FEDERAL UNIVERSITY OF AMAZONAS
FACULTY OF AGRICULTURAL SCIENCES
GRADUATE PROGRAM IN ANIMAL SCIENCE AND FISH RESOURCES

OCCURRENCE AND PHYSIOLOGICAL PARAMETERS OF FRESHWATER STINGRAYS FROM THE UATUMÃ RIVER BASIN IN THE AMAZON

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MANAUS
2024

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Dissertation presented to the Graduate Program in Animal Science and Fishery Resources - PPGCARP of the Federal University of Amazonas – UFAM, as a requirement to obtain the title of Master in Animal Science and Fishery Resources.

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Area of Concentration: Sustainable Use of Fishery Resources

Research Line: Management and Conservation of Fishery Resources

Financing: FAPEAM (Notice No. 007/2021 – BIODIVERSA) and CAPES

MANAUS

2024

Ficha Catalográfica

Ficha catalográfica elaborada automaticamente de acordo com os dados fornecidos pelo(a) autor(a).

G633o Gomes, Maria Fernanda da Silva
Ocurrence and physiological parameters of freshwater stingraysfrom the Uatumã river basin in the Amazon / Maria Fernanda da Silva Gomes . 2024
66 f.: il.; 31 cm.

Orientador: Adriano Teixeira de Oliveira
Dissertação (Mestrado em Ciência Animal e Recursos Pesqueiros) - Universidade Federal do Amazonas.

1. Blood. 2. Conservation. 3. Dorsal coloration. 4. Elasmobranchs.. 5. Elasmobranchs.. I. Oliveira, Adriano Teixeira de. II. Universidade Federal do Amazonas III. Título

MARIA FERNANDA DA SILVA GOMES

OCORRÊNCIA E PARÂMETROS FISIOLÓGICOS DE ARRAIAS DE ÁGUA DOCE DA BACIA DO RIO UATUMÃ NA AMAZÔNIA

Dissertação apresentada ao Programa de Pós-Graduação em Ciência Animal e Recursos Pesqueiros da Universidade Federal do Amazonas, como requisito para obtenção do título de Mestre em em Ciência Animal e Recursos Pesqueiros, área de concentração em Uso Sustentável de Recursos Pesqueiros.

Aprovada no dia 17 de julho de 2024.

BANCA EXAMINADORA

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ACKNOWLEDGMENTS

Thank you, God, for granting me life, supporting me in difficult times, giving me strength to pursue my dreams, and blessing me so much.

My family, especially my mother, Zilma Gomes, who is my example of strength and kindness and never spared efforts to meet my needs, always encouraged me to study and fight for my dreams. My sister, Maria Yasmin Gomes, for always helping me when I needed it, and my father, Ernandes Gomes (*in memoriam*), was always present in my heart and gave me strength.

My advisor, Professor Adriano Oliveira, who is fundamental to my academic and professional growth, has been with me since the third period of my degree and is my most outstanding example of a good teacher/advisor/researcher. Thank you for choosing me, for believing and trusting in me, for always showing me that I would succeed when I thought I wouldn't, for introducing me to science, for your patience, advice, and teachings and especially for introducing me to the fantastic world of freshwater stingrays and physiology.

To my research group, the Núcleo de Estudos de Invertebrados e Vertebrados da Amazônia - NEIVA, for all the teachings and contributions during the weekly meetings.

To my undergraduate and now master's companion Rayana Paixão, for all her help with laboratory analyses, for being my support, for all the conversations, for believing in me and always encouraging me and calming me down in moments of despair, for all the laughter and all the moments we shared.

To my field companions, Maiko Ribeiro, Cristiane Guimarães and Tiago Cabral, for agreeing to venture along the beaches of the Uatumã River; I couldn't have better partners. Despite the tiredness and sleepless nights, the collections were always fun. Thank you for the conversations, laughter and all the help during the collections.

To my friends at the Fish Technology Laboratory for welcoming me to the laboratory and helping me with the analyses.

To the friends I made during my master's degree, Márcia Simões, Ana Clara, Adriene Núzia, Cristiane Guimarães, Tiago Cabral, Maiko Ribeiro, Alexandre Barai, and Rayana Paixão, for all the moments shared in the activities of the research group, in the laboratory, and during the coffee that made the master's degree more special.

To Ph.D. Marcio Quara is responsible for teaching blood tests in the laboratory.

To Ph.D. Paulo Aride for his assistance and availability whenever requested.

To Ph.D. Maria Lúcia Araújo, Ph.D. Gabriela Tomas and Ph.D. Jelmir Almeida, thank you for your contributions to the qualification class.

To Diego Valente, thank you for his help in creating the maps.

To the staff of the Paraíso da Fisheries Sportive Lodge in Presidente Figueiredo and the Hotel Amazonas in Itapiranga for their assistance during the field collections, especially to the fishermen Mario and Otávio Marques "Dunga" for capturing the stingrays and guiding us through the Uatumã river.

The Amazonas State Research Support Foundation (FAPEAM) provided the financial support essential for the development of this study.

The Coordination for the Improvement of Higher Education Personnel (CAPES) for the master's scholarship was granted.

To the Graduate Program in Animal Science and Fisheries Resources (PPGCARP) for the opportunity.

The Federal University of Amazonas (UFAM) and the Federal Institute of Education, Science and Technology of Amazonas (IFAM) are responsible for the infrastructure.

To everyone who contributed to the development of this research.

ABSTRACT

Freshwater stingrays are naturally endemic to South America, and the Amazon region is home to the most incredible diversity; however, areas still lack biological information about this group of elasmobranchs, including the Uatumã River, Amazonas, Brazil. The present work aims to establish the occurrence and physiological parameters of four freshwater stingrays from the Uatumã river basin, Amazonas, Brazil. To this end, stingray collections were carried out in three municipalities bathed by the Uatumã River: Presidente Figueiredo, Itapiranga and São Sebastião do Uatumã. The stingrays were captured through fishing using a hand net, and after capture, the rays were anesthetized, and blood was collected to determine erythrogram and plasma biochemistry values. After blood collection, the captured specimens were identified based on the disc's dorsal and ventral color pattern, the presence or absence of the labial groove and the row of spines on the tail; they were also measured, weighed and sexed. In total, 69 freshwater stingrays were captured, 59 of which were from the *Potamotrygon* genus and ten from the *Paratrygon* genus. Most of the individuals captured were young, and the most common species was *Potamotrygon orbignyi*, which had a reticulated color pattern. Regarding physiological parameters, the values found for the erythrogram and plasma biochemistry were similar to those found in other studies in the Amazon basin. This study provides essential information about the biology of four freshwater stingrays found in the Uatumã river basin, contributing to scientific knowledge, management and conservation of these species, which are fundamental to the Amazon aquatic ecosystem.

Keywords: Blood. Conservation. Dorsal coloration. Ecology. Elasmobranchs.

RESUMO

As arraias de água doce são naturalmente endêmicas da América do Sul, e a região Amazônica abriga a maior diversidade, porém, ainda existem áreas carentes de informações biológicas sobre esse grupo de elasmobrânquios, dentre esses locais, encontra-se o rio Uatumã, Amazonas, Brasil. O presente trabalho tem como objetivo estabelecer a ocorrência e os parâmetros fisiológicos de quatro arraias de água doce da bacia do rio Uatumã, Amazonas, Brasil. Para isso foram realizadas coletas de arraias em três municípios banhados pelo rio Uatumã: Presidente Figueiredo, Itapiranga e São Sebastião do Uatumã. As arraias foram capturadas por meio de pescarias usando rede de mão e após a captura, as arraias foram anestesiadas e foi realizada a coleta de sangue para posterior determinação dos valores do eritrograma e da bioquímica plasmática. Após a colheita de sangue os exemplares capturados foram identificados com base no padrão de coloração dorsal e ventral do disco, na presença ou ausência do sulco labial e na disposição da fileira de espinhos na cauda, além disso, foram medidas, pesadas e sexadas. No total, foram capturados 69 espécimes de arraias de água doce, sendo 59 do gênero *Potamotrygon* e 10 do gênero *Paratrygon*. A maioria dos indivíduos capturados eram jovens e a espécie mais comum foi a *Potamotrygon orbignyi* com o padrão de colorido reticulado. Em relação aos parâmetros fisiológicos, os valores encontrados para o eritrograma e bioquímica do plasma foram semelhantes aos encontrados por outros estudos na bacia Amazônica. Este estudo fornece informações importantes sobre a biologia de quatro espécies de arraias de água doce encontradas na bacia do rio Uatumã, contribuindo para o conhecimento científico, para o manejo e a conservação dessas espécies que são fundamentais para o ecossistema aquático amazônico.

Palavras-chave: Colorido dorsal. Conservação. Ecologia. Elasmobrânquios. Sangue

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LIST OF ACRONYMS

DW: Disk width

TL: Total length

Ht: Hematocrit

Hb: Hemoglobin concentration

RBC: Red Blood Cell Count

MCV: Mean Corpuscular Volume

MCH: Mean corpuscular hemoglobin

MCHC: Mean corpuscular hemoglobin concentration

CONCEA: Council for the Control of Animal Experimentation

EDTA: Ethylenediaminetetraacetic Acid

HPP: Hydroelectric Power Plant

SUMMARY

ORGANIZATION OF THE MASTER DISSERTATION

Even with all the difficulties, the master's thesis is structured into two chapters, the first chapter was published in the journal Fishes (factor impact 2,3) and is the first characterization of four species in the Uatumã river basin, Amazonas, Brazil, unprecedented fact in scientific literature.

The second chapter was submitted to the Annals of the Brazilian Academy of Sciences in August 2024 and addresses for the first time erythrogram values and plasma biochemistry of freshwater stingrays collected in the Uatumã River, Amazonas, Brazil.

1 INTRODUCTION

Freshwater stingrays belong to the subfamily of Potamotrigonines, which emerged from a marine ancestor that invaded the freshwater environment through marine incursions around 15-23 million years ago, through massive movements from marine waters to the upper part of the Amazon Region (Lovejoy, 1998; Lovejoy, Albert and Crampton, 2006). Since then, these organisms have diversified, especially in the Amazon region, as new geographic barriers emerge, becoming a specific group for certain river basins since the network of rivers that make up the Amazon basin provides an aquatic system that favors speciation and the development of diversity (Lasso et al., 2013; Santos and Ferreira, 1999). This group stands out for being the only group of elasmobranchs capable of exclusively inhabiting freshwater environments and playing an essential role in the energy flow between trophic levels in their environments (Shibuya et al., 2016).

The Amazon region contains the most extraordinary diversity of Potamotrigonines species, some with a wide distribution and others with a restricted distribution (Lasso et al., 2013). In their natural environment, Potamotrigonines explore diverse habitats, ranging from beaches, small coves with rocky or muddy bottoms, leaf litter, lakes and flooded forests (Oliveira et al., 2017). Thus, according to the characteristics of the environment, some species may have greater affinity according to the physical and chemical properties of the water (Oliveira et al., 2017).

Among the existing biological tools, the assessment of blood parameters provides valuable information on how freshwater stingrays respond to conditions in the natural environment and the changes caused naturally by the flood pulses and biological aspects, such as sex and ontogeny. (Santos et al., 2024; Oliveira et al., 2021; Oliveira et al., 2017; Oliveira et al., 2016). On the other hand, when evaluating the properties of blood in the *Potamotrygon magdalena* stingray kept in captivity, Perez-Rojas et al., (2022) pointed out valuable information on the health conditions of stingrays kept in captivity, thus being fundamental for management in aquarium conditions.

Among the various rivers in the Amazon region, the Uatumã River, located in northern Brazil, is part of the hydrographic network of the Amazon basin (Baldisseri, 2005). Its springs are located in the Precambrian shield of the Guianas, from where it transports black, acidic waters with a low amount of sediment and nutrients; thus, the Uatumã River is an ecosystem that houses an incredible biodiversity of aquatic organisms (Baldisseri, 2005; Laques et al., 2018) including freshwater stingrays.

