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ECOMORFOLOGIA, MORFOMETRIA E ALIMENTAÇÃO DE
PEIXES DA RESERVA DE ITAMACAOCA, BACIA DO RIO MUNIM,
LESTE MARANHENSE.

ELIOENAI DA SILVA OLIVEIRA

SÃO LUÍS – MA
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ECOMORFOLOGIA, MORFOMETRIA E ALIMENTAÇÃO DE
PEIXES DA RESERVA DE ITAMACAOCA, BACIA DO RIO MUNIM,
LESTE MARANHENSE.

Dissertação apresentada ao Programa de
Pós-Graduação em Biodiversidade e
Conservação – PPGBC/UFMA, como
requisito parcial para a obtenção do grau de
Mestre em Biodiversidade e Conservação.

Linha de Pesquisa: Diversidade Animal e
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Orientador: Prof. Dr. Felipe Polivanov Ottoni
Coorientadora: Dra. Josie South

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ECOMORFOLOGIA, MORFOMETRIA E ALIMENTAÇÃO DE PEIXES DA
RESERVA DE ITAMACAOCA, BACIA DO RIO MUNIM, LESTE MARANHENSE


Esta dissertação foi julgada e aprovada como requisito para a obtenção do grau de
Mestre em Biodiversidade e Conservação.

São Luís, MA, _____ de _____de 2021.


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SÃO LUÍS – MA
2021

*À minha família, por tudo que sou.*
*Pois, "a força do lobo está na alcateia e a força da alcateia está no lobo."*

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**APRESENTAÇÃO**

A Mata de Itamacaoca é uma área urbana de proteção ambiental criada pela CAEMA (Companhia de Saneamento Ambiental do Estado do Maranhão), localizada no Município de Chapadinha, estado do Maranhão, Bacia do Alto Rio Munim. O bioma predominante na região é o Cerrado e está, aproximadamente, à 90 metros acima do nível do mar. A Mata de Itamacaoca foi reconhecida como Área de Relevante Interesse Ecológico para a conservação da fauna e flora pelo Decreto Municipal nº 05/2018 e contém cerca de 460 hectares consistindo de um grande mosaico de árvores ao longo das nascentes e riachos. A Mata de Itamacaoca é uma das poucas áreas conservadas da região, entretanto, apesar da área de estudo está em processo moderado de conservação, pouco se sabe sobre a ictiofauna local. Em face dessa lacuna, este estudo propôs fornecer um inventário e analisar os padrões morfológicos e tróficos da comunidade de peixes da Mata de Itamacaoca.

Esta Dissertação é composta por cinco capítulos. O **Capítulo I**, é composto por uma introdução geral e referencial teórico que buscou-se contextualizar sobre a importância dos Riachos de Cabeceiras para a ictiofauna, adaptação morfológica em peixes e estudos morfológicos como ferramentas para refletir adaptações em peixes. O **Capítulo II** teve como objetivo principal fornecer um inventário de espécies de peixes da Mata da Itamacaoca e comparar as espécies listadas com as espécies listadas nos demais trabalhos publicados realizados no Bacia do Alto Rