a síntese e liberação dos hormônios: CRH (hormônio liberador de corticotrofina), ACTH (hormônio adrenocorticotrófico) e corticosterona, resposta primária de estresse fisiológico. (Sheriff et al. 2011).

Ao longo de captura e manipulação, a glicose passa a ser uma das mais importantes fontes de energia para sustentar a via anaeróbica durante o esforço muscular associado à contenção física dos crocodilianos (Jessop et al. 2003). Com isso, a ativação do metabolismo anaeróbico resulta na produção excessiva de ácido láctico, um produto final indesejável, caso seja acumulado nos tecidos (Jackson et al. 2003, Hartzler 2006, Duncan et al. 2022).

Sabe-se que os crocodilianos são especialmente sensíveis à elevada concentração de ácido láctico, isto pode resultar na morte do animal. No entanto, há pouca informação sobre o efeito do estresse de captura nos níveis de corticosterona e lactato plasmático em crocodilianos amazônicos submetidos à interação turística. Apenas valores de referências oriundos do primeiro estudo que avaliou estresse fisiológico de captura intensiva em *M. niger* e *C. crocodilus* (Duncan et al. 2022). Portanto, a quantificação dos teores de corticosterona e de lactato no sangue de jacarés pode ajudar a estabelecer limites e protocolos de captura, manipulação e obtenção de fotografias com uso de flash durante a interação com humanos.

Do ponto de vista clínico, apesar de diferentes esforços realizados para entender a biologia de *M. niger* e *C. crocodilus* (Marioni et al 2021), até os dias de hoje sequer foram estabelecidos na literatura científica valores de referência de parâmetros plasmáticos destas espécies amazônicas. Tais valores são usados na clínica veterinária para interpretar o estado de saúde, bem-estar, condição corporal, diagnósticos clínicos entre outros (Uhart et al. 2003, Friedrichs et al. 2012, Nieto-Claudín et al. 2021). No entanto, estabelecer estes dados em literatura científica é de relevância biológica, conservacionista e clínica devido o parco conhecimento produzido até hoje sobre esta temática, o que leva profissionais da área utilizar dados de espécies mais próximas.

Assim sendo, este foi um momento oportuno e singular de avaliar os impactos do turismo sobre jacarés e estabelecer de parâmetros bioquímicos de referência do sangue de jacarés no intuito de gerar informações que oferecem embasamento biológico e científico às tomadas de decisões no que confere à conservação, e principalmente considerando as escassas abordagens que avaliam efeitos fisiológicos em crocodilianos, quanto mais aos submetidos ao turismo de interação.

Nesse contexto, a presente tese investigou o efeito fisiológico e implicações do turismo de interação, e avaliou 11 parâmetros bioquímicos do sangue de *M. niger* e *C. crocodilus* no PARNA Anavilhanas, Amazônia Central. Os resultados alcançados encontram-se organizados em dois capítulos:

Capítulo 1 avalia o efeito fisiológico direto do turismo de interação com *M. niger* e *C. crocodilus*;

Capítulo 2 estabelece intervalos de referência de 11 parâmetros sanguíneos para *M. niger* e *C. crocodilus*.

CAPÍTULO 1

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Conservation implications of tourism and stress for Amazonian caimans

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ABSTRACT Ecotourism is a strategy for biodiversity conservation, but it involves possible negative effects on animal health and welfare. Large predators such as crocodilians are one of the great public attractions sought after for tourist interactions. Interactions with wild animals and humans can hyperstimulate the hypothalamic-pituitary-adrenal axis in the short term, in the case of crocodilians showing an increased corticosterone level, which is indicative of physiological stress. Between September and December 2019, we simulated interactions between tourists and Amazonian crocodilians at Anavilhanas National Park in Central Amazonia, Brazil to evaluate the effects of handling and use of photographic flashes on black caiman (*Melanosuchus niger*) and spectacled caiman (*Caiman crocodilus*) on circulating corticosterone and lactate. Corticosterone levels increased 1.7-fold during handling and 2.7-fold when exposed to photographic flashes in black caiman but not in spectacled caiman. Increased corticosterone concentrations in black caiman were characterized by an increase caused by handling and were more intense after flashes than in controls, but the combination of handling and flash had no effect. During handling in simulated tourist interactions, anaerobic respiration increased lactate in black caiman but not in spectacled caiman. The effect of simulated tourist interactions with Amazonian crocodilians was dependent on the handling and especially on flash use in black caiman. The results can assist management, conservation programs, and public policies, especially in programs based on tourism interaction with Amazonian crocodilians.

KEYWORDS Amazon ecotourism, *Caiman crocodilus*, corticosterone, crocodilian conservation, *Melanosuchus niger*, plasma lactate

INTRODUCTION

Ecotourism is recognized as a global biodiversity conservation strategy (Stronza et al. 2021). Wildlife interactions are among the attractions of ecotourism, and the opportunity to interact with iconic animals like apex predators can sensitize tourists to wildlife conservation (Duffus and Dearden, 1990, Macdonald et al 2017, Miranda et al. 2021). But negative effects of wildlife interaction and tourism can result in physiological stress primarily indicated by increased glucocorticoid hormones and altered plasma parameters that are indicative of animal health and welfare (Green and Giese 2004, Bateman and Fleming 2017).

Interaction tourism has contributed to the conservation of several species. In Brazil, a country with ample natural beauty and with much of it still in a good state of conservation, there is a demand for interaction tourism with fauna (Tischer et al. 2013, Macdonald et al. 2017, Tortato and Izzo 2017). Examples include sea turtle tourism on the coast (Pegas and Stronza 2010), Amazon river dolphin (*Inia geoffrensis*) tours (Vidal et al. 2020, 2021), jaguar (*Panthera onca*) viewing in the Pantanal (Peralta 2012, Nassar et al. 2013), and harpy eagle (*Harpia harpyja*) watching (Miranda et al. 2021). In the Amazon, ecotourism with black caiman (*Melanosuchus niger*) and spectacled caiman (*Caiman crocodilus*), the most abundant and widely distributed of Amazonian crocodilian species in central Amazonia (Marioni et al. 2021), has great potential to allow tourists to learn about, observe, and interact with caimans in Amazonian flooded forests (Rosenblatt et al. 2021).

Amazonian crocodilian interaction tourism usually includes nocturnal targeting with the use of spotlight, capture, handling, and flash photography (D`Cruze et al. 2017). The effects of this activity on Amazonian crocodilians are unknown. One way to assess this effect on vertebrates is to measure stress-related hormones to assess welfare, disease, and environmental challenges (Jessop et al. 2003). These effects are often measured by the body's response to internal and external stimuli (stressors) characterized by hyperstimulation of the hypothalamic-pituitary-adrenal (HPA) axis, which increases adrenal gland activity and the synthesis of glucocorticoids (Sapolsky et al. 1986, Guillette et al. 1995, Romero 2002, Sheriff et al. 2011, Cockrem 2013, Dantzer et al. 2014). Wildlife species subjected to increases in glucocorticoid may be susceptible to ecological failures and changes in behavior, reproduction, and survival (Sapolsky et al. 2000, Jessop et al. 2003) and the corticosterone (CORT) levels are a good indication of stress.

Capture and handling stimulates muscular effort via anaerobic pathways (Wingfield et al. 1995, Jessop et al. 2003) and can result in excessive production of lactate, an undesirable product if it accumulates in tissues (Gleeson 1991, Franklin et al. 2003). Crocodilians are especially sensitive to high lactate concentration that can cause profound metabolic acidosis and may lead to death (Seymour et al. 1987). Physiologically the body compensates for this pH disturbance by producing buffers such as bicarbonate and calcium to neutralize the lactic acidosis (Seymour 1985, Jackson et al. 2003).

Approaching and handling are major human disturbances to animals undergoing interaction tourism, which may alter behavior (Lott and McCoy 1995, Bateman and Fleming 2017, Carder et al. 2018, Szott et al. 2019*a*) like increasing wariness in crocodilians subject to a tourism area (Paquier et al. 2006) and physiological parameters that can interfere with the health of individuals in the wild and in captivity (Assis et al. 2015, Szott et al. 2019*b*). Wildlife tourism induces stress in some species (Bateman and Fleming 2017) more than others (Romero and Wikelski 2002). For example, tourist observation of Galapagos marine iguanas (*Amblyrhynchus cristatus*) does not stress the animals (Romero and Wikelski 2002).

Although the effects of interaction ecotourism have been demonstrated for some vertebrate groups, especially mammals, few studies have been conducted on reptiles (Bateman and Fleming 2017) and none with crocodilians. Even though tourism with crocodilians is common place with some species in some regions, such as the saltwater crocodile (*Crocodylus porosus*) in Australia and Malaysia (Ryan 1998, Macdonald et al. 2017, Hassan et al. 2018), American alligator (*Alligator mississippiensis*) in the United States (Jones 2015, Riordan et al. 2020), and black caiman and spectacled caiman in Guyana, French Guiana, and Brazil (Paquier et al. 2006, D`Cruze et al. 2017, Rosenblatt et al. 2021), there have been no studies of stress triggered by interaction tourism with crocodilians. Cohen (2019) reported the 1-sidedness of current studies on encounters between tourists and crocodilians, which prioritize people experiences but do not consider any biological effects on crocodilians.