However, despite its ecological relevance, information on the biological aspects, including the occurrence and physiological parameters of freshwater stingrays in several basins in the Amazon region, is non-existent, such as in the Uatumã river basin. The lack of specific data on the biological characteristics of these South American elasmobranchs prevents accurate assessments for determining extinction risk (Vazquez and Lucifora, 2023). In addition, most Potamotrigonines are listed as data deficient on the list of threatened species from the IUCN due to the lack of information on population rates and geographic dispersion (Shibuya et al., 2022).

Therefore, freshwater stingrays face significant challenges regarding their conservation, mainly due to their reproductive aspects that result in low rates of population renewal and the increasing pressure from human activities on their habitat (Lucifora et al., 2017; Vazquez and Lucifora, 2023). These factors make species more vulnerable to the impacts caused by fishing exploitation and environmental changes (Carrier et al., 2004; Lameiras et al., 2013; Lucifora et al., 2017), and measures aimed at management and conservation are fundamental to These elasmobranchs are native to South America and make up Amazonian biodiversity.

2 LITERATURE REVIEW

2.1 Uatumã River

The Uatumã river basin is part of the complex hydrographic network of the Amazon basin that covers an area of 6,112,00 km², equivalent to 57% of the Brazilian territory (Baldisseri, 2005). The Uatumã River is a tributary of the north bank of the Amazon River, and its total area is around 70,600 km2 (Baldisseri, 2005). This river is formed by black, acidic waters with a low amount of sediment and nutrients, aspects that make its waters have low ions (Junk et al., 2015; Baldisseri, 2005). The Uatumã River runs in a north-south direction (Baldisseri, 2005), passing through the municipalities of Presidente Figueiredo, Itapiranga, São Sebastião do Uatumã and Urucará, respectively, until it flows into the Amazon River.

Notably, since the 19th century, the Uatumã River has been undergoing transformations linked to exploiting its natural resources (Baldisseri, 2005). This made it the scene of intense conflicts and questionable government actions, the consequences of which profoundly affected its dynamics and, consequently, the production and reproduction of space by its various agents. (Baldisseri, 2005). Uatumã is a case of how managing natural resources can significantly impact the ecosystem, as the Balbina Hydroelectric Plant (UHE) was installed on this river between 1983 and 1987, which flooded an area of more than 3000 km² of water. forests (Assahira et al., 2017). Of the Uatumã basin area of 70,600 km2, around 18,862 km2 represent the contribution basin of the Balbina HPP (Baldisseri, 2005). In the flooded area, the flooded vegetation is still decomposing, compromising water quality in the region (Fearnside, 2015).

The installation of hydroelectric plants causes significant changes in water flow dynamics, reducing high flow and increasing low flow, causing negative impacts on river biodiversity and associated ecosystems (Pekel et al., 2016; Assahira et al., 2017). The construction of the Balbina HPP resulted in significant impacts downstream due to the reduction of the annual flood pulse during the flood season. In the first 45 km downstream of the Balbina dam, there was a period when the river was dry during the dam's filling phase. When the dam was complete, the water released from the spillways and turbines contained low levels of oxygen, which caused mass fish deaths in the river below the dam over a substantial distance (Fearnside, 2019).

This oxygen deficiency also inhibits the re-establishment of fish populations (Fearnside, 2019), in addition to blocking migration, reducing fish stocks along the entire stretch of the dam (Fearnside, 2019), damaging connectivity between populations, reducing genetic diversity and, in the case of freshwater stingrays, causing the loss of color variability (Palmeira-Nunes et al., 2022).

Currently, the Uatumã River has two conservation units (UC), the Uatumã Biological Reserve (REBIO) in the municipality of Presidente Figueiredo, created by federal decree No. 99,277 of June 6, 1990, to compensate for the environmental impact caused by the implementation of the Hydroelectric Balbina (ISA, 2023) and the Uatumã Sustainable Development Reserve (RDS) in the municipalities of São Sebastião do Uatumã and Itapiranga, created in 2004 by decree nº 24,295 (ISA, 2023).

Regarding the region's ichthyofauna, according to the State Secretariat for the Environment (2015), in the Uatumã River, there are species common to the other areas of the Amazon basin, such as the jaraqui (*Semaprochilodus* spp.), the Jatuarana (*Brycon* spp.), pirarucu (*Arapaima* spp.) and species of ornamental interest, such as *Copella nigrofasciata*, *Carnegiella strigata* and *Nannostomus marginatus* and cichlids, such as discus angelfish (*Symphysodon* spp.). In the area influenced by the Balbina HPP, the abundance of species that do not need to carry out migrations and have benefited from the change in the environment was reported, such as peacock bass (*Cichla* spp.) and black piranha (*Serrasalmus rhombeus*) (Santos; Santos, 2005). Regarding elasmobranchs, there is no information about the presence and diversity of this group in the Uatumã River. Hence, data collection and dedicated scientific studies are necessary to understand better the biology and distribution of these elasmobranchs in the Uatumã River, which, in turn, will contribute to the conservation of these species and their habitat in the region.

2.2 Freshwater Stingrays

Freshwater stingrays are cartilaginous rays belonging to the subclass Elasmobranchii (Bonaparte, 1839), order Myliobatiformes (Compagno, 1973) and family Potamotrygonidae (Garman, 1877), arising from a marine ancestor that invaded the freshwater environment through marine incursions during the fourth epoch of the Cenozoic era, approximately 15-23 million years ago, through massive movements of marine waters to the upper part of the Amazon Region, which resulted in the formation of saltwater lakes, which over time they were desalination and the marine ancestor was adapting and diversifying (Lovejoy, 1998; Lovejoy, Albert and Crampton, 2006; Ribeiro-Neto, 2021). Since then, freshwater stingrays have differentiated as new geographic barriers emerge, making them a diverse and specific group for some river basins (Lasso et al., 2013).

Among the central morphophysiological adaptations to living in freshwater are the inability to retain urea, the reduction of the rectal gland that in marine individuals is used to excrete excess salt and changes in the ampullae of Lorenzini for their functioning in freshwater (Rosa, Charvet-Almeida and Quijada, 2010). Until recently, it was believed that the Potamotrygonidae family was made up only of species exclusive to freshwater. However, in 2016 a taxonomic review was carried out on two marine/euryhaline species of the genus *Himantura* of the Dasyatidae family; two species of stingrays (*H. pacifica* and *H. schmardae*) that were assigned to *Himantura* were reassigned to a new genus and subfamily, the genus *Styracura* and the subfamily Styracurinae, which was placed in the family Potamotrygonidae as a sister group to the subfamily Potamotrygoninae, a subfamily of freshwater stingrays (Carvalho, Loboda and Silva, 2016).

Therefore, the Potamotrygoninae subfamily became the only taxon within the Elasmobranchii subclass adapted to survive and reproduce exclusively in freshwater environments. Currently, the group is composed of 45 species divided into four genera (Figure 1): the genus *Paratrygon* (Duméril, 1865), which is considered a species complex, with ten species, *Plesiotrygon* (Rosa, Castelo, Thorson, 1987) and *Heliotrygon* (De Carvalho & Lovejoy, 2011) with two species each and *Potamotrygon* (Garman, 1877) which is the most diverse genus with 31 species (Loboda, 2016; Loboda et al., 2021).

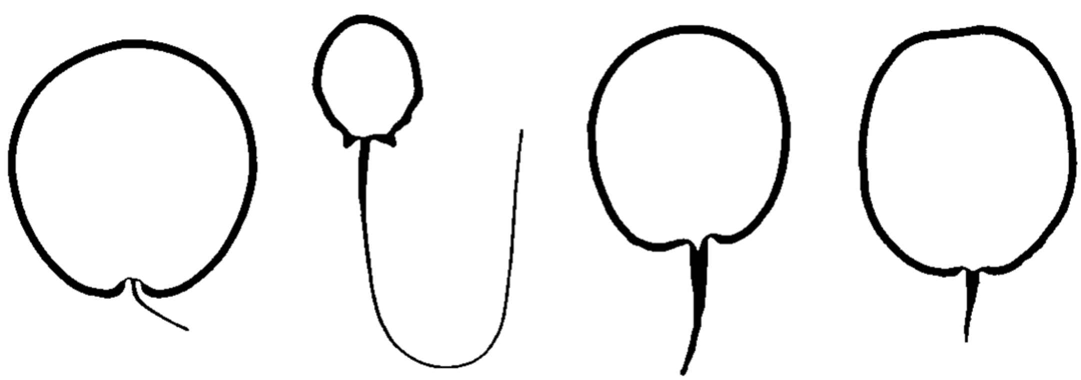

Figure 1: Genus *Heliotrygon*, genus *Plesiotrygon*, genus *Potamotrygon* and genus *Paratrygon*, respectively.

The Potamotrigonines are endemic to the rivers of South America; they are found in almost all countries except for the rivers of Chile, with Brazil standing out for having the most excellent richness (Fontenelle, Carvalho, 2017). Potamotrigoníneos can be found in all rivers that flow into the Atlantic Ocean and the Caribbean Sea, except in rivers belonging to the São Francisco, Prata and Patagonian River basins (Duncan, 2005; Compagno; Cook, 1995; Rosa et al., 2010).

Some species present high phenotypic plasticity and wide distribution in the Amazon basin and can be found in all types of waters (black, clear, white and intermediate types), such as *Paratrygon aiereba* (Müller & Henle, 1841), *Potamotrygon motoro* (Müller & Henle, 1841) and *Potamotrygon orbignyi* (Castelnau, 1855), other species have specialized physiological mechanisms to interact with the environment where they live, and therefore have a geographical distribution limited to watercourses, such as the species *Potamotrygon wallacei* (Carvalho, Rosa and Araújo, 2016), which is endemic to the Rio Negro basin, *Potamotrygon leopoldi* (Castex & Castello, 1970) native to the Xingu river, and *Potamotrygon henlei* (Castelnau, 1855) native to the Tocantins river basin ((Duncan, 2016; Carvalho et al., 2003; Araújo, 2004; Carvalho, 2016; Carvalho et al., 2016).

Stingrays have a dorsoventrally flattened body, which is why the mouth and eyes are positioned on opposite surfaces (Figure 2), they can live in lentic habitats, such as lakes and igapós, and lotic habitats, including strong currents and in the most diverse types substrate, such as rocky, sandy, muddy or leaf litter bottoms (Shibuya et al., 2016; Almeida et al., 2009; Rosa et al., 2010). Despite the wide variety of habitats, stingrays have a preference for specific areas; the species *P. aiereba* has a preference for areas with sandy substrates; on the other hand, the species *P. motoro* can live in intermediate areas between igapós and beaches, with muddy bottoms. (Oliveira et al., 2017).

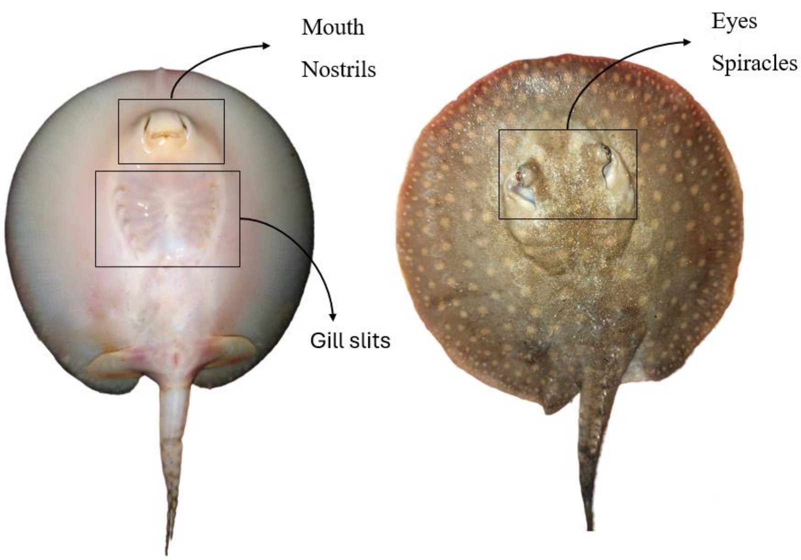

Figure 2: External anatomy of the body of a freshwater stingray from the subfamily Potamotrygoninae.

Freshwater stingrays are benthic inhabitants, and most of their prey inhabit the region close to the bottom, often buried in the substrate or camouflaged under plant debris that falls from surrounding forested areas (Shibuya et al., 2016). Elasmobranchs are predators in almost all environments where they live and play an essential role in the energy flow between trophic levels (Shibuya et al., 2016). They are predominantly carnivorous and have a diet that encompasses a wide variety of prey, including crustaceans, mollusks, fish and, notably, a diverse range of insects, which represent a unique category of prey found in this riverine environment (Shibuya et al., 2016). There are species, such as *P. orbignyi*, that have a specialized feeding habit for consuming insects (Rincon, 2006), while other species, such as *P. motoro*, have more remarkable dietary plasticity, consuming different types of prey depending on local prey availability (Shibuya et al., 2016).

Regarding reproductive aspects, stingrays present internal fertilization and matrotrophic viviparity without the development of a placenta and with the development of a trophoneme and lipid secretion for the nutrition of the embryos (Garrone Neto, 2010). They have biological characteristics similar to marine elasmobranchs, such as low fecundity, slow growth, great longevity, complex reproduction patterns and late sexual maturation. These aspects result in low rates of population renewal and greater vulnerability to overfishing and environmental changes (Carrier et al., 2004; Lameiras et al., 2013; Lucifora et al., 2017). This finding emphasizes the need to implement population management strategies in freshwater ecosystems.

2.2.1 *Potamotrygon orbignyi* (Castelnau, 1855)

The *Potamotrygon orbignyi* stingray is widely distributed in the north of South America and can be found in up to 7 countries: Brazil, Venezuela, Colombia, Guyana, French Guiana, Peru and Suriname, with its occurrence recorded throughout the entire Amazon basin, a basin of the Orinoco, the Approuague River and the Essequibo River basin (Lasso et al., 2013). It occurs in various environments, including rivers (lotic), lakes and other bodies of still water (lentic). In addition, in the Amazon region, it can be found in all three types of water (black, white and transparent) (Lasso et al., 2013). In the Paranã River, the species' preferred area is close to the banks at points of moderate and weak current, where it finds the most incredible abundance of its prey on the muddy or sandy bottom (Rincon, 2006), in the Uatumã River basin, the species also showed a preference for areas with moderate and weak currents, with sandy substrate (Gomes et al., 2024).

P. orbignyi (Figure 3) is a species of medium-sized stingray (Araújo et al., 2009) in the Paranã River; the majority of the population is between 14 and 28 cm in disc width and 15 and 37 cm in length total for males and 15 and 46 cm for females (Rincon, 2006), in the Tocantins River in a study carried out by Silva (2010) specimens ranged from 11 to 47 cm in disc width for male individuals and 9 to 61 cm for females. In Colombia, in individuals from the Bita, Orinoco and Orotoy rivers, the most incredible disc width recorded for females was 53 cm with a weight of 6.3 kg and for males, it was 31 cm, with a weight of 1.5 kilograms (Lasso et al., 2013).

Figure 3: Young specimen of *P. orbignyi* (24 cm DW; 40 cm TL), with reticulated dorsal color pattern.

P. orbignyi presents high polychromatism with several patterns in the dorsal region of the disc, with a predominance of some patterns in the specific areas or basins (Rincon, 2006). Thus, *P. orbignyi* is distinguished from other species of *Potamotrygon* by presenting the bottom of the dorsal region of the disc dark brown to black and although it can show different patterns, such as the reticulate, ocellate, modified rosette, dotted, vermiculated, spot and patterns mixed, the most predominant pattern is formed by beige, brown and black reticules, forming hexagonal or rounded spots (Figure 3), in addition to the tail with dorsal spines generally organized in a single regular row, rarely irregular and in two rows and the labial groove is well developed (Lasso et al., 2013; Rincon, 2006).

Regarding food, *P. orbignyi* has an entomophagous habit (Lasso et al., 2013), but it can also adapt to the availability of food resources present in the environment, depending on the compatibility of the items with the predatory capacity and morphology of its digestive system (Rincon, 2006). In the region of the middle Rio Negro, Amazonas River, Parnaíba River and the Tocantins/Araguaia River basin, *P. orbignyi* had a predominantly insectivorous diet, with the main items being Ephemeroptera larvae, Diptera and Trichoptera larvae (Shibuya, 2009; Rincon, 2006;

Moro et al., 2011; Lasso et al., 2013), on the other hand, in Baiá de Marajó, crustaceans from the order Isopoda were the main items found in the diet of *P. orbignyi* (Bragança et al., 2004).

Regarding the reproduction of *P. orbignyi*, the maturation age is estimated at five years and the maximum longevity at ten years (Rincon, 2006); in the Rio Negro basin, a gestation period of 11 months was observed, with an ovarian fecundity ranging from 3 to 7 eggs and an average embryonic fecundity of 2 embryos (Charvet-Almeida et al., 2005).

2.2.2 *Potamotrygon motoro* (Müller & Henle, 1841)

The *P. motoro* stingray can be found in almost all of South America, being present throughout nearly the entire Amazon basin (except the highest areas of the tributaries of the Solimões river), Orinoco River, Essequibo River, Mearim, Paraguay-Paraná and in Uruguay River, occurring in Brazil, Bolivia, Colombia, Ecuador, Guyana, Paraguay, Peru, Uruguay and Venezuela (Loboda et al., 2016). *P. motoro* can be found in both clear, white and black water, and can inhabit rivers, streams and flood zones (Lasso and Sánchez-Duarte, 2012), and differs from other species of Potamotrigonines by presenting a gray, brown or beige coloration of the disc and pelvic fins, with the predominant ocellate pattern, with the ocelli being large, medium or small. Generally, the ocelli have a central yellow spot, an orange ring in the middle and a peripheral black ring (Figure 4). The tail typically has circular spots from the base to the stinger (Loboda et al., 2016; Rosa and Carvalho, 2016).

The dorsal coloration pattern varies among Amazonian populations. In the Negro River basin, the ocelli are slightly darker and can be found united at the margin of the disc. High chromatic variability is also observed in the Orinoco River basin in Colombia. The species found in Colombia have patterns similar to those found in the Brazilian Amazon, with three color patterns observed: 1) ocelli with a large, central spot, which may be yellow or orange, surrounded by a black ring; 2) a small, central black spot surrounded by a large yellow or orange ring that is also surrounded by another black ring; 3) a large black spot with two to five smaller yellow spots (Loboda et al., 2016).

Figure 4: Young specimen of *P. motoro* (21 cm DW; 27 cm TL), with ocellate dorsal color pattern.

P. motoro appears to be the Potamotrigonine with the most remarkable trophic plasticity, exhibiting the most varied diets (Almeida et al., 2010). In the Curiaú River floodplain in Amapá, Brazil, crustaceans, fish, insects and mollusks were found in its diet. The species presented seasonal feeding behavior, changing its preferred item depending on availability in the environment, with mollusks and fish being most consumed during the flood season and crustaceans and insects most consumed during the dry season (Vasconcelos and Oliveira, 2011). In the region of the middle Negro River and the lower Amazon River, a significant participation of fish in the diet of *P. motoro* was identified (Shibuya et al., 2016). On the other hand, in the Orinoco River in Colombia, the most identified items were insects of the order Diptera and Ephemeroptera (Lasso et al., 2013).

Regarding reproduction, in the El Pañuelo and Caño Dagua lagoons in Colombia, Lasso et al. (2013) recorded three females with ovarian fertility ranging from one to four eggs, while in the Inírida River, the researchers recorded two females with three and six embryos, respectively. P. motoro in the Brazilian Amazon can reach an average of 40 cm in DW and TL of 65 to 70 cm, weighing between 10 and 20 kg; in Ecuador, an average size of 32 cm in DW is reported for males and an average weight of 17.8 kg, and 28 cm in DW for females weighing 16.5 kg (Lasso et al., 2013). In the Colombian, Ecuadorian and Peruvian Amazon, 77 specimens were examined; for males, a maximum DW of 52 cm and a weight of 7.3 kg was recorded; for females, a weight of 11 kg and a maximum DW of 62.5 cm was recorded (Lasso et al., 2013).

2.2.3 *Potamotrygon scobina* (Garman, 1913)

Stingrays of the species *Potamotrygon scobina* are challenging to find and capture (Lasso et al., 2016). They have a more limited distribution when compared to the species *P. orbignyi* and

P. motoro. They can be found in two countries, Brazil and Colombia, in the basins of the Solimões River, Pará River, the lower part of the Tocantins River and the Orinoco River basin (Rosa et al., 2013). *P. scobina* differs from other species of the genus *Potamotrygon* in that its dorsal disc coloration varies between brown, dark brown or dark gray, with small circular white or light yellow ocellated spots or numerous spots grouped in rosettes or around a central ocellus (Figure 5) (Fontenelle and Carvalho, 2017). Pelvic fins dorsally have the same coloration pattern as the disc, with a long tail and a regular row of spines with a large oval base at the back of the tail (Rosa et al., 2013).

Figure 5: Adult specimen of *P. scobina* (24 cm DW; 47 cm TL), with rosette dorsal color pattern.

P. scobina does not have a preference for a particular type of habitat; the species is common in sandy or muddy bottoms and in areas with different current velocities, white water, clear water and black water (Rosa et al., 2013; Gomes et al., 2024). Regarding feeding, the species has a carnivorous diet, feeding on crabs, fish and insects (Rosa et al., 2013; Lasso et al., 2016) in Marajó Bay. Bragança et al. (2004) found crustaceans of the order Isopoda and Decopoda mainly.

Regarding reproduction, in the Parazinho Biological Reserve in Amapá, Brazil, the gonads of five females of *P. scobina*, ranging from 29.4 to 57.9 cm in disc width, were analyzed at the end of the dry period. Only one was pregnant and contained four embryos in the uterus, with the smallest female measuring 29.4 cm in DW (Rosa et al., 2013). Specimens with DW smaller than 16 cm are considered neonates, between 16 and 20 cm are considered juveniles, and above 20 cm are adults (Acosta-Santos et al., 2016).

2.2.4 *Paratrygon* (Duméril, 1865)

Paratrygon is currently considered a species complex; however, for many years, the genus was considered monotypic, with only *Paratrygon aiereba* (Loboda, 2016). The discussion about whether *P. aiereba* is not just one species was addressed by Rosa (1991), Rosa et al. (2010) and Carvalho et al. (2003), but the species was only separated after a taxonomic and morphological review of the genus carried out by Loboda (2016) that resulted in eight more species. Furthermore, Loboda et al. (2021) described two new species for the genus endemic to the Orinoco River basin: *Paratrygon parvaspina* and *Paratrygon orinocensis.*

Although the scientific community accepts that Paratrygon is a species complex, the species are not yet well elucidated, and most of the information in the literature addresses the biological characteristics of *P. aiereba*. Therefore, the present study uses the biological aspects described for *P. aiereba* as a reference for the genus as a whole.