Handling and artificial brightness caused by photographic flashes could result in activation of the HPA axis. The biological effects of artificial light on vertebrates have been studied for decades (Wurtman 1968). The use of artificial light to locate, capture, and obtain photos is indispensable in Amazonian crocodilian interaction tourism. Artificial light as a source of stress was reported in some vertebrates (Ishida et al. 2005, Morgan and Tromborg 2007) such as tree swallows (*Tachycineta bicolor*) and zebra finches (*Taeniopygia guttata*) in which CORT concentrations increased with exposure to artificial light (Alaasam et al. 2018, Injaian et al. 2021). Although some studies have reported on photographic selfies in tourist activities with wild animals (Osterberg and Nekaris 2015, D`Cruze et al. 2017, Carder et al. 2018), they did not address the effect of photographic flashes on glucocorticoid concentrations as an indicator of stress.

The studies that evaluated physiological parameters in free-living Amazonian crocodilians were limited to records of blood parasites in black caiman (Oliveira et al.

2017), reference values for glucose, triglycerides, and lactate (Barão-Nóbrega et al. 2018), and a single study that evaluated the physiological stress of capture and restraint in black caiman and spectacled caiman. Duncan et al. (2022) used intense capture (3 min in snare) and restraint, which is different from handling experienced during interaction tourism activities, where the capture procedures are more lenient without intense mechanisms such as snares and cables. Therefore, evaluation of the direct effects of tourist interaction on Amazonian crocodilians can help decision making regarding the regulation and planning of interaction tourism.

We evaluated the effects of interactions with tourism on stress-related physiological processes in Amazonian crocodilians. We hypothesized that interaction tourism would cause stress in the short-term in Amazonian crocodilians and therefore predicted that CORT and plasma lactate levels would increase during handling and flashes.

STUDY AREA

This research was undertaken between September and December 2019 in the Anavilhanas Archipelago in Anavilhanas National Park (Figure 1) on the lower course of the Negro River, in the state of Amazonas, between 03°03'13" S and 60°25'15" W, about 40 km northeast of Manaus, Brazil. The climate at the study sites is a tropical rainforest climate, characterized by high moisture and rainfall, with a mean monthly temperature ranging 25–28° C (Salati and Marques 1984). In this area the annual variation of precipitation was 2,000–2,200 mm (Sombroek 2001), and the river level reaches up to 10 m. The area comprises 350,000 ha of unflooded terra firme forest where medium and large-sized mammal assemblages (Tardio and Da Silveira 2015) occur with large trees causing forest structure simplification (Gachot et al. 1966). About 100,000 ha of islands with hydromorphic soils form an archipelago that includes hundreds of lakes and channels. Channels occupy approximately 33.5% of the total area, the islands (levees) make up 40.6%, and lakes and pools cover 25.9% (Latrubesse and Stevaux 2015). The landscape encompasses the most extensive areas of igapó forest, where vegetation is seasonally flooded, subjecting the forest to extended inundation periods of up to 8 months. This dynamic imposes strong restrictions on plant assemblages (Montero et al. 2014) as black-water rivers carry low loads of suspended matter and solutes, resulting in the relatively poor nutrient status (Ayres 1983, Pires and Prance 1985, Junk et al. 2011, Latrubesse and Stevaux 2015, Fleischmann et al. 2022). The canopy height averages about 15–20 m, and species richness was 79 species/ha, with a few emergent trees up to 30 m, but woody lianas are almost absent. It is one forest kind with most one of the low index of species richness in the Amazon (Montero and Latrubesse 2013). The Negro River sustains a rich fish diversity of over 1,000 species (Chao 2001) and other aquatic vertebrates including aquatic mammals (Crema et al. 2020, Vidal et al 2021). In the park there were no inhabitants, it is used for ecotourism activities, but hunting, fishing, and logging in the park occur illegally (Hallwass et al. 2020).

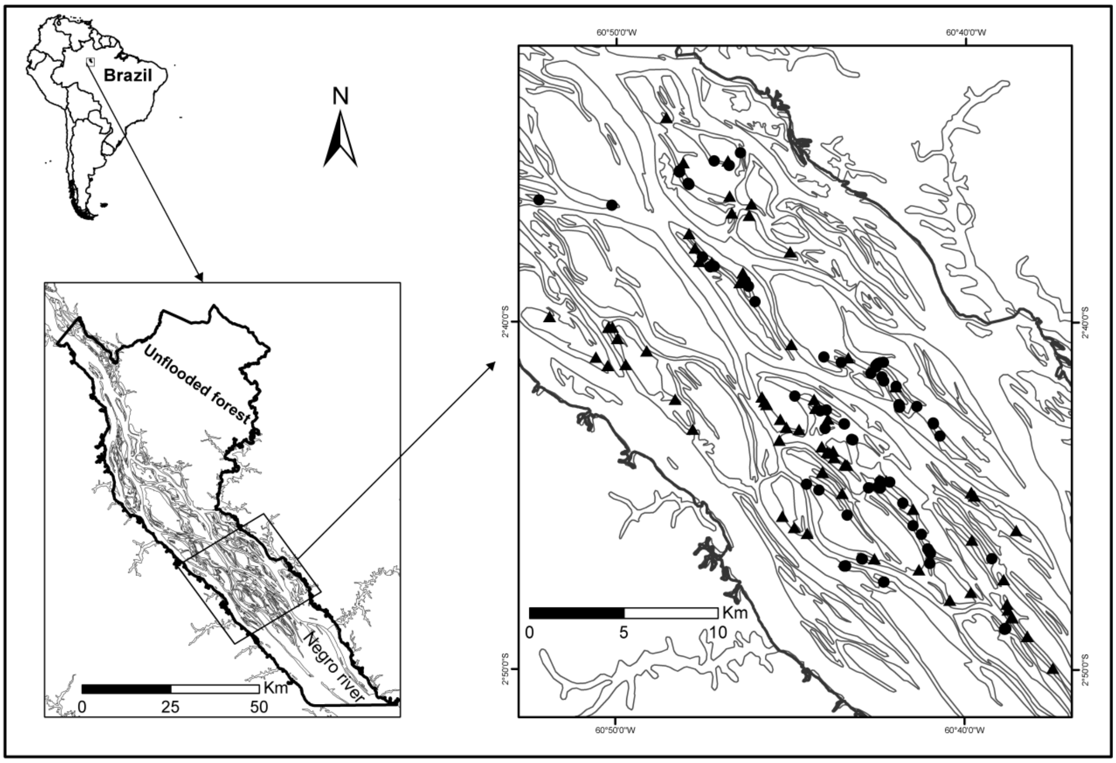

Figure 1. Anavilhanas National Park, Brazil and location of the 63 black caimans (circles) and 58 spectacled caimans (triangles) subjected to experimental handling and photography between September and December 2019.

METHODS

Capture and experimental procedures

We located free-living black and spectacled caimans by their eye shines when illuminated during nocturnal spotlight surveys with observers in a motorized aluminum canoe (Da Silveira et al. 1997). We focused capture efforts on the sizes most targeted by tourism in the area (20 cm ≤ snout-vent length [SVL] ≤ 60 cm; Table 1). We captured smaller individuals (SVL < 40 cm) using 1.80-m reptile tongs (Midwest Tongs®, Greenwood, MO, USA), with broad convex jaws to ensure maximum holding pressure with minimal risk of damage to the reptile. We captured larger crocodilians (40 cm < SVL ≤ 106 cm) with an animal restraining pole (ketch-All®, San Luis Obispo, CA, USA). We captured caimans between 1900 and midnight to standardize corticosterone levels in relation to the circadian cycle (Lance and Lauren 1984). We tried to minimize the stress of capture before subjecting the animals to experimental procedures, as follows. For baseline measurements (control), we captured caimans and collected blood within 30 seconds of capture. To simulate tourist capture (handling), we manipulated the caimans and made hand-to-hand exchanges over a 2-minute period. To simulate the use of photographic flash (flash), we applied 2 photographic flashes of a smartphone (~40–50 lumens) at 20 cm in front of the crocodilian´s eyes every 15 seconds, 8 times (2 min). We simulated the combination of flash exposure and handling (handling + flash) by subjecting the crocodilians to tourist capture with handling and hand-to-hand exchange and applied 2 flashes 20 cm from the animal’s eyes every 15 seconds 8 times, for 2 minutes of combined stimulus. For both caiman species, we collected blood <1 minute after simulations.

Table 1. Snout-vent length (SVL) and mass of black caiman (BC) and spectacled caiman (SC) submitted to the simulated interaction tourism experiment in Anavilhanas National Park, Brazil, 2019.