Paratrygon is widely distributed throughout the Amazon basin, being present in the three types of water in the region (Duncan, 2016), and can be found in Brazil, Bolivia, Colombia, Ecuador, Peru and Venezuela, in the Orinoco basin, the Amazon River, the Solimões River, the Xingu River, the Tapajós River, the Negro River, the Napo River, the Putumayo River, the Apure River and the Yanayacu River (Lasso et al., 2013). *Paratrygon* differs from other Potamotrigonine genera by having a flattened, discoid body in the shape of an apple cut in half, pelvic fins covered dorsally by the disc, and a short, filiform tail in young individuals (Figure 6) (Lasso et al., 2013).

Figure 6: Young specimen of the genus *Paratrygon* (22 cm DW; 44 cm TL).

Paratrygon inhabits the main channel of large rivers and streams and is not found in floodplain areas (Lasso et al., 2013). The genus prefers areas with sandy bottoms (Oliveira et al., 2017). Regarding its diet, *Paratrygon* is ichthyophagous-carcinopharyngeal (Lasso et al., 2013). In a study by Barbarino and Lasso (2005) with specimens from the Apure River in Venezuela, Paratrygon consumed exclusively fish. In the Orinoco River, it was found mainly fish and shrimp (Lasso et al., 1996). In the Negro River basin and the Paranã River, fish and insects were found, although insects were found less frequently (Rincon, 2006; Shibuya et al., 2009; Shibuya et al., 2016).

The species *P. aiereba* is large and can reach up to 130 cm in disc width (Oliveira et al., 2017). Regarding the reproductive biology of the genus, in the Apure River in Venezuela, it was observed that males reached sexual maturity from 44 cm DW and females from 37 cm. In addition, it was also observed that females more significant than 61 cm DW were fully mature (Lasso et al., 2013). In addition, sexual and spatial segregation is observed according to size, in which young and subadult individuals of both sexes tend to remain aggregated in the same area. On the other hand, adult males and females are isolated in different regions (Oliveira et al., 2017; Charvet-Almeida et al., 2005).

2.3 Freshwater stingray fishing

Historically, freshwater stingrays have been exploited in the Amazon basin for ornamental purposes since the 1960s (Santos, 2022). Currently, stingray fishing for ornamental and aquarium purposes is regulated by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) through Normative Instruction 204/08, which authorized the commercialization of some species of the genus *Potamotrygon* through an annual quota system for the States of Amazonas and Pará, Brazil (Table 1).

Table 1: Quotas and standards for the exploration, for ornamental and aquarium purposes, of live specimens of native stingrays from continental waters in Brazil.

Stingray	Scientific name	Maximum diameter (cm)	Quotas		
			Amazonas	Pará	Totally

Motoro	*Potamotrygon motoro*	30	4.000	1.200	5.200
Cururu	*Potamotrygon wallacei*	14	6.000	-	6.000
Schroederi	*Potamotrygon schroederi*	30	1.000	-	1.000
Orbignyi	*Potamotrygon orbignyi*	30	1.200	1.200	2.400
Henlei	*Potamotrygon henlei*	30	-	1.000	1.000
Leopoldi	*Potamotrygon leopoldi*	30	-	5.000	5.000
Totally			12.200	8.400	20.600

Source: Adapted from Ibama (2008).

Recently, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) added some species of freshwater stingrays to its second appendix, which includes species that, although not threatened with extinction, may reach that situation and therefore require greater control (CITES, 2023). This data came into force on May 21, 2023. Since IBAMA uses CITES data to evaluate and issue licenses (IBAMA, 2022), changes are likely to occur in regulation 204/08, mainly due to the inclusion of *P. wallacei*, *P. leopoldi* and *P. henlei*, in which trade is legalized in Brazil (Table 2).

Table 2. Freshwater stingrays are included in the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Species	Appendix	Country	Inclusion
Paratrygon aiereba	III	Bolívia, Brazil, Colombia, Ecuador, Peru and Venezuela	Since 2017
Potamotrygon spp.	II	Too many countries	-
	III	Brazil (species that are not in Appendix II)	Since 2017
Potamotrygon albimaculata	II	Brasil	Since 2023
Potamotrygon amandae	III	Argentina, Bolivia, Brazil and Paraguay	Since 2017
Potamotrygon brachiura	III	Argentina, Brazil, Paraguay and Uruguay	Since 2017

Potamotrygon constellata	III	Brazil and Colombia	Since 2017
Potamotrygon falkneri	III	Argentina, Bolivia, Brazil, Paraguay and Peru	Since 2023
Potamotrygon henlei	II	Brazil	Since 2023
Potamotrygon histrix	III	Argentina, Bolivia, Brazil and Paraguay	Since 2023
Potamotrygon humerosa	III	Brazil	Since 2023
Potamotrygon jabuti	II	Brazil	Since 2023
Potamotrygon leopoldi	II	Brazil	Since 2023
Potamotrygon limai	III	Brazil	Since 2023
Potamotrygon magdalenae	III	Colombia	Since 2017
Potamotrygon marquesi	II	-	Since 2023
Potamotrygon motoro	III	Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guiana, Paraguay, Peru, Singapore, Suriname, Thailand, Uruguay and Venezuela	Since 2017
Potamotrygon ocellata	III	Brazil	Since 2023
Potamotrygon orbignyi	III	Brazil and Colombia	Since 2017
Potamotrygon pantanensis	III	Bolivia, Brazil and Paraguay	Since 2023
Potamotrygon rex	III	Brazil	Since 2023
Potamotrygon signata	II	Brazil	Since 2023

Potamotrygon wallacei	II	Brazil	Since 2023
Potamotrygon schroederi	III	Colombia	Since 2023
Potamotrygon scobina	III	Colombia	Since 2023
Potamotrygon yepezi	III	Colombia	Since 2023

For commercial fishing, no specific legislation or fishing statistics characterize this type of fishing for stingrays (Santos, 2022). Although in the Amazon region, the consumption of stingrays is not yet a common practice, according to Batista (2008), stingrays began to be part of fishing landings in Manaus in 2004, and most of these stingrays are sold to states in the northeast and southeast of the country. This is due to the scarcity of marine stocks already in overexploitation conditions, which has caused the gradual replacement of the trade of marine stingrays by freshwater stingrays (Batista, 2008).

Currently, in the states of Amazonas and Pará, there is an increasing exploration and commercialization of freshwater stingray fillets. In some locations, such as the Rio Negro basin region, freshwater stingray fishing for consumption purposes already occurs, with the species *Paratrygon aiereba* and *Potamotrygon motoro* being the most captured species, as they have a more extensive weight range and a higher meat yield (Duncan, 2010; Santos, 2022). In a study carried out by Santos (2022) in which the production of stingray fishing in three municipalities in the State of Amazonas, Tefé, Parintins and Manacapuru, was evaluated between 2015 and 2020, it was found that during this period, approximately 76,913.95 kg of stingrays were traded. Due to the reproductive aspects of stingrays, such as low fecundity, slow growth and late sexual maturation, if exploitation continues to grow, the local populations of these species may be vulnerable or in danger of extinction (Duncan et al., 2010; Lucifora et al., 2017).

Recent analyses of spatial estimates of fishing pressure on freshwater stingrays indicated a population decline of species of the genus Potamotrygon found in the Paraná Basin, with abundance decreasing by 15% annually between 2005 and 2016 (Lucifora et al., 2017). In addition to capture, the constant accidents and injuries involving stingray stingers have driven the practice of damaging fishing, which consists of capturing animals for mutilation purposes by removing their tails or sacrificing them (Oliveira et al., 2015b).

Another threat is illegal trade. In a study conducted by Andrade et al. (2023), a new analytical approach was used, using spectral and chemical reflectance to authenticate freshwater stingray meat samples from the natural environment and supermarkets. A total of 202 samples were collected from the natural environment in municipalities in the state of Amazonas, including 84 individuals of *Potamotrygon orbignyi*, 87 of *Potamotrygon motoro*, and 31 of *Paratrygon aiereba*. From the supermarket, 150 samples of stingray fillets sold as *Potamotrygon* spp. were analyzed. The study results showed that some samples from the supermarket (*Potamotrygon spp.*) were similar to *P. aiereba*, indicating that stingray meat may be sold illegally. The species *P. aiereba*, which is classified as critically endangered by the Ministry of the Environment (2022), is being traded unlawfully as if it were the species *P. motoro* and *P. orbignyi*. According to Oliveira et al. (2017), *P. aiereba* is often traded under the name of different species in Amazonas. These data reflect the difficulty inspection agents from the competent agencies have in identifying the species (Oliveira et al., 2017). This confusion in identifying the species compromises conservation efforts and the protection of endangered species, requiring inspection techniques and trade regulation improvements.

2.4 Physiological parameters

2.4.1 Blood physiology

Blood is composed of a liquid medium, plasma, in which three types of blood cells are suspended: erythrocytes, leukocytes and thrombocytes, which make up the formed elements of blood (Ranzani-Paiva et al., 2013; Tavares-Dias and Moraes, 2004). Plasma comprises 90% water, 7% proteins, and various solutes, such as metabolites, hormones, and enzymes (Ranzani-Paiva et al., 2013). Erythrocytes are the most abundant cells in the bloodstream, and their primary function is to transport gases by combining hemoglobin with oxygen, forming oxyhemoglobin in the respiratory organs and subsequent exchange for tissue carbon dioxide (Ranzani-Paica et al., 2013).

Thrombocytes assist in blood clotting and the body's defense function through phagocytic activity, which can be hemostatic and homeostatic (Tavares-Dias et al., 2002). Leukocytes are defense cells in the blood whose primary function is to act in the immune system by fighting diseases and infections (Zaminhan et al., 2017). Leukocytes have proteins in both the nucleus and the cytoplasm, emphasizing enzymes, which play a fundamental role and are associated with cell granulations (Oliveira, 2013). These proteins in the granules play an essential role in defense against microorganisms, causing their death when released after the rupture of these cells (Oliveira, 2013).

Blood circulates through almost all organic tissues, which makes it strategic in the analysis of the homeostatic framework since the composition and quantity of standard elements in the blood are directly related to the functional state of the animal. It also protects the biological system, eliminates metabolic waste, and transports gases and nutrients, which causes variations in its components to influence endogenous characteristics such as the nutritional balance of animals (Ranzani-Paiva et al., 2013). The information analyzed in blood physiology forms the blood count. The blood count is the set of analyses determining the number of cells in the blood, the volume occupied by the red cells, and the quantity of hemoglobin within them. This information is divided into three parts: erythrogram, leukogram, and thrombogram (Ranzani-Paiva et al., 2013).

The erythrogram quantitatively and qualitatively analyzes the red blood cells (erythrocytes) and consists of counting the erythrocytes (RBC) determining the hematocrit (Ht) and the hemoglobin level (Hb). From these data, it is possible to calculate the hematimetric indices: the mean corpuscular volume (MCV), which evaluates the volume of the erythrocytes; the mean corpuscular hemoglobin (MCH), which determines the amount of hemoglobin present in each erythrocyte, and the mean corpuscular hemoglobin concentration (MCHC), which is related to the concentration of the hematic pigment in the erythrocytes (Ranzani-Paiva et al., 2013).

The leukogram and the thrombogram are techniques used to identify, classify and evaluate the morphology of circulating leukocytes and thrombocytes, respectively (Ranzani-Paiva et al., 2013). By assessing plasma biochemistry, it is possible to obtain information about the levels of glucose, proteins, triglycerides, cholesterol, urea, chlorides, calcium, sodium, phosphorus and potassium. These plasma components are related to dietary conditions (Oliveira et al., 2017; Brito et al., 2015) and are an important biological tool for assessing ionic regulation and stress-related problems (Brinn et al., 2012). Analyzing these parameters makes diagnosing issues related to anemia and other diseases possible, which plays a crucial role in promoting health and well-being.