		Control			Handling			Flash			Handling + flash		
Parameters	Species	*n*	$\overline{X}$	SD	*n*	$\overline{X}$	SD	*N*	$\overline{X}$	SD	*n*	$\overline{X}$	SD
SVL (cm)	BC	21	26.4	6.1	17	27.6	6.9	14	30.3	10.9	11	27.7	5.8
	SC	22	44.0	24.5	9	31.6	10.1	11	32.9	8.2	13	36.1	10.3
Mass (kg)	BC	21	0.4	0.5	17	0.5	0.4	14	0.8	1.1	12	0.4	0.3
	SC	22	3.5	6.1	9	0.8	0.8	11	0.8	0.7	13	1.2	1.0

To obtain blood samples, we captured 63 black caiman (37 males, 22 females) and 57 spectacled caiman (29 males, 27 females) and restrained each one by firmly grasping the neck and tail. We sampled blood from each animal by puncturing the occipital venous sinus in the post-occipital region to access the spinal venous sinus through the interarcuate space between the atlas and axis (Myburgh et al. 2014) using 5-mL syringes with a 25×7-mm needle containing lithium heparin as an anticoagulant. We collected blood samples (~3 mL) within 1 minute of each experimental procedure, refrigerated samples and kept them in a cooler for <4 hours before processed them at the field base in Anavilhanas Archipelago. We centrifuged the samples at 5,000 rpm for 5 minutes and separated the plasma in cryogenic tubes; we then kept samples in liquid nitrogen and stored them at −80°C in an ultra freezer for 4 months until we conducted the biochemical analyses.

Biochemical analyses

We analyzed primary (CORT) and secondary (lactate) parameters in plasma as indicators of physiological stress from interaction tourism. We quantified CORT levels with an enzyme-linked immunosorbent assay (ELISA) method using a commercial kit from Cayman Chemical (Ann Arbor, MI, USA). The CORT extraction protocol followed the manufacturer's specifications. Finger et al. (2018) previously validated the use of this kit for crocodilians. Briefly, the assay protocol consisted of adding 50 µL of the resuspended samples to wells with the addition of 50 µL of the corticosterone-acetyl cholinesterase conjugate, followed by the inclusion of 50 µL of CORT-specific ELISA antibody. We sealed the plates, incubated them at room temperature with gentle shaking for 1 hour, and washed them 5 times in wash buffer. Then we added 200 µL of Ellman's reagent to each well and incubated the plate again at room temperature for 120 minutes. We read the absorbencies (ng/mL) at 450 nm using a spectrophotometer.

We determined lactate levels using commercial enzymatic colorimetric Labtest™ kits (LabtestDiagnóstica S.A., Lagoa Santa, MG, Brazil). We added plasma samples containing lactate to the assay reagent containing hydrogen peroxide, N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline, aminoantipyrine, and the enzymes lactate oxidase and peroxidase. We read the absorbencies (mmol/L) at 550 nm. We performed all assays in duplicate.

Statistical analysis

We evaluated normality and homoscedasticity of the data using the Shapiro Wilk and Bartlett tests, respectively. When the assumptions of analysis of variance were not met, we used a nonparametric Kruskal Wallis test followed by the Dunn test for paired contrasts. We used analysis of variance followed by Tukey's *post hoc* tests ($P < 0.05$) to evaluate the effects of stimulation caused by handling, flash, and the combined effect of both on stress indicators. We performed statistical analyses for each species separately and we considered 4 factors (control, handling, flash, and combined handling and flash), with the response variables CORT and lactate level. We conducted all statistical analyses in program R version 4.2.1 (R Core Team 2022).

RESULTS

The CORT concentration in spectacled caiman did not vary as a function of treatments ($\chi^2 = 5.299$; $P = 0.15$; Figure 2A). The stress sources involving handling or flash increased CORT in black caiman, except for the combined treatment of handling and flash ($F = 24.018$; $P < 0.001$; Figure 2B). In black caiman, handling increased the concentration of CORT relative to the control ($P = 0.016$) 1.7-fold and the flash ($P < 0.001$) increased CORT 2.7-fold (Figure 2B). Use of the flash alone resulted in CORT levels 1.6 times higher than handling ($P < 0.001$; Figure 2B) and twice as high as the combined handling and flash ($P < 0.001$; Figure 2B). The CORT levels were similar to the control (Figure 2B) when handling and flash were combined.

In spectacled caiman, lactate concentration, the result of increased H^+ ion due to muscle activity during anaerobic respiration, also did not vary statistically significantly as a function of the treatments ($\chi^2 = 6.526$; $P = 0.08$; Figure 2C), though the low probability indicates the possibility of a type- II error. In black caiman, lactate concentration increased in all handling treatments ($\chi^2 = 24.119$; $P < 0.001$; Figure 2D). In this species, the lactate concentration produced in handling (Figure 2D) was 2.34 times higher than that recorded in the control ($P < 0.001$) and 1.6 times higher than in the flash treatment ($P = 0.029$). The combined effect of handling and flash increased lactate concentration 2.55-fold over the control ($P = 0.001$; Figure 2D) but was similar to the handling procedure alone.

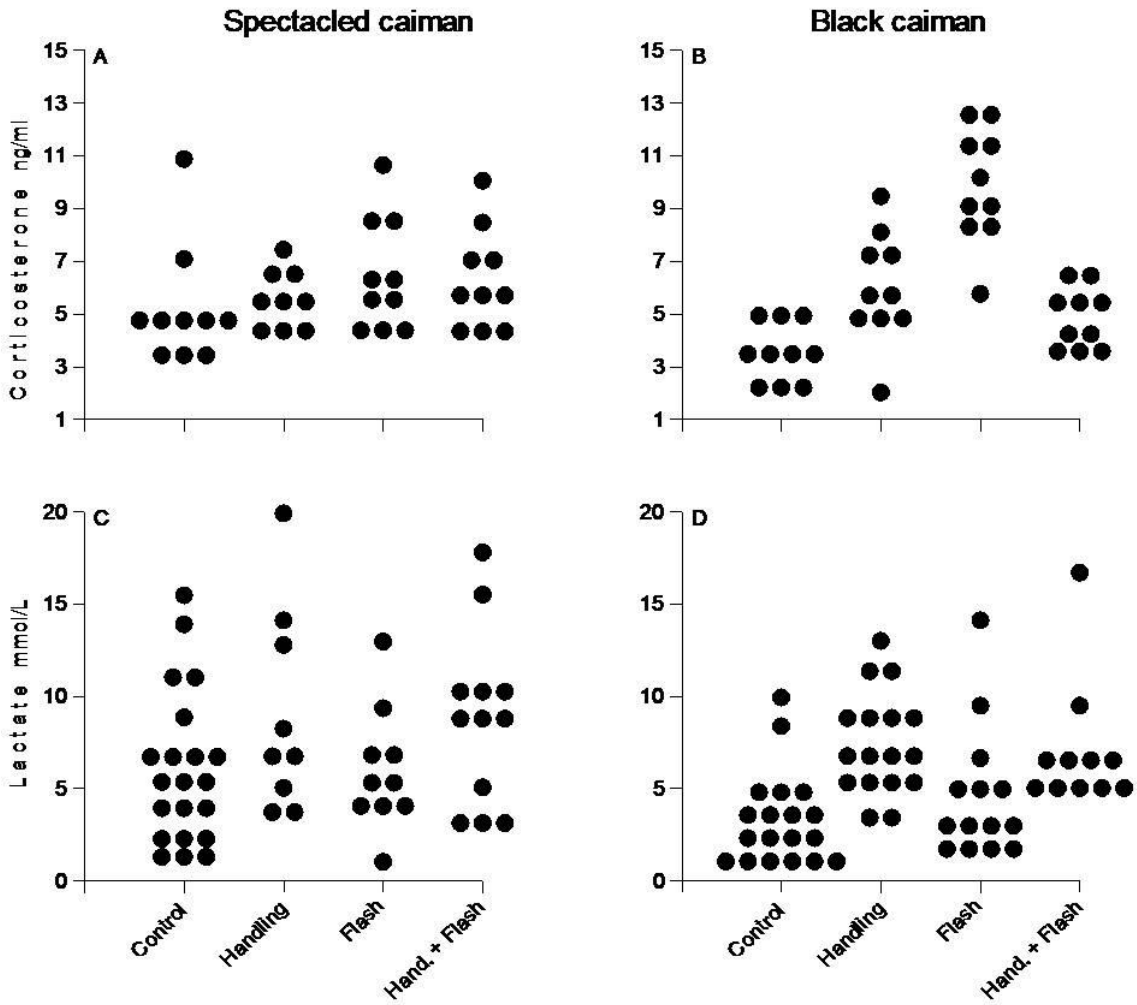

Figure 2. Variance of plasma levels of corticosterone (A, B) and lactate (C, D) levels in spectacled caiman (left column) and black caiman (right column) within 30 seconds of capture (control) and after 2 minutes of touristic simulation (handling, flash, or handling + flash) in Anavilhanas National Park, Brazil, 2019. Each point represents one Amazonian crocodilian.

DISCUSSION

The simulation experiment conducted in the Anavilhanas Archipelago induced distinct physiological responses in the 2 species of Amazonian crocodilians evaluated. While the spectacled caiman was largely indifferent to the stressors (handling, flash), the black caiman was affected by both. In this species, handling and photographic flashes hyperstimulated the HPA axis, as has also been shown for American alligators (Lance and Elsey 1999*a*, *b*). The CORT concentration of black caiman in the flash treatment was 1.6 times higher than in the handling treatment, and 2.7 times higher than the control group. Handling increased CORT to a lesser extent (1.7-fold), maintaining the same pattern observed in the American alligator (Hamilton et al. 2018) and the saltwater crocodile (Franklin et al. 2003).