2.4.2 Blood physiology of freshwater stingrays

Freshwater stingrays have unique physiological characteristics, differentiating them from marine representatives and teleost fish. They cannot accumulate urea in their tissues, have a non-functional rectal gland, and have a smaller amount of erythrocytes in their blood. However, the size of this cell type is more significant than that observed in teleost fish (Wilhelm Filho et al., 1992; Duncan, 2016). About other cells present in the blood, erythroblasts, mature erythrocytes, thrombocytes, lymphocytes, monocytes, heterophils, and basophils can be found in the blood of stingrays. There are no eosinophils, which suggests that heterophils are essential in immune defense (Oliveira et al., 2021). Freshwater elasmobranchs exhibit a wide variation in their physiological profiles, which may result from the influence of external factors, such as habitat diversity, feeding habits and physicochemical properties of the water (Brito et al. 2015; Oliveira et al. 2017).

Studies have already demonstrated the interaction between biological, ecological and hematological characteristics and water properties in freshwater stingrays, indicating that the physicochemical parameters of the water influence the distribution and ecophysiological parameters. Oliveira et al. (2017) carried out a study in the Mariuá archipelago, in the Negro River basin region, with neonates and young individuals of three species of freshwater stingrays: *P. wallacei*, *P. aiereba* and *P. motoro*, ecologically correlating the physicochemical parameters of the water and the hematological parameters, and concluded that *P. wallacei* has a lower red series index than the stingray *P. aiereba*, while *P. motoro* has an intermediate red series pattern, varying between the two species, indicating that the *P. motoro* species is widely distributed in the environment; in contrast, the *P. aiereba* species interacts more closely. The authors observed distinct habitat preferences among three species: *P. wallacei* showed a propensity for igapós, which are submerged vegetation typical of the Amazon; *P. aiereba* showed restricted distribution due to favorable water properties, such as sandy soil areas; *P. motoro* inhabited intermediate areas between igapós and beaches, classified as muddy bottom areas; *P. wallacei* was distributed in areas where water properties were unfavorable, mainly in places with leaf litter (biomass). This study demonstrates a significant integration between biological, ecological and hematological characteristics and water properties in the regions where freshwater stingrays live in the Amazon basin.

Due to this interaction and the variation in physiological characteristics between freshwater stingray species and the variations present in the environment, it isn't easy to use data acquired in a given basin for a given species in another species (Santos et al., 2024; Brito et al., 2015; Oliveira et al., 2015). For this reason, characterizing the cellular components generating hematological and biochemical reference information is essential to establish the physiological state of freshwater populations, which can assist in the diagnosis of health status and improve care and management protocols, both in natural and artificial environments, enabling comparison between these same species under controlled conditions (Pérez-Rojas et al., 2022; Oliveira et al., 2021).

Therefore, understanding how rays react to environmental changes is fundamental to their physiological strategies. In the aquatic environment, there are extreme variations in water parameters, and fish have developed numerous techniques to ensure their survival (Ranzani-Paiva et al., 2013).

3 OBJECTIVES

3.1 General Objective

To establish the occurrence and physiological parameters of four freshwater stingrays from the Uatumã river basin, Amazonas, Brazil.

3.2 Specific Objective

- Record the species and biometric parameters of freshwater stingrays found in the Uatumã River;
- Characterize the Erythrogram and plasma biochemistry of captured stingrays;
- Determine the physical-chemical parameters of the water where the stingrays were captured.

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Article published in May 2024 in the international journal Fishes (Impact factor 2.3)

CHAPTER 1

Occurrence of Four Freshwater Stingrays (Chondrichthyes: Potamotrygoninae) in the Uatumã River Basin, Amazon Region: A Field Study

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Abstract: The Amazon region has the largest diversity of freshwater stingrays; however, there are still places where information about this group is scarce. The present work aims to record the occurrence of freshwater stingrays in the Uatumã river basin, Amazonas, Brazil. For this, collections were carried out in three municipalities bathed by the Uatumã River, Presidente Figueiredo, Itapiranga, and São Sebastião do Uatumã. The rays were collected through fishing using a hand net and, after capture, were identified through the pattern of the dorsal and ventral color of the disc, the absence or presence of the labial groove, and the organization of the row of spines on the tail, and were also measured, weighed, and sexed. In total, 69 specimens of Potamotrygonines were captured, 59 of which were from the genus *Potamotrygon* and ten individuals from the genus *Paratrygon*. The present study provides preliminary information on the characteristics of freshwater stingray species that may occur in the Uatumã River. Furthermore, new research is necessary to improve the identification of specimens to determine the diversity and elucidate aspects related to the biology of elasmobranchs in the Uatumã River Basin, Brazil.

Keywords: biodiversity; geographic distribution; Potamotrigoníneos; Amazonian; stingrays; Brazil

Key Contribution: Add knowledge to characterize the freshwater stingray population in the Uatumã River, Amazon region, Brazil, contributing to increasing knowledge regarding the geographic distribution of freshwater stingrays.

1 Introduction

Stingrays of the subfamily Potamotrygoninae are the only taxon within the subclass of Elasmobranchii adapted to survive and reproduce exclusively in freshwater environments. They are endemic to the rivers of South America and are found in almost all countries, except for rivers in Chile, where Brazil stands out for having more richness [1]. Currently, the group is composed of 45 species divided into four genera: the genus *Paratrygon* (Duméril, 1865), which is considered a species complex, with ten species; *Plesiotrygon* (Rosa, Castelo, Thorson, 1987) and *Heliotrygon* (De Carvalho & Lovejoy, 2011), with two species each; and *Potamotrygon* (Garman, 1877), which is the most diverse genus with 31 valid species [2,3].

Some species present high phenotypic plasticity and a wide distribution in the Amazon basin, and they can be found in all types of water (black, clear, white, and intermediate types), such as South American freshwater stingrays *Potamotrygon motoro* (Müller & Henle, 1841) and Smooth back river stingray *Potamotrygon orbignyi* (Castelnau, 1855), other species have specialized physiological mechanisms to interact with the environment where they live, and therefore have a geographical distribution limited to water courses, such as the species Cururu ray *Potamotrygon wallacei* (Carvalho, Rosa and Araújo, 2016), which is endemic to the Rio Negro basin, and the White-blot ched river stingray *Potamotrygon leopoldi* (Castex & Castello, 1970) native to the Xingu river [4–8].

Stingrays can be found in different habitats and substrates, with their occurrence recorded in both lentic and lotic habitats, as well as rocky, sandy, muddy, or leaf litter bottoms [9,10]. However, despite the wide variety of habitats, stingrays have a preference for specific areas; the species *Paratrygon aiereba* has a preference for areas with sandy substrates; on the other hand, *Potamotrygon motoro* can live in intermediate areas between igapós and beaches, with muddy bottoms [11]. Elasmobranchs are predators in almost all environments where they live and play an essential role in the energy flow between trophic levels [12]. Regarding reproductive aspects in Amazonian elasmobranchs, the seasonal hydrological cycle of river floods and droughts influences reproduction [13] but, in general, they have similar biological characteristics to marine elasmobranchs, such as low fecundity, slow growth, great longevity, complex reproduction patterns, and late sexual maturation; these aspects result in low rates of population renewal and greater vulnerability to overfishing and environmental changes [14,16]. Furthermore, constant accidents and injuries involving stingray stingers have driven the practice of harmful fishing, which consists of capturing animals for mutilation purposes by removing the tail or sacrificing it [17,18].

In recent years, there have been significant advances in studies of freshwater stingrays, including studies on contaminants [19], the determination of stingray meat using low-cost analytical tools [20], blood physiological assessment in a natural environment [21–23], and blood parasite records [24,25]. Still, despite these studies, most Potamotrygonines are listed as Data Deficient on the IUCN List of Threatened Species due to a lack of information on population rates and geographic dispersion [26]. This lack of information occurs because these are regions of difficult access and complexity, where the transport of people and cargo are mainly carried out via waterways [27]; among these locations is the Uatumã River basin, Amazonas, Brazil. The Uatumã River is part of the complex hydrographic network of the Amazon basin that covers an area of 611,200 km2, which is equivalent to 57% of the Brazilian territory [28].

The Uatumã River is a tributary of the Amazon River, and its total area is around 70,600 km2; its sources are located in the Precambrian shield of the Guianas, from which it transports black, acidic waters with a low amount of sediment and nutrients [28]. Uatumã is a case of how managing natural resources can significantly impact the ecosystem, as the Balbina Hydroelectric Plant (BHP) was installed on this river between 1983 and 1987, which flooded an area of more than 3000 km^2 of forests [29]. Of the Uatumã basin area, around 18,862 km2 represents the contribution basin of the HPP [28]. Due to the scarcity of information on the presence of elasmobranchs in the Uatumã River, the present work aims to record the occurrence of freshwater stingrays in the Uatumã River basin to expand knowledge about their geographic distribution. More specifically, it aims to (i) determine the diversity and biometrics of captured freshwater stingrays; (ii) assess developmental stage; and (iii) identify intraspecific phenotypic variability.

2 Materials and Methods

This research was carried out upon release from the Biodiversity Authorization and Information System (SISBIO) following Normative Instruction Ordinance ICMBio n◦748/2022. Number: 76127-4 and by the Ethics Committee on the Use of Animals of the Federal Institute of Education, Science and Technology of Amazonas (2019/010.02.0905). This study was developed following the regulations of the ethical principles in animal experimentation considered by the National Animal Control Council Animal Experimentation—CONCEA.

The collections took place in the Uatumã River basin, the northeastern region of the State of Amazonas, Brazil, with points in the municipalities of Presidente Figueiredo (PF), Itapiranga (ITA), and São Sebastião do Uatumã (SSU) (Figure 1). The collections followed the direction of the river that goes from north to south, starting in Presidente Figueiredo, then Itapiranga, and finally, São Sebastião do Uatumã, which was the closest collection point to the mouth of the Uatumã River, on the Amazon River. In Presidente Figueiredo, collections took place in an area that was impacted by the construction of the Balbina Hydroelectric Plant.

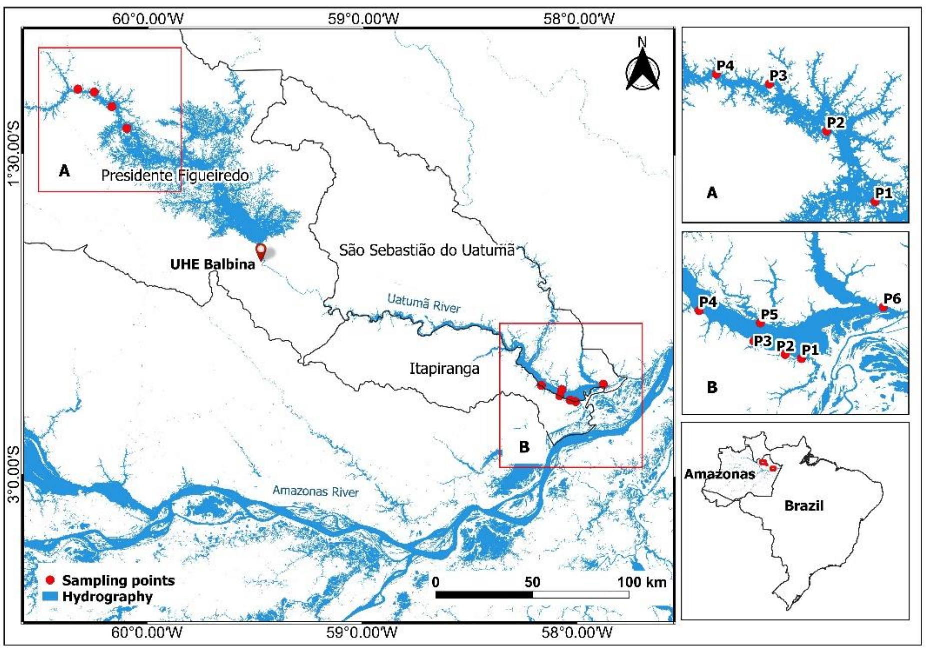

Figure 1: Geographic location of the Uatumã River with an indication of sampling points in the municipalities of Presidente Figueiredo, Itapiranga, and São Sebastião do Uatumã, a northeastern region of Amazonas, Brazil.

The capture of stingrays occurred through night fishing with a hand net, made of micro mesh with a 20 × 20 mm mesh (Figure 2), at a depth of 0.5 to 1.5 m, following the recommendations of Oliveira et al. [30]. Stingrays were collected in November 2022 (dry), May 2023 (flood), and September 2023 (dry).

Figure 2. *Potamotrygon scobina* captured with a hand net being introduced into an immersion bath with eugenol, Uatumã River, northeastern Amazonas, Brazil.

After capture, to avoid possible accidents with stingers, the rays were anesthetized through immersion baths in plastic pockets with eugenol (200 μL·L −1) and then mechanical containment of the stinger was performed using forceps; subsequently, procedures were carried out to obtain biometric data, the disc width—DW and total length—TL were measured using a measuring tape (Figure 3), and the weight was checked using a portable scale.

Figure 3. Measurement of biometric parameters in *Paratrygon* spp. collected in the Uatumã River basin, northeastern Amazonas, Brazil. (a) Measurement of total length; (b) measurement of disc width.

The developmental stage of the captured stingrays was determined from the values recorded for the disc width—DW,; for the *Potamotrygon motoro* stingray, the recommendations of Araújo [31] were followed; for *Potamotrygon scobina* and *Potamotrygon orbignyi*, the recommendations of Acosta-Santos et al. [32] and Lasso et al. were followed [33], respectively; for specimens of the genus *Paratrygon*, data from the species *Paratrygon aiereba* described by Araújo [34] were used.

For identification, the key proposed by Rosa and Carvalho [35] was used, verifying five characteristics: 1) disc shape; 2) pattern of the ventral color of the disc; 3) dorsal color pattern of the disc; 4) absence or presence of the labial groove; 5) distribution pattern of spines on the tail. In addition, sexing was also carried out based on observation of the presence or absence of claspers.

The biometric data were organized in the statistical program R, which was evaluated based on mean and standard deviation.

3 Results and Discussion

3.1. Diversity and Biometric

In total, 69 specimens of Potamotrygonines were captured, 59 of which were from the genus *Potamotrygon* and 10 individuals from the genus *Paratrygon*. Of the 59 individuals of the genus *Potamotrygon*, 44 were of the species *P. orbignyi*, 12 *P. motoro*, and 3 *P. scobina* (Figure 4).

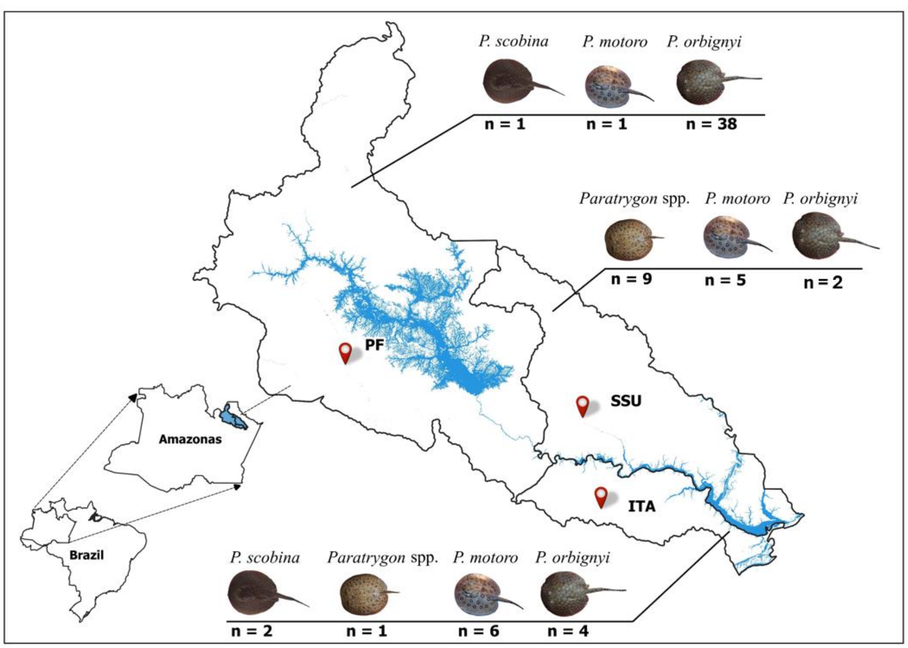

Figure 4. Indication of stingrays captured in the municipalities of Presidente Figueiredo (PF), São Sebastião do Uatumã (SSU), and Itapiranga (ITA) in the Uatumã River basin, northeastern region of Amazonas, Brazil.

Data for biometric parameter values and developmental stages are found in Table 1. All neonatal individuals were captured during the high water season of the Uatumã River in May. This fact may be related to the influence of the hydrological period of Amazon rivers on the reproduction of stingrays [13]. It is essential to highlight that, in this collection, only neonatal individuals were captured, and no individuals were found in other stages of development. In addition to being related to reproduction, this may also be related to the increase in the volume of water, the periodic expansion of the stingrays' habitat, and the reduction in transparency, which causes low visibility and makes capture difficult [36,37]. All other specimens captured at different stages of development (juveniles, subadults, and adults) were collected during the Uatumã River's dry period between September and November. At this time of year, the volume of water is smaller, the habitat is reduced, and there is an increase in transparency, making capturing these individuals easier [4,17]. The total length of the neonate of *P. motoro* captured at point ITA was not determined, as it was captured without the tail, demonstrating the presence of harmful fishing.

Table 1. Values of biometric parameters of stingrays captured in the Uatumã River basin, northeastern Amazonas region, Brazil.

Locality	Species	Development Stage	Sex	n	DW (cm)	TL (cm)	Weight (g)
PF	*P. orbignyi*	Young	M	18	19.0 ± 2.69	33 ± 2.79	384 ± 101.9
			F	15	20.0 ± 2.27	34 ± 3.69	420 ± 139.7
		Adult	M	2	24.0 ± 0.0	38.5 ± 0.70	625.0 ± 7.07
			F	3	24.0 ± 0.0	42.0 ± 1.52	683.0 ± 110.1
	P. motoro	Young	M	1	21.0	32.0	570.0
	P. scobina	Adult	M	1	23.0	44.0	470.0
ITA	*P. orbignyi*	Neonate	F	1	7.5	13.0	270.0
		Young	F	3	24.7 ± 3.5	45.5 ± 3.5	660.0 ± 266.3
	P. motoro	Neonate	M	1	8.0	-	310.0
		Young	M	1	24	43	530

			F	3	21.7 ± 3.05	41 ± 9.64	433.5 ± 179.2
		Adult	M	1	41.5	51.0	570.0
	P. scobina	Neonate	F	1	11.0	21.5	590.0
		Adult	M	1	24.8	47.0	480.0
	Paratrygon spp.	Subadult	M	1	45.0	57.0	3,100.0
SSU	*P. orbignyi*	Young	F	2	20.0 ± 4.2	37.0 ± 4.2	310.0 ± 169.7
	P. motoro	Young	M	2	23 ± 1.0	47.3 ± 6.07	436.6 ± 49.3
			F	3	31 ± 2.82	53.05 ± 13.4	1075 ± 233.3
	Paratrygon spp.	Young	M	2	23 ± 1.41	40.75 ± 12.3	435 ± 35.35
			F	6	23.21 ± 1.54	45 ± 8.83	425 ± 103.2
		Subadult	F	1	33.0	44.0	1330.0

n: Number of samples, **DW:** disc width, **TL:** total length.

3.2 Diagnosis of *Potamotrygon orbignyi* (Castelnau, 1855)

The most common freshwater stingray in the Uatumã River basin was *P. orbignyi*, with the highest incidence in the portion located in the Presidente Figueiredo municipality. This portion of the Uatumã River was impacted by the construction of the Balbina hydroelectric plant, and the flooded vegetation is still decomposing, compromising water quality in the region [38]. The occurrence of *P. orbignyi* in the hydrographic areas impacted by hydroelectric plants was also recorded for the Parnaíba, Tocantins, and Araguaia river basins, highlighting the high adaptability of *P. orbignyi* [39,40].

A total of 38 specimens of *P. orbignyi* were captured at the site. Although this species presents high polychromatism in the dorsal region of the disc [40], all individuals presented the same reticulated pattern (Figure 5a,b). This may have occurred due to the construction of the hydroelectric plant, which intensified the impact of population fragmentation within and between tributaries, resulting in a reduction in genetic diversity and the loss of color variability [39]. The captured species presented the typical pattern of spines on the tail, organized in a row, with a well-developed labial groove, and the majority presented a rounded spot of variable size and color in the ventral region (black and dark gray) in the portion located between and below the slits.

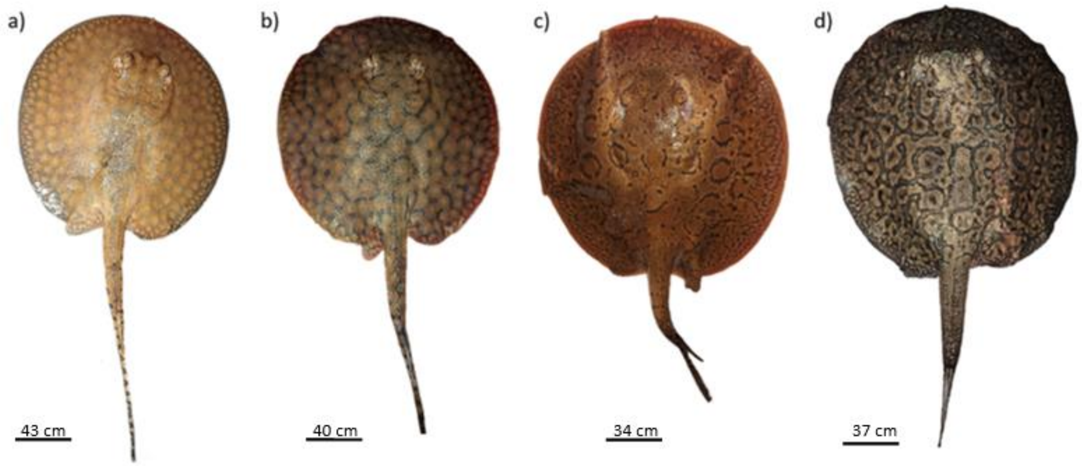

Figure 5. Specimens of *P. orbignyi* were collected in the Uatumã River, northeastern Amazonas, Brazil. (a), (b) *P. orbignyi* young with a reticulated color pattern; (c) *P. orbignyi* young with a brownish background; (d) *P. orbignyi* young with a black background.

Other factors that may explain the abundance of *P. orbignyi* are the effect of the transformation of the river, a lotic environment into a lentic climate, as well as the increase in the flooded area, which consequently increased the biomass of invertebrates, such as insects, favoring the diet of *P. orbignyi*. *P. orbignyi* is predominantly insectivorous [40–42].

In other stretches of the Uatumã River, the occurrence of *P. orbignyi* was lower. Still, in addition to presenting the reticulated dorsal color pattern (Figure 5a,b), species with other patterns, such as a brownish background a black background (Figure 5d), were also found (Figure 5c).

3.3. Diagnosis of *Potamotrygon motoro* (Müller & Henle, 1841)

The *P. motoro* stingray occurred at all the points analyzed in this study, but unlike *P. orbignyi*, *P. motoro* showed a higher incidence in the area outside the influence of the Balbina hydroelectric plant, with only one specimen captured in this region. The specimen presented a variation in the color pattern not found at other points in this study, with small, bicolored ocelli with a yellow center and black outer ring arranged on a dark brown background and without the presence of ocelli on the tail (Figure 6 b).