Baseline values of CORT in black caiman have not yet been established. At comparable sizes (SVL ≤ 55.2 cm), the CORT levels we recorded ($\bar{X}$ = 6.1 ng/mL, SD = 2.86) across all treatments (handling, flash, combined effect of both) were 3-fold lower than those recorded ($\bar{X}$ = 19.6 ng/mL, SD = 15.32) in another stress experiment conducted in the Brazilian Amazon (Duncan et al. 2022). The mean CORT value we obtained across all SVLs ($\bar{X}$ = 3.6 ng/mL, SD = 1.24) was even lower and is the best available estimate of baseline values for free-living black caiman.

For spectacled caiman (SVL ≤ 70 cm) included in the control treatments, the difference in CORT levels compared to other studies was even more striking. Duncan et al. (2022) reported a mean of 68.2 ± 28.12 (SD) ng/mL, whereas we recorded a mean value of only 5.9 ± 1.93. We regard this as the best available estimate of baseline values for free-living spectacled caiman. Notably, the caimans studied by Duncan et al (2022) were closer to an urban center (Manaus, the largest city in the Brazilian Amazon), whereas the caiman populations examined in this study live in a protected area (Anavilhanas National Park) far from large cities.

The combined stimuli (handling + flash) showed no significant difference in CORT values from the control for both species. The increase in CORT in black caiman during simulated tourist interactions, particularly that promoted by the flash, corroborates evidence of initial stress after 2 minutes of stimulus recorded in other vertebrates (Romero and Reed 2005). It is likely that vertical pupil combined with a multifocal optical system (Malmström and Kröger 2006) gives crocodilians vision well-adapted to dark environments, with high light sensitivity and visual acuity (Nagloo et al. 2016). These factors may have contributed to the flash effect in black caiman, as the vision of this species is more similar to that of the American alligator (Sillman et al. 1991) and species of caiman appear to have less spectral variation than species of alligator and crocodiles (Karl et al. 2018). Thus, it may be that the photoreceptors of black caiman were more sensitive to flash, resulting in the increase of CORT. Previous research has shown in free-living zebra finches exposure to artificial light maintained higher levels of nocturnal activity without increasing food consumption and without losing body mass (Alaasam et al. 2018). However, in fish artificial light (Brüning et al. 2015) and photographic flashes (Knopf et al. 2018) do not increase cortisol.

Visitation to free-living western lowland gorillas (*Gorilla gorilla gorilla*) causes increased cortisol concentrations, with gorillas newly habituated to contact with humans showing higher cortisol concentrations (Shuttet al. 2014). Higher visitor numbers to populations of wildcats (*Felis silvestris*; Piñeiro et al. 2012), African elephants (*Loxodonta africana*; Szottet al. 2019*b*), and red deer (*Cervus elaphus*; Dixon et al. 2021) increase cortisol concentrations. The same pattern occurred in simulated tourism interaction with black caiman, indicating that the short-term interaction with this

Amazonian crocodilian is stressful. Knowledge of the effects of this activity on the crocodilians is important for planning tourism and possibly for the conservation of the target species. Crocodilians with elevated CORT become more vulnerable by compromising performance in intra- and interspecific relationships (Jessop et al. 2003, Dantzer et al. 2014), foraging, and growth (Morici et al. 1997), and individuals may become more vulnerable to disharmonic interactions such as cannibalism (Campos et al. 2021).

All handling treatments activated anaerobic metabolism by elevating the circulating lactate levels indicative of metabolic acidosis (H^+ increment) in black caiman but not in spectacled caiman. Lactate acidosis in reptiles is naturally common during physical effort or when they are handled or restrained (Gleeson 1991) as demonstrated in saltwater crocodiles and juvenile American alligators (Franklin et al. 2003, Hartzler 2006). Although common, lactic acidosis may be a metabolic problem that can compromise systems such as cardiac muscles (Seymour et al. 1987) and take more than 12 hours to recover to baseline levels (Jackson et al. 2003, Hartzler 2006). Black caimans subjected to capture with intense effort reached peak lactate after 0.5 hours and took 6 hours to recover to initial levels (Duncan et al. 2022). Based on this assumption and considering that the time to plasma calcium mobilization in black caimans has been reported as 6 hours after intense capture (Duncan et al. 2022), operators should be cautious about handling this species for tourist-interaction purposes. Lactic acidosis in juvenile saltwater crocodiles caused by physical restraint or handling can reduce basking and feeding and cause them to exhibit various disorders associated with escape behavior (e.g., pacing or swimming in stereotypic patterns) or fight with other individual interspecifics (Olsson and Phalen 2013).

Interaction tourism with Amazonian crocodilians in Brazil (D`Cruze et al. 2017, Carder et al. 2018) and neighboring countries (Paquier et al. 2006, Rosenblat et al. 2021) is a positive alternative in face of a long history of destructive use of these species (Smith 1981, Best 1984, Da Silveira and Thorbjarnarson 1999, Mendonça et al. 2016). It provides economic benefit to the local community (Rosenblat et al. 2021), encourages conservation of Amazonian crocodilians by involving local communities, and decreases human–caiman conflict (Pierre et al. 2022).

MANAGEMENT IMPLICATIONS

This study indicates that brief handling and exposure to flashes during simulated tourist interactions increase the levels of CORT and lactate in black caimans. Interaction tourism with Amazonian crocodilians is focused on juvenile individuals and this study indicates that the black caiman is probably more vulnerable to interaction tourism than the spectacled caiman. Continuous sessions of tourist interaction may trigger chronic stress, which could lead to negative consequences of cumulative CORT on the health and survival of individuals. Handling and flash photography from interaction tourism can induce short-term stress-related physiological reactions in black caiman but not in spectacled caiman, which suggests that it would be better to focus interactive tourism on spectacled caimans. Some tourist establishments engage in intense and frequent repeated recaptures, with a consequent increase in the exposure time of the same individual to handling and flashes, which could increase stress or could reduce it through habituation. Until the effects of repeated interventions have been evaluated, it would be best to concentrate on spectacled caimans and distribute tourist activities over a wide area to avoid repeated interaction of the same individuals.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

The license for crocodilian capture and manipulation was granted by the Instituto Chico Mendes de Conservação da Biodiversidade - Chico Mendes Institute for Biodiversity Conservation (authorization number 67559-1). The experimental procedures were approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Amazonas (protocol 001/2020), according to guidelines of the Brazilian Council for the Control of Animal Experimentation.

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CAPÍTULO 2

Washington C. S. Mendonça, Wallice P. Duncan, Marcelo D. Vidal, William E. Magnusson e Ronis Da Silveira. **Blood Biochemical Reference Intervals of Amazonian Black Caiman and Spectacled Caiman** Artigo aceito para publicação no periódico *Journal of Wildlife Diseases* em 25 de setembro de 2023.

RRH: SHORT COMMUNICATIONS

Blood Biochemical Reference Intervals of Black Caimans (*Melanosuchus niger*) and Spectacled Caimans (*Caiman crocodilus*) in the Brazilian Amazon region

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ABSTRACT

Reference intervals for physiologic parameters, crucial for assessing the health status of animals, have been documented for various crocodilian species across the globe. Nonetheless, the establishment of plasma biochemical reference intervals specific to Amazonian crocodilians remains incomplete. In an effort to address this gap, we procured blood samples from 65 black caimans (Melanosuchus niger) and 58 spectacled caimans (Caiman crocodilus) during the period of September–December 2019 within the Anavilhanas National Park in the Brazilian Amazon region We aimed to define reference intervals for 11 key plasma variables measured, namely glucose, triglycerides, total cholesterol, calcium, magnesium, sodium, potassium, albumin, total protein, uric acid, and urea. In general, the determined blood reference intervals aligned closely with those established for other crocodilian species. Some specific measurements, such as total cholesterol, sodium, and magnesium, exhibited distinct variations based on the species. Furthermore, female black caimans showcased elevated cholesterol levels compared with their male counterparts. Within the spectacled caimans, disparities related to sex were evident solely in the case of electrolytes sodium and potassium, with males demonstrating higher levels compared with females. These reference intervals not only provide data for assessing potential fluctuations in the health of wild or captive Amazonian crocodilians but also hold value for veterinary management.

Keywords: Amazonia, Anavilhanas National Park, *Caiman crocodilus*, *Melanosuchus niger*, Plasma references

INTRODUCTION

Five species of crocodilians occur in the Amazon basin, including the black caiman (*Melanosuchus niger*) and the spectacled caiman (*Caiman crocodilus*), which are commercially exploited (Antunes et al. 2016). These two species occur in sympatry in many habitats throughout the Amazon basin, and have been subject to intense legal and illegal commercial pressure for their skins and meat since the 1950s (Antunes et al. 2016; Da Silveira and Thorbjarnarson 1999; Mendonça et al. 2016).