In total, four different patterns were found in the color of *P. motoro* in the Uatumã River, and the pattern present in stingrays a and b (Figure 6 a, b) is the most widespread and comprehensive in the Amazon basin region [43].

Patterns a and c were found both at the point in ITA and the point in SSU. Pattern a is formed by large, tricolor eyespots with a yellow and orange background and a black outer ring arranged on a brown background, while pattern c is formed by yellowish ocelli arranged on a light brown background (Figure 6 a, c). The pattern of stingray d (Figure 6 d) is less common for this species, being found in more restricted areas. Only one specimen with this pattern was collected at the point in ITA and, to date, this pattern has only been recorded in three regions, two in Peru and one in Brazil on the Tarauacá River in the state of Acre [43]. All the captured specimens had a circular disc, poorly developed labial furrow, and a single row of spines on the tail.

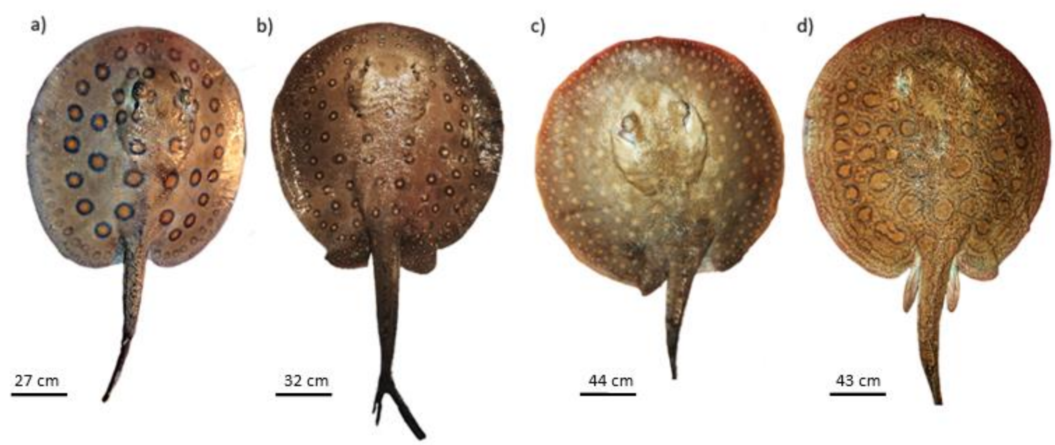

Figure 6. Specimens of *Potamotrygon motoro* collected in the Uatumã River basin, northeastern Amazonas, Brazil. (a) *P. motoro* young with tricolor ocelli captured in ITA and SSU; (b) *P. motoro* young with bicolor ocelli captured in PF; (c) *P. motoro* young with yellow ocelli captured in ITA and SSU; (d) *P. motoro* young with a less common pattern for the species, collected at the point in ITA.

3.4. Diagnosis of *Potamotrygon scobina* (Garman, 1913)

P. scobina was the species found least frequently; its occurrence was recorded at two collection points, PF and ITA (Figure 3), with two specimens at the ITA point and one at the PF point. The specimens from the ITA point presented a brownish disc with numerous clustered light spots distributed throughout the disc and tail with a single row of spines (Figure 7a).

The PF specimen had a brownish disc with small, light eyespots and a tail with spines arranged irregularly at the base and organized in a single row on the rest of the tail in addition to spots spread throughout the tail (Figure 7b).

Figure 7. Specimens of *Potamotrygon scobina* collected in the Uatumã River basin, northeastern Amazonas, Brazil. (**a**) Specimen adult collected in ITA; (**b**) specimen adult collected in PF.

All the captured individuals had a subcircular disc with a more prominent central portion, a robust tail up to the base of the stinger that became thinner from that point onwards, a poorly developed labial groove, a poorly exposed pelvic fin, and a light ventral region, showing a white color.

3.5. Diagnosis of *Paratrygon* spp. **(Dúmeril, 1865)**

Until recently, the genus *Paratrygon* was believed to be the only monotypic genus in the family Potamotrygonidae, with only the species *Paratrygon aiereba* [2].

Although *P. aiereba* is one of the first species of freshwater stingrays described, there is still no extensive and specific literature about it [3]. The discussion about gender diversity had already been addressed by Rosa [44], Rosa et al. [10], and Carvalho et al. [5], but the species was only dismembered by Loboda [2] after carrying out a taxonomic and morphological review of the genus, which resulted in eight more species.

However, although it is currently known that *Paratrygon aiereba* is a complex species, the characteristics of the species are not yet well elucidated, which is why individuals of *Paratrygon* are referred to only by the generic epithet in the present work.

In the Uatumã River, three color patterns were found for *Paratrygon*, with patterns b and c (Figure 8 b, c) being the most similar and predominant. For pattern a (Figure 8 a), only one specimen was found at point SSU. In Figure 8, stingray d is the standard of stingray c at another stage of development, with stingray c being a young individual and stingray d a subadult.

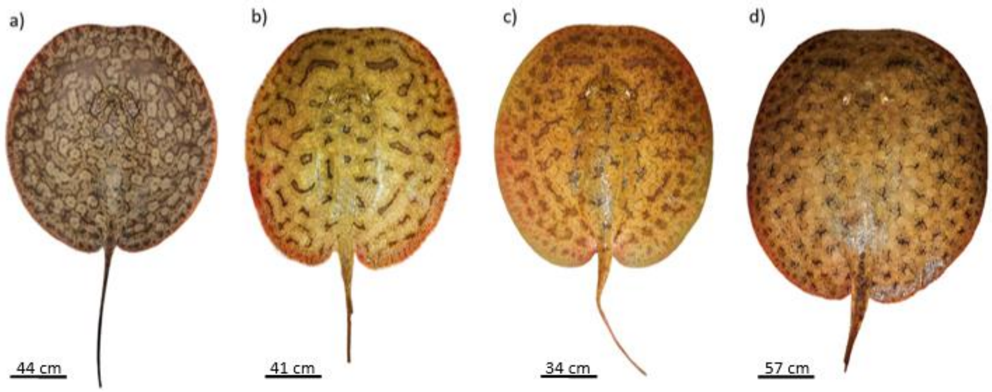

Figure 8. Specimens of the genus *Paratrygon* captured in the Uatumã river basin, northeastern Amazonas, Brazil. (a) Specimen young captured at the SSU point; (b) specimen young collected in ITA and SSU; (c) specimen young collected in ITA and SSU; (d) subadult specimen collected in ITA and SSU.

All the captured specimens presented characteristics already described in the literature: an absence of labial groove; slightly concave shaped disc; a long, thin tail without the presence of spines in young individuals and subadult individuals; a short, robust tail with long spines organized irregularly throughout the tail.

All the captured individuals of the *Paratrygon* spp. were captured in areas with a sandy substrate, corroborating what Oliveira described [11] about the preference of these stingrays for beaches. This preference may be related to the absence of *Paratrygon* spp. at the PF point.

4. Conclusions

In general, the present work adds knowledge about the characterization of the stingray population in the Uatumã River region and the Amazon region, contributing to an increasing collection of knowledge related to the geographic distribution of freshwater elasmobranchs.

Although only four species were collected, the high degree of polychromatism found in the color pattern of the rays was notable since the color was related to adaptation processes related to the characteristics of the species, such as eating habits, hunting, and escaping from threats [45].

Furthermore, the high rate of young individuals collected reflects the capture method used in this study.

This study provides preliminary information on the characteristics of freshwater stingray species that may occur in the Uatumã River. Furthermore, new research is necessary to improve the identification of specimens to determine the diversity and elucidate aspects related to the biology of elasmobranchs present in the Uatumã River Basin.

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Article submitted to the Annals of the Brazilian Academy of Sciences in August 2024

CHAPTER 2

Physiological parameters of freshwater stingrays from the Uatumã River Basin, Amazon, Brazil

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ABSTRACT

Knowledge of freshwater stingrays' physiological parameters is crucial for conserving elasmobranch diversity. However, many regions lack this data, including the Uatumã River in the Amazon. This study aims to characterize the erythrogram and plasma biochemistry of freshwater stingrays from the Uatumã River basin for the first time. Specimens were captured in Presidente Figueiredo, São Sebastião do Uatumã, and Itapiranga. Blood samples were collected from 69 stingrays and processed to obtain blood values. The results were similar across locations, species, and existing data from the Amazon basin.

Keywords: Erythrogram. Conservation. Hematology. Plasma biochemistry. Potamotrygoninae.

1 INTRODUCTION

Stingrays of the subfamily Potamotrygoninae are a unique group of freshwater cartilaginous stingrays that evolved from marine ancestors around 15-23 million years ago, adapting to freshwater environments in the Amazon region (Lovejoy, 1998; Lovejoy, Albert, and Crampton, 2006). Over time, they diversified as new geographic barriers emerged, leading to the current recognition of four genera: *Paratrygon*, *Potamotrygon*, *Heliotrygon*, and *Plesiotrygon* (Loboda, 2016; Loboda et al., 2021). These stingrays differ from their marine relatives by lacking the ability to accumulate urea and having a non-functional rectal gland; they also have larger erythrocytes and lower blood parameter values compared to teleost fish, reflecting their adaptation to freshwater (Duncan, 2016; Wilhelm Filho et al., 1992).

Understanding the physiological parameters of these species is challenging due to the difficulty of capturing them and the risks associated with their sting (Oliveira et al., 2015; Santos et al., 2020). However, establishing hematological and biochemical reference values in natural environments is crucial for assessing their health and improving care protocols in the wild and captivity (Pérez-Rojas et al., 2022; Oliveira et al., 2021). Since blood composition is directly linked to the animal's functional state, knowledge of how stingrays respond to environmental changes is essential for understanding their survival strategies (Ranzani-Paiva et al., 2013). This study aims to characterize, for the first time, the erythrogram and plasma biochemistry of freshwater stingrays from the Uatumã River basin, Amazonas, Brazil.

2 MATERIAL AND METHODS

2.1 Ethics in Research

The collection of stingrays was carried out with the necessary authorization from SISBIO - Biodiversity Authorization and Information System, by ICMBio Ordinance nº 748/2022, under number 76127-4. Ethics Committee of the Federal Institute of Education, Science and Technology of Amazonas, number: 1955.0905/2023.

2.2 Study area

The research was carried out in the Uatumã river basin, with collections of stingrays from the Potamotrygoninae subfamily in the municipalities of Presidente Figueiredo, São Sebastião do Uatumã and Itapiranga, Amazonas, Brazil (Figure 1).