Amazonian crocodilians are also threatened by deforestation, hydroelectric dams, illegal gold mining, and water pollution (Campos 2019; Oliveira et al. 2021). Information on crocodilian physiology, such as blood biochemical intervals, is an important and minimally invasive tool for assessing individual and population health, and for veterinary science (Friedrichs et al. 2012; Nieto-Claudínet al. 2021). However, plasma biochemistry reference intervals for Amazonian crocodilians have been measured in individuals from outside of the Amazon basin and were determined using limited numbers of animals: 12 black caiman (Caixeta et al. 2015) and 30 spectacled caiman (Monteiro 2015).

Recent studies have documented the effect of stresses such as nesting (Barão-Nóbrega et al. 2018) and capture (Duncan et al. 2022) on certain biochemical parameters. This highlights the need for reference intervals which can be used to interpret biochemistry findings as part of health assessment of wild and captive individuals. The aim of our study was to develop such reference intervals.

METHODS

Caimans were captured at night, between 19:00 and 00:00 h, during the dry season, September to December 2019, in 43 lakes and canals in the archipelago (100,000 hectares) of the Anavilhanas National Park (03°03'13" S and 60°25'15" W), an important protected area located on the lower course of the Negro River in central Amazonia, Brazil. The Negro River is the second largest tributary of the Amazon River, and the Anavilhanas Archipelago is one of the most complex and intricate fluvial island systems in the world (Latrubesse and Stevaux 2015). The capture and blood collection procedures were approved by the Animal Use Ethics Committee of the Federal University of Amazonas (Protocol CEUA No. 001/2020), according to the guidelines of the Brazilian Council for the Control of Animal Experimentation. The license for capture and data collection was granted by the Chico Mendes Institute for Biodiversity Conservation (ICMBio, License No. 67559-1).

We focused mainly on individuals with snout-vent length (SVL) between 20-60 cm. Age of individuals was estimated based on age-size relationships for species from the Anavilhanas Archipelago provided by Da Silveira et al. (2013). Sex of individuals was determined by visual inspection of the (Webb et al. 1984) penis.

We collected blood samples by puncturing the occipital venous sinus within 2 min of capture using a 25x0.7 mm needle and a 5 mL syringes containing heparin diluted 1:100 (~50 U/mL). Blood samples (3 mL) were stored in an insulated box with ice until processed within 4 h of collection. Plasma was separated by centrifugation at 2,800 G-force for 5 min, resuspended in liquid nitrogen, then stored at -80ºC in an ultra-freezer (Sanyo Corp., New York, New York USA) until biochemical analyses were performed.

Biochemical Analyses

We measured 11 plasma variables. Parameters evaluated included glucose, triglycerides, total cholesterol, total protein, albumin, urea, uric acid, magnesium (Mg^{2+}), calcium (Ca^{2+}), potassium (K^{+}), and sodium (Na^{+}). Total protein concentration was determined by the Bradford spectroscopic assay (Labtest Diagnóstica S.A., Lagoa Santa, Minas Gerais, Brazil). Metabolites such as glucose, uric acid, and urea levels were determined by commercial enzymatic colorimetric methods, while albumin, Ca^{2+}, and Mg^{2+} concentrations were estimated by a colorimetric assay provided by commercial kits (Labtest Diagnóstica S.A., Lagoa Santa, Minas Gerais, Brazil). Triglyceride and total cholesterol levels were measured using commercial enzymatic colorimetric kits (Vida Biotecnologia, Belo Horizonte, Minas Gerais, Brazil). All assays were performed in duplicate at their respective wavelengths using a Multiskan Go spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA); the mean of the duplicate was used to calculate reference intervals. Plasma electrolyte Na^{+} and K^{+} levels were analyzed by flame photometry using a Digimed DM-62 (Digicrom Analitica LTDA, São Paulo, São Paulo, Brazil).

Statistical analyses

Descriptive statistics were used to analyze the data. Normality was assessed using the Kolmogorov-Smirnov test. When the data had a Gaussian distribution, the Student's t-test was used to compare the two groups (caiman species or sex). The Mann-Whitney *U* test was used for variables that did not meet the assumption of normal distribution. Analysis of covariance ANCOVA was used to evaluate possible effects of size on variables. All statistical and graphical analyses were performed in Systat 8.0 (Systat Software, Chicago, Illinois, USA).

RESULTS

We captured 65 black caimans (21.0 cm $\leq$ SVL cm $\leq$ 55.2) and 58 spectacled caimans (20.0 cm $\leq$ SVL cm $\leq$ 106.8), of which 42/65 (65%) and 30/58 (52%) were males, respectively.

In black caimans of both sexes, total cholesterol (*t*-test, $P = 0.02$; Fig. 1A) and Na^{2+} (U=736.5, P<0.001; Fig. 1C) concentrations were lower than in spectacled caimans. The Mg^{2+} concentration (U=2855.5, P<0.001; Fig. 1D) was higher in black caimans. Other blood biochemical parameters (glucose, triglycerides, Ca^{2+}, albumin, total protein, uric acid, and urea) were similar between the two species (Table 1).

Table 1. Blood biochemical reference intervals for juvenile free-living black caiman (*Melanosuchus niger*) and spectacled caimans (*Caiman crocodilus*) sampled September to December 2019 in the Anavilhanas National Park, Brazilian Amazon. region.

	Black caiman			Spectacled caiman		
Parameter	N	Min-Max	Median or Mean	N	Min-Max	Median or Mean
Glucose (mmol/L)	63	1.4-6	3.6[b]	56	1.3-6.5	3.8[b]
Triglycerides (mmol/L)	64	0.02-4	0.18[a]	55	0.04-1.6	0.21[a]
Total cholesterol (mmol/L)	61	0.6-4.9	2.3[a]	54	1.1-6	3[a]
Total protein(g/L)	64	25.3-55.2	45.2[a]	56	29.0-53.2	44.5[a]
Albumin(g/L)	61	6.7-32.5	13.7[a]	56	4.9-27.4	13.6[a]
Acid uric (mmol/L)	61	28.2-618.2	112.1[a]	55	5.3-664.1	111.2[a]

Urea (mmol/L)	61	0.8-8.4	2.2[a]	56	0.5-5.5	2.44[a]
Ca^{2+} (mmol/L)	64	1.1-3.3	2.2[a]	56	1.1-3.4	2.1[b]
Na^{2+}(mmol/L)	59	82-135	121[a]	56	104-197	127.5[a]
K^{+} (mmol/L)	59	2.2-5.2	3.7[b]	56	2-7.4	3.9[b]
Mg^{2+} (mmol/L)	61	0.7-1.6	0.9[a]	56	0.5-0.9	0.8[a]

[a]median of data non-normally distributed.
[b]mean of data normally distributed.

In black caimans, only total cholesterol was higher in females than in males (*t*-test, *P*= 0.02; Fig. 1A). In the spectacled caiman, Na^+ (*U* = 512.5, *P*=0.04; Figure 1C) and K^+ (*t*-test, *P*=0.002; Fig. 1B) were higher in males than in females.

Total cholesterol (F = 9.717, *P*=0.003), glucose (F = 7.448, *P*=0.008), and urea (F = 5.570, *P*=0.02) had size effects in black caimans. In spectacled caimans, only total cholesterol (F = 13.316, *P*=0.001) was affected by size.

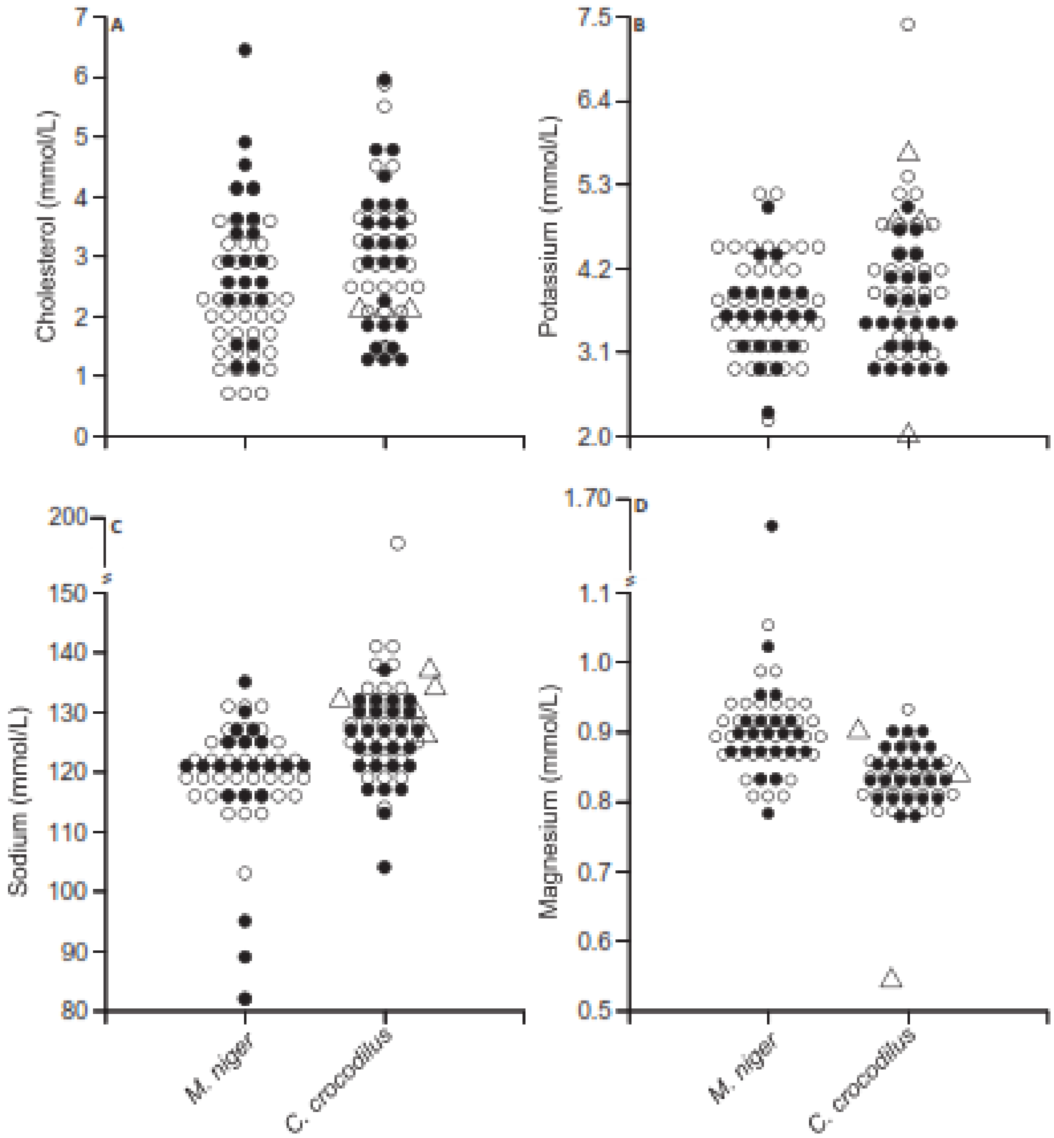

Figure 1. Total cholesterol (A), potassium (B), sodium(C) and magnesium (D) levels in male (open circles) and female (filled circles) black caiman(B C, *Melanosuchus niger*)

and spectacled caiman (S C, *Caiman crocodilus*) sampled September to December 2019 in the Anavilhanas National Park, Brazilian Amazon region. The triangles indicate spectacled caiman individuals with SVL ≥ 60 cm.

DISCUSSION

The 11 blood biochemical parameters analyzed for two species of Amazonian crocodilians were within the range found for other crocodilian species (Olsson and Phalen 2013; Hamilton et al. 2016; Scheelings et al. 2016; Adelakun et al. 2019; Nóbrega et al. 2021; Balaguera-Reina et al. 2022). However, our results established reference intervals for sympatric free-living individuals, including juvenile and subadult black caiman (SVL ≤ 55 cm) with estimated ages (Da Silveira et al. 2013) ranging from 0.5 to 5 years, and all sizes of spectacled caiman (SVL ≤ 107 cm) with estimated ages (Da Silveira et al. 2013) ranging from 1 to19 years.

Cholesterol results in black caiman were close to those found in a *Varzea* habitat in central Amazonia (4.2 mmol/L, Duncan et al. 2022) and similar (2.2 mmol/L) to those found in the same species in southern Amazonia, midwestern Brazil (Caixeta et at. 2015). Total cholesterol in black caiman females (mean = 3.06 ± 1.31 mmol/L) was higher than in black caiman males (mean = 2.33 ± 0.95), suggesting that females may use cholesterol as a primary precursor for steroid hormone synthesis. We do not believe that the higher cholesterol in females was due to reproductive activity as the sizes of the individuals sampled do not correspond to caimans of reproductive age. In addition, positive feedback suggests that cholesterol content is up-regulated in females likely due to the influence of estrogen (Lance et al. 2001; Lavoie 2016). The other authors did not evaluate the variation of this blood variable between the sexes for this species.

Na^+ concentration was lower in black caimans than in spectacled caimans. In addition, Na^+ and K^+ concentrations were higher in males than in females of the spectacled caiman. Higher Na^+ concentrations were also found in wild juvenile males of *Alligator mississippiensis* (Faulkner et al. 2021). In *Crocodylus niloticus*, K^+ concentration was higher in females than in males (Lovely et al. 2007). The slightly higher and significant difference in electrolyte (Na^+ and K^+) intervals may be related to the concentration of androgen hormones and different dietary habits (Birukawaet al. 2005) or hydration status (Faulkner et al. 2021) due to their role in hydro electrolytic balance. These results suggest that the hydro electrolyte profile may vary between species and between sexes and should be investigated in the future. Mean Mg^{2+} concentrations were similar in black caiman and spectacled caiman, but the variance of concentrations was higher in black caimans. For the albumin measurement using bromocresol green is not accurate in most non-mammalian species and therefore is likely not accurate in these species (Cray 2021).

The interspecific differences in biochemical levels and sex suggest different physiological mechanisms in black caiman and spectacled caiman individuals of similar size/age in the same season and habitat. Furthermore, these differences suggest caution in applying reference intervals from one species to another, even when they live in sympatry.

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CONSIDERAÇÕES FINAIS

De modo geral, os resultados obtidos nesta tese contribuíram para o conhecimento sobre os efeitos do turismo de interação com jacarés e sobre a bioquímica do sangue de *Melanosuchus niger* e *Caiman crocodilus* ampliando as informações sobre estes temas até então pouco abordados, mas de ampla importância para conservação e manejo dos crocodilianos amazônicos.

No Capítulo 1, foi realizado o primeiro estudo que avaliou o estresse ocasionado pelo turismo de interação com crocodilianos. Neste estudo foi simulado o a interação entre turistas e jacarés, no qual envolveu a manipulação e flash fotográfico, o qual revelou que uso de flash fotográfico estressa quase duas vezes mais que a manipulação o *M. niger*, enquanto *C. crocodilus* não apresentou efeito, o que implica esta espécie ser mais indicada no caso do turismo de interação. Estes resultados embasarão tomadores de decisão quanto à regulamentação e recomendações do turismo com jacarés. Também se sugere estudos futuros para aumentar dimenssão temporal, a fim de avaliar os efeitos da espacialidade temporal sobre os níveis de cortiscosterona.

No Capítulo 2, o manuscrito descreve pela primeira vez 11 intervalos de referência para parâmetros bioquímicos plasmáticos de *M. niger* e de *C. crocodilus* machos e fêmeas na Amazônia brasileira, estes resultados são de suma importância para compreensão das interferências do ambiente sobre os jacarés. Assim como para avaliação do estado de saúde, condição corporal animal e diagnóstico clínico veterinário tanto jacarés de vida livre quanto de criadouros.

Por fim, os resultados encontrados nesta tese poderão embasar decisões que direcionem o regulamento e protocolos de manejo sustentável não destrutivo de jacarés, no que diz respeirto ao turismo de interação com estes animais, também auxiliarão veterinários e biólogos da vida silvestre nos diagnósticos clínicos e de avaliação da condição física de jacarés. Espero que esta tese incentive outras pesquisas como desdobramentos dos resultados aqui apresentados.

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Tabela de dados de 13 parâmetros Bioquímicos do plasma de *Melanosuchus niger* (MN) e *Caiman crocodilus* (CC) usados nas análises deste estudo.

SPP/cód.	Glicose (mmol/L)	Colesterol (mmol/L)	Lactato (mmol/L)	CORT (ng/mL)	TGC (mmol/L)	Ca (mmol/L)	ProtTot (g/L)	Albu. (g/L)	Mg (mmol/L)	Urato (mmol/L)	Uréia (mmol/L)	Na (mmol/L)	K (mmol/L)
MN07	.	.	.	.	.	.	.	.	.	.	.	114	3.3
MN01	3.07	1.28	8.37	.	0.25	2.69	46.51	17.43	0.86	75.99	2.30	121	3.3
MN02	4.94	6.45	5.43	.	4.09	2.10	49.96	13.12	1.62	351.09	8.43	124	3.6
MN03	4.24	1.29	9.48	.	0.29	3.12	48.88	12.88	0.90	152.45	2.25	121	4.1
MN04	3.62	1.53	4.79	.	0.95	3.25	48.24	17.93	0.90	80.46	2.91	120	3.5
MN05	2.53	1.34	16.69	.	0.53	1.86	44.50	15.73	0.91	124.70	2.76	127	4.2
MN06	3.33	2.23	3.93	.	0.13	2.54	45.32	18.87	0.91	152.91	2.45	.	.
MN13	4.28	4.90	9.92	.	0.76	2.29	50.14	18.65	0.96	186.81	2.73	120	3.9
MN14	1.99	1.05	4.91	.	0.25	2.99	46.08	20.41	0.89	33.43	2.79	126	3
MN16	2.00	0.60	1.07	.	0.19	1.49	38.28	.	.	.	.	.	.
MN19	3.66	2.19	6.38	5.14	0.20	1.39	52.74	16.24	0.81	618.27	1.37	103	2.8
MN20	3.75	3.49	11.38	4.54	0.16	1.14	45.95	.	.	.	.	.	.
MN21	3.80	4.17	4.81	.	1.03	3.38	49.85	13.82	1.02	191.56	3.22	121	3.8
MN22	3.12	1.60	14.10	8.02	0.43	3.23	53.65	14.76	0.87	381.82	1.40	119	3.6
MN23	3.44	3.41	2.31	4.64	0.43	2.98	51.76	13.30	0.84	187.48	1.95	127	4
MN24	2.40	2.90	12.99	.	0.16	2.28	49.63	13.26	0.91	64.54	2.49	124	4.5
MN25	2.74	1.41	4.56	12.61	0.50	2.22	50.62	15.55	0.92	188.86	2.64	115	3.4
MN26	3.27	1.13	5.76	.	0.18	1.99	41.06	.	.	.	.	.	.
MN27	3.30	2.96	6.49	3.75	0.29	2.31	55.24	18.04	0.95	97.32	1.16	95	3.2
MN28	3.46	2.98	1.24	.	0.09	1.96	40.46	14.07	0.83	91.56	1.83	125	3.9
MN29	4.21	3.28	6.64	5.75	0.09	2.77	51.10	10.66	0.92	96.48	1.58	120	4.3
MN30	4.10	3.55	9.35	7.22	0.23	2.93	49.91	12.31	0.82	500.74	1.65	120	4.6
MN31	3.74	4.52	9.48	4.23	0.25	3.32	54.80	18.33	0.92	119.11	1.93	120	4.4
MN32	5.51	2.53	2.75	2.21	0.10	2.45	44.37	18.14	0.91	191.94	1.49	122	3.7