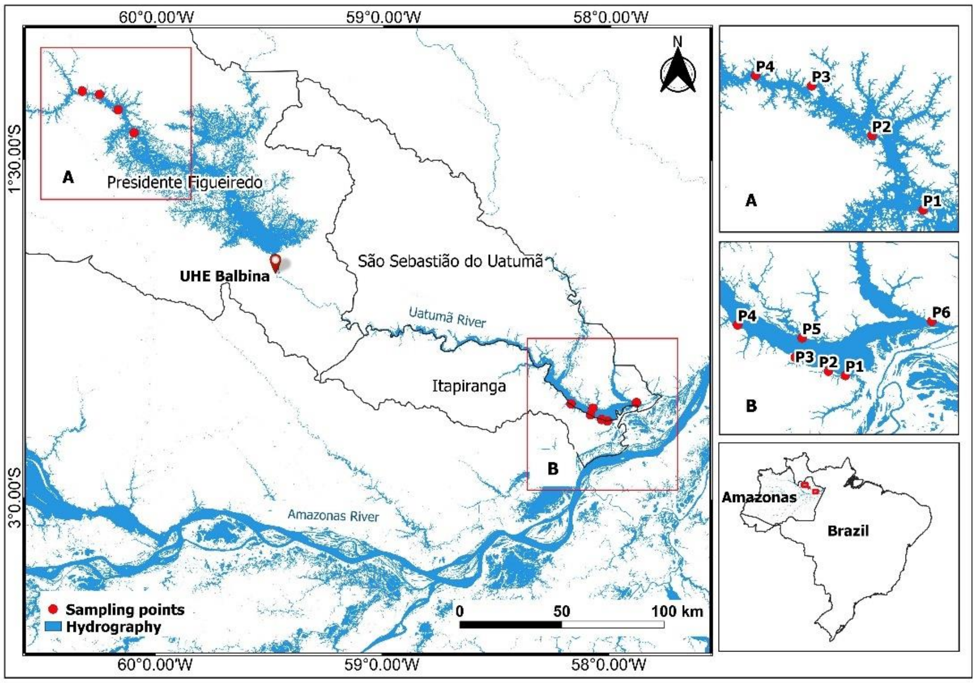

Figure 1: Geolocation of the Uatumã River, highlighting the collection points located in the Northeast region of Amazonas, Brazil. **Source:** Gomes et al., (2024).

2.3 Stingray capture

The stingrays were captured by night fishing with the aid of flashlights for illumination; the fishing instrument used was a hand net made of 20x20 mm micromesh, and the capture occurred at a depth of 0.5 to 1.5 meters, following the recommendations of Oliveira et al., (2012). After capture, the individuals were anesthetized with eugenol (200 μL.L-1), according to Oliveira et al. (2015), each individual remained immersed in the eugenol bath in plastic bags for 4 minutes. After this period, the stinger was mechanically restrained with Foerster forceps to avoid possible accidents. A total of 69 stingrays were captured.

2.4 Blood collection

Blood collection was performed by puncturing the gill vessel, which is the most recommended method for freshwater stingrays, as it is less invasive and poses less risk to the animal (Oliveira et al., 2012). Each captured stingray collected 1.0 to 2.0 mL of blood with syringes containing around 15 μL of 10% EDTA anticoagulant. The samples were kept at a low temperature (-4 °C) until inclusion in the reagents and plasma centrifugation.

3 RESULTS AND DISCUSSION

In the three investigated locations, four species of the genus *Potamotrygon*, *43 P. orbignyi*, 11 *P. motoro*, 3 *P. scobina* and 2 *P. marquesi* and 10 specimens of the genus *Paratrygon* were identified.

In the physiological parameters of the stingrays captured in Presidente Figueiredo, the similarity was observed in both the red blood series (Ht, Hb, RBC, MVC, MHC and HCMC) and plasma biochemistry (total cholesterol, glucose, triglycerides, total proteins and albumin) between the development stages and between the species investigated (Table I).

Table I. Erythrocyte and biochemical parameters of freshwater stingrays in the Uatumã River in Presidente Figueiredo, Amazonas, Brazil.

Parameters	**Species**			
	P. orbignyi		*P. motoro*	*P. scobina*
	Young	Adult	Young	Adult
Ht (%)	18.17 ± 3.31	18.10 ± 4.04	17	13
Hb ($g.dL^{-1}$)	3.18 ± 0.70	3.17 ± 0.71	3.11	2.16
RBC ($millions.\mu L^{-1}$)	0.33 ± 0.07	0.33 ± 0.03	0.25	0.31
MCV (fL)	558 ± 97.62	551.25 ± 100.60	693.88	419.35
MCH (pg)	97.57 ± 21.49	96.63 ± 18.39	126.93	69.70
MCHC ($g.dL^{-1}$)	17.43 ± 1.82	17.52 ± 0.94	18.29	16.62
Cholesterol ($mmol.L^{-1}$)	30.15 ± 20.85	28.30 ± 11.78	38.71	16.46
Glucose ($mmol.L^{-1}$)	25.51 ± 8.24	39.34 ± 28.93	32.77	10.42
Triglycerides ($mmol.L^{-1}$)	84.09 ± 47.08	64.63 ± 57.81	50.09	37.84
Total Proteins ($g.L^{-1}$)	1.19 ± 0.46	0.92 ± 0.37	0.98	0.69
Albumin ($g.dL^{-1}$)	0.25 ± 0.22	0.12 ± 0.08	0.27	0.38

Itapiranga was the only location where neonates and individuals of *P. marquesi* were captured. At this stage, the *P. orbignyi* and *P. scobina* stingrays presented lower blood profiles than those found in young, adult and subadult individuals (Table II).

Table II. Erythrocyte and biochemical parameters of freshwater stingrays in the Uatumã River in Itapiranga, Amazonas, Brazil.

Parameters	**Species**								
	P. orbignyi		*P. motoro*			*P. scobina*		*Paratrygon* spp.	*P. marquesi*
	Neonate	Young	Neonate	Young	Adult	Neonate	Adult	Subadult	Young
Ht (%)	12	21 ± 7.07	10	20 ± 2.82	22	17	20	52	16.75 ± 3.88
Hb (g.dL^{-1})	3.37	4.68 ± 1.64	2.61	4.46 ± 0.12	4.86	3.37	3.53	4.33	4.25 ± 0.8
RBC (millions.µL^{-1})	0.33	0.33 ±0.10	0.27	0.34 ± 0.07	0.39	0.46	0.34	0.67	0.28 ±0.07
MCV (fL)	369.36	624 ± 13.2	370.4	585.3 ± 49.9	564.1	369.96	588.24	776.12	599.8 ± 12.57
MCH (pg)	124.85	138 ± 4.98	96.67	133.7 ± 33.69	124.6	73.25	103.82	64.63	153.03 ± 9.85
MCHC (g.dL^{-1})	34.33	22.2 ± 0.32	26.10	22.57 ± 3.82	22.09	19.82	17.65	8.33	25.50 ± 1.10
Cholesterol (mmol.L^{-1})	23.99	56.95 ± 12.77	15.60	45.3 ± 6.37	80.99	13.01	38.53	69.88	41.43 ± 7.04
Glucose (mmol.L^{-1})	14.59	86.05 ± 54.96	5.43	77.73 ± 9.81	58.77	18.88	35.08	22.87	154.76 ± 10.79
Triglycerides (mmol.L^{-1})	10.81	93.68 ± 58.14	69.10	32.03 ± 16.41	69.40	20.36	141.58	124.9	30.58 ± 6.75
Total Proteins (g.L^{-1})	0.46	1.17 ± 0.59	0.91	1.35 ± 0.37	1.03	0.87	0.77	0.52	1.37 ± 0.16
Albumin (g.L^{-1})	0.24	3.70 ± 0.88	0.17	2.92 ± 0.34	3.66	0.26	3.16	2.17	2.67 ± 0.244

In São Sebastião do Uatumã, only juveniles and subadults were captured, with the latter stage being identified only in *Paratrygon* spp. (Table III). Thus, the values were considered low for the erythrogram of juveniles of *P. orbignyi*. However, for plasma biochemistry, the values were higher than those of the other individuals in this location (Table III).

Table III. Erythrocyte and biochemical parameters of freshwater stingrays in the Uatumã River in São Sebastião do Uatumã, Amazonas, Brazil.

Parameters	Species			
	P. orbignyi	*P. motoro*	*Paratrygon* spp.	
	Young	Young	Young	Subadult
Ht (%)	20.5 ± 0.7	28.1 ± 6.54	26.71 ± 7.65	39.5
Hb (g.dL^{-1})	3.36 ± 0.25	3.27 ± 0.25	2.31 ± 0.52	5.46
RBC (millions.µL^{-1})	0.41 ± 0.10	0.41 ± 0.10	0.41 ± 0.08	0.57
MCV (fL)	501.17 ± 93.9	690.5 ± 72.7	648.7 ± 117.6	692.98
MCH (pg)	83.30 ± 30.5	83.24 ± 17.1	58.1 ± 17.25	95.79
MCHC (g.dL^{-1})	16.33 ± 3.0	12.06 ± 2.20	9. 27 ± 3.28	13.82
Cholesterol (mmol.L^{-1})	54.98 ± 1.7	46.5 ± 18.3	56.9 ± 13.59	37.82
Glucose (mmol.L^{-1})	45.27 ± 7.5	20.81 ± 12.3	15.94 ± 4.57	16.00
Triglycerides (mmol.L^{-1})	115.20 ± 74.6	81.8 ± 36.87	93.59 ± 49.33	51.36
Total Proteins (g.L^{-1})	1.75 ± 0.7	1.41 ± 0.49	1.57 ± 0.60	2.01
Albumin (g.L^{-1})	3.10 ± 0.03	3.18 ± 0.22	2.86 ± 0.38	2.86

Blood parameters in freshwater stingrays have been established for some species in different regions of the Amazon basin. Oliveira et al. (2016, 2017, 2021) studied the species *Potamotrygon wallacei*, *P. motoro*, and *Paratrygon aiereba* in the middle Rio Negro region. Oliveira et al. (2015) investigated *P. orbignyi* and *P. schroederi*. Pérez-Rojas et al. (2022) determined the hematological and biochemical profile of the stingray *Potamotrygon magdalenae* in captivity in Colombia. Santos et al. (2024) analyzed the blood parameters of *P. motoro* and *P. orbignyi* in the lower Rio Solimões, Amazonas, Brazil.

Duncan et al. (2010) pointed out that the physicochemical properties of water limit the geographic distribution of freshwater stingrays in the Amazon. Furthermore, Santos et al. (2024) indicated that ontogeny, not sexual dimorphism, influences physiological parameters, such as erythrogram, in *P. orbignyi*. Plasma biochemistry, related to diet and stress, suggests good biochemical conditions in the Uatumã River stingrays, despite the environmental changes caused by the Balbina HPP (Oliveira et al., 2016; Santos et al., 2024). This study adds crucial data to the physiology of freshwater stingrays and supports conservation strategies, particularly in the Uatumã

River, highlighting the need for more research in the Amazon basin (Santos et al., 2024).

It is concluded that the available literature on the blood physiology of freshwater stingrays has little data available, so the present study adds information that can be used in strategies to manage and conserve freshwater stingrays, especially in the Uatumã River. Considering the changes that may occur between species and in the ontogeny of the taxon, as well as those caused by the flood pulse, future studies should be conducted to evaluate these factors, as well as to understand better the blood physiological responses of the stingrays of the Uatumã River. In addition, studies should also be conducted with several species of Potamotrigonines found in the Amazon River systems that have not yet been explored to assist regulatory agencies and the commercial chain in Brazil and other countries.

4 CONCLUSION

This is a pioneering study on the physiological parameters of freshwater stingrays in the Uatumã River. It provides essential information on their health status in an environment that has impacted human activities, such as the Balbina Hydroelectric Power Plant construction.

The study provides a basis for future research on stingrays in the Uatumã River basin. It encourages continued research, both in the Uatumã River and in other basins in the Amazon region, to better monitor changes in the physiological conditions of stingrays due to environmental changes that are increasingly intensifying, contributing to their preservation by evaluating the effectiveness of implemented conservation measures, minimizing negative impacts on these organisms.

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DISSERTATION CONCLUSION

The occurrence of freshwater stingrays in the Uatumã River basin suggests that the site provides suitable conditions for their survival and reproduction. The physiological parameters found were similar to those found for these species in other basins in the Amazon region.

The species presented differences in dorsal coloration patterns according to the collection sites, especially in Presidente Figueiredo when compared to the other two sites. In addition, the size and development stage of the captured stingrays were influenced by the fishing gear used.

This study provides important information on the occurrence and physiological parameters of freshwater stingrays in the Uatumã River basin, contributing to scientific knowledge and conservation of these species. These data benefit future studies to expand understanding of the ecology and importance of stingrays for the Amazonian aquatic ecosystem.