MN34	5.25	3.73	5.48	11.63	0.20	2.30	44.60	14.98	0.88	106.63	2.35	132	3.9
MN35	4.53	1.52	9.30	8.09	0.07	2.56	44.60	15.19	0.91	127.21	2.92	130	3.7
MN36	3.44	1.00	20.61	6.24	0.13	2.68	47.81	32.56	0.90	198.27	0.84	126	3.6
MN37	4.68	3.62	3.09	1.92	0.18	2.57	41.55	10.18	0.78	148.14	1.53	125	3.5
MN38	3.57	2.23	3.74	9.08	0.13	2.31	41.97	7.46	0.91	73.38	1.29	118	3.5
MN42	3.37	1.40	2.96	8.33	0.02	2.04	46.83	9.36	0.88	59.79	2.15	120	3.3
MN43	5.14	2.31	6.59	5.41	0.14	2.01	42.75	9.95	0.89	92.85	2.25	120	4.4
MN46	4.32	2.12	2.67	.	0.75	1.36	45.52	15.85	0.93	60.63	1.56	124	3.4
MN47	3.32	1.83	3.02	.	0.55	2.00	40.17	14.40	0.91	178.99	2.45	121	2.9
MN48	4.72	2.86	4.62	4.31	0.49	2.52	41.82	11.91	0.91	114.17	2.14	82	2.2
MN49	2.20	3.26	3.16	.	0.86	2.54	51.10	16.68	0.93	70.03	1.69	117	3
MN50	4.62	3.60	2.57	8.73	2.01	2.38	45.97	14.30	1.05	153.06	3.47	125	3.8
MN51	4.70	1.49	9.52	4.90	0.12	2.38	38.99	8.63	0.90	196.41	3.70	119	5.2
MN52	6.00	2.47	7.30	6.02	0.14	1.28	41.58	15.83	0.91	87.73	3.04	122	5
MN53	4.36	1.90	6.12	3.29	0.43	1.48	39.90	20.58	0.94	107.10	3.07	117	3.9
MN54	4.76	1.91	1.91	3.48	0.09	2.46	43.57	12.84	0.86	270.96	2.09	117	3.3
MN55	4.74	3.46	1.32	12.27	0.15	1.23	44.51	14.21	0.89	109.61	2.56	135	3.7
MN56	4.15	3.62	6.49	.	0.14	2.11	42.95	13.42	0.86	261.78	2.13	126	4.4
MN57	3.42	4.23	4.76	5.63	0.23	2.09	25.34	13.73	0.84	353.99	2.74	130	4.5
MN58	3.06	3.20	3.46	3.61	0.14	1.29	43.74	18.74	0.98	131.96	2.42	125	4.5
MN59	1.41	2.80	5.66	.	0.10	2.60	51.30	7.54	0.91	69.66	1.08	119	3.1
MN60	2.76	0.85	5.55	2.01	0.13	1.89	43.73	13.05	0.82	28.20	1.67	125	4.5
MN61	2.13	2.97	4.84	5.46	0.71	3.34	49.75	10.96	0.93	193.52	2.22	121	3.8
MN62	3.01	1.56	2.85	.	0.09	2.06	42.32	12.76	0.90	110.91	2.25	117	3.1
MN63	2.28	2.34	8.41	5.33	0.13	2.86	50.88	9.30	0.87	422.36	1.24	117	4.1
MN64	2.80	2.45	5.35	3.98	0.18	1.95	44.32	12.05	0.88	109.61	1.46	82	2.8
MN65	2.58	2.59	1.36	.	0.08	1.83	41.64	8.07	0.86	64.44	1.29	115	3.3
MN66	4.17	4.04	2.70	10.17	0.17	1.57	44.43	13.90	0.90	110.17	2.17	89	2.3

MN67	4.27	0.81	5.56	9.45	0.10	1.46	45.57	9.93	0.83	169.02	2.35	120	3.6
MN68	4.01	2.17	6.39	5.80	0.05	2.10	43.33	12.50	0.87	284.88	1.82	122	4
MN69	2.80	2.41	1.81	5.46	0.13	1.66	41.52	9.18	0.89	151.70	2.36	122	3.7
MN70	3.60	1.66	2.18	11.08	0.06	1.70	44.81	6.71	0.80	201.48	1.81	113	3.3
MN72	3.60	2.17	6.35	.	0.39	1.63	41.46	13.39	0.89	321.71	3.15	118	5.1
MN73	4.28	2.26	5.37	6.17	1.46	1.70	45.52	16.46	0.94	130.28	4.63	.	.
MN74	2.69	1.97	1.52	2.65	0.86	1.59	42.28	13.34	0.99	140.62	4.02	.	.
MN74	4.83	.	2.72	.	1.39	1.59	42.82	.	.	.	.	122	2.8
MN75	3.30	4.02	10.95	7.32	1.44	1.34	40.58	12.80	0.94	92.57	3.43	121	3.9
MN76	4.64	1.84	0.65	3.35	0.19	1.36	44.86	16.01	0.83	76.62	2.15	119	3.7
MN77	4.61	2.19	1.37	.	0.77	1.52	43.47	15.10	0.94	113.34	4.56	125	4.2
MN78	3.30	2.82	2.34	.	0.30	1.37	41.89	13.32	0.88	78.13	1.77	122	3.7
MN79	.	.	.	.	.	.	.	11.44	0.93	101.69	2.61	112	3
MN80	.	.	.	3.19	0.13	1.32	45.64	14.52	0.89	46.84	2.08	115	3.2
CC01	3.44	1.96	14.11	.	1.43	2.20	52.25	13.76	0.88	67.98	4.52	129	3.4
CC02	2.65	2.93	12.95	4.27	0.21	1.92	52.53	16.08	0.89	111.47	2.26	128	5.2
CC06	3.54	3.75	4.95	.	0.49	2.96	50.49	21.61	0.86	232.60	2.89	131	4.1
CC08	3.12	2.25	4.74	6.29	0.25	2.88	49.26	27.47	0.82	52.11	2.45	137	3.7
CC09	2.23	1.18	8.24	5.65	0.05	3.18	47.60	13.63	0.80	398.05	2.72	131	3
CC10	3.98	3.54	21.74	8.46	0.21	2.60	51.77	12.55	0.86	81.33	3.21	131	3.8
CC11	1.35	2.38	6.42	4.85	0.11	3.21	48.63	16.11	0.82	25.48	2.88	137	5.4
CC13	2.34	3.62	21.15	5.26	0.34	1.78	52.51	19.85	0.83	93.90	1.86	133	4.2
CC14	2.89	2.76	20.77	5.18	0.25	2.55	46.46	14.43	0.80	53.59	2.47	140	4.7
CC16	4.80	3.98	17.79	7.06	0.71	3.33	53.26	18.44	0.86	142.17	0.73	120	3.5
CC17	2.60	2.50	5.12	8.24	0.06	2.87	51.31	14.46	0.81	70.05	0.56	130	4.2
CC18	3.49	1.43	10.22	4.33	0.21	3.40	51.78	20.14	0.82	108.33	1.87	124	3
CC20	3.73	4.68	6.83	8.98	0.96	3.36	50.80	17.58	0.88	61.73	1.60	132	4.7
CC21	3.94	3.46	19.90	4.18	0.79	2.11	47.25	15.75	0.83	82.81	1.38	117	3.2

CC22	3.23	.	3.07	.	0.37	2.15	43.62	.	.	.	.	.	.
CC24	2.96	3.36	10.62	.	0.48	2.33	52.74	11.63	0.85	96.49	1.50	123	3.6
CC25	2.78	1.75	3.65	5.33	0.12	2.43	49.51	13.77	0.80	104.81	2.44	129	3.6
CC26	4.61	4.53	15.50	.	1.66	2.56	51.10	14.25	0.93	94.74	2.86	127	3.1
CC27	4.82	.	12.77	4.21	0.95	3.00	50.95	15.62	0.85	383.67	2.69	131	4
CC28	4.31	3.77	3.47	5.43	0.24	2.66	51.86	14.14	0.80	486.36	1.72	123	3
CC29	4.01	2.63	3.53	.	0.11	1.82	40.78	13.30	0.82	59.32	2.40	129	5.1
CC30	3.40	2.60	6.81	10.64	0.65	2.46	46.34	13.72	0.84	5.33	3.04	129	4.9
CC31	4.86	3.34	3.01	5.49	0.10	2.39	39.58	15.70	0.82	74.02	1.35	129	3.2
CC32	.	1.46	.	.	.	.	.	10.64	0.83	18.73	3.34	129	3.2
CC33	6.60	4.50	6.30	.	0.07	2.02	43.51	13.73	0.79	412.15	3.00	129	3.3
CC34	3.28	3.60	9.35	4.52	0.11	2.33	48.86	16.01	0.84	59.14	2.63	132	4
CC35	5.28	2.56	6.34	4.07	0.12	2.58	43.23	.	.	.	.	.	.
CC45	2.52	2.17	6.94	.	0.23	2.18	49.16	15.41	0.83	26.78	2.57	124	4.2
CC46	5.22	2.01	8.38	.	0.20	1.29	43.05	15.33	0.81	118.09	1.69	125	3.3
CC47	2.86	3.61	2.33	3.64	0.04	2.02	35.26	9.17	0.77	44.53	1.99	120	2.9
CC49	3.93	3.04	9.84	5.78	0.07	2.56	41.82	13.33	0.89	394.16	2.26	127	4.7
CC51	3.57	3.46	1.57	5.11	0.47	2.15	41.62	12.55	0.87	111.19	1.98	127	4.4
CC53	5.48	5.87	3.65	5.83	0.60	1.16	44.52	12.39	0.78	144.62	1.30	121	4.1
CC54	4.95	5.95	7.40	4.35	0.22	2.21	44.56	13.31	0.85	419.48	0.95	104	3.4
CC55	5.22	1.28	10.03	10.05	0.05	1.60	41.96	11.13	0.85	373.10	3.21	128	3.1
CC56	4.59	4.81	3.87	.	0.19	1.77	45.77	11.27	0.83	417.16	3.41	126	4
CC57	4.88	4.33	1.02	.	0.11	1.58	42.39	12.28	0.83	422.63	2.48	121	2.9
CC59	4.72	3.18	8.89	5.42	0.09	1.90	41.63	9.05	0.84	397.40	1.54	197	7.4
CC61	4.61	5.50	3.32	7.02	0.67	1.32	35.72	10.38	0.83	172.26	1.98	120	4.4
CC62	4.35	3.02	4.51	7.43	0.10	1.63	42.43	8.98	0.85	107.42	3.27	114	4.4
CC63	4.31	2.95	5.54	7.08	0.23	1.83	41.81	11.28	0.87	407.79	3.94	122	4.2
CC64	5.28	3.12	4.90	4.09	0.48	1.22	42.81	11.02	0.86	415.03	2.53	122	3.5

CC65	4.15	2.80	5.03	6.22	0.08	1.21	43.52	10.92	0.79	94.06	2.63	130	5
CC66	2.66	1.49	2.72	5.81	0.08	1.72	40.16	9.76	0.82	5.84	1.75	125	3.1
CC67	3.94	1.98	4.27	5.06	0.31	1.57	41.75	9.02	0.84	422.45	2.62	113	2.8
CC68	3.88	2.36	15.47	.	0.36	1.73	43.56	15.42	0.83	76.07	0.78	137	4.8
CC69	3.62	3.87	1.30	10.87	0.13	1.85	40.84	10.92	0.90	90.11	2.52	124	3.8
CC70	3.85	1.49	2.27	.	1.13	1.94	41.11	10.49	0.85	245.27	2.04	120	4.3
CC71	3.06	1.98	3.86	4.60	0.12	1.84	40.00	12.99	0.85	426.07	2.30	126	3.9
CC72	5.41	1.96	11.37	3.79	0.08	1.47	47.23	15.78	0.83	248.05	0.83	132	4.7
CC73	2.15	1.40	8.85	.	0.38	1.23	29.03	10.33	0.84	56.96	2.34	130	3.7
CC74	4.65	2.03	13.89	4.47	0.83	1.34	47.14	16.23	0.90	664.10	2.64	134	5.7
CC75	3.06	3.25	6.45	.	0.30	1.25	40.92	9.06	0.82	141.93	3.57	128	4.8
CC76	4.45	3.16	3.86	.	0.70	1.30	38.14	13.18	0.83	127.75	2.80	118	4.7
CC77	1.65	.	0.89	3.15	.	1.59	32.65	4.92	0.54	.	4.08	126	2
CC78	4.10	3.76	8.92	6.75	0.07	1.33	47.71	22.43	0.85	103.15	3.57	125	3.9
CC79	4.46	2.95	5.06	.	0.28	1.34	44.50	15.71	0.83	137.85	1.29	120	3.9
CC85	.	.	.	.	.	.	.	13.80	0.83	407.51	1.35	138	4

Poder Executivo
Ministério da Educação
Universidade Federal do Amazonas
Comissão de Ética no Uso de Animais

UFAM

CERTIFICADO

Certificamos que a proposta intitulada **"Avaliação das tendências populacionais e do estresse ocasionado pelo turismo com populações de jacarés (*Crocodylia, Alligatoridae*) no Parque Nacional de Anavilhanas, Amazônia Central Brasileira"**, sob a responsabilidade do pesquisador **Washington Carlos da Silva Mendonça** (doutorando PPGZOO/ICB/UFAM) sob a orientação do professor Dr. **Ronnis Da Silveira** (docente ICB/UFAM) – que envolve a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica – e por encontrar-se de acordo com os preceitos da Lei n. 11.794, de 8 de outubro de 2008, do Decreto n. 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), após análise pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA UNIVERSIDADE FEDERAL DO AMAZONAS, foi aprovada *ad hoc* sob o N. 001/2020.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	De Março/2020 à março/2023
Espécie/linhagem/raça	*Crocodylia, Alligatoridae*
N. de animais	80
Peso/Idade	1 a 15 kg
Sexo	Machos e fêmeas
Origem	Animal de vida livre – Parque Nacional de Anavilhanas, Novo Airão/AM. Licença SISBIO N. 67559-1

Manaus, 06 de março de 2020.

Profa. Dra. Cinthya Iamile Frithz Brandão de Oliveira
Presidente do CEUA-UFAM

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 67559-1	Data da Emissão: 14/01/2019 12:05:49	Data da Revalidação*: 14/01/2020
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Marcelo Derzi Vidal	CPF: 563.917.652-00
Nome da Instituição: Instituto Chico Mendes de Conservação da Biodiversidade	CNPJ: 08.829.974/0001-94

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Pesquisa socioambiental	03/2019	03/2020
2	Coleta/transporte de amostras biológicas	08/2019	08/2021
3	Captura de animais silvestres	08/2019	08/2021

Equipe

#	Nome	Função	CPF	Nacionalidade
1	Ronis Da Silveira	Pesquisador	109.037.738-05	Brasileira
2	WALLICE LUIZ PAXIUBA DUNCAN	Pesquisador	304.905.962-15	Brasileira
3	Washington Carlos da Silva Mendonça	Pesquisador	743.901.632-72	Brasileira
4	Jean Francisco Venturin Samonek	Pesquisador	008.027.509-58	Brasileira
5	Priscila Maria da Costa Santos	Pesquisadora	003.695.737-22	Brasileira

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Observações e ressalvas

1	[illegible]
2	[illegible]
3	[illegible]
4	[illegible]
5	[illegible]
6	[illegible]
7	[illegible]
8	[illegible]

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Parque Nacional de Anavilhanas	AM	Amazônia	Não	Dentro de UC Federal

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Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Captura de animais silvestres in situ	Melanosuchus niger	-
2	Coleta/transporte de amostras biológicas in situ	Melanosuchus niger	-
3	Captura de animais silvestres in situ	Caiman crocodilus	-
4	Coleta/transporte de amostras biológicas in situ	Caiman crocodilus	-
5	Captura de animais silvestres in situ	Paleosuchus trigonatus	-
6	Coleta/transporte de amostras biológicas in situ	Paleosuchus trigonatus	-
7	Captura de animais silvestres in situ	Paleosuchus palpebrosus	-
8	Coleta/transporte de amostras biológicas in situ	Paleosuchus palpebrosus	-

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Répteis)	Sangue
2	Método de captura/coleta (Répteis)	Captura manual, Laço com cabo de aço

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	Universidade Federal do Amazonas	Laboratório

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Ministério do Meio Ambiente - MMA

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Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

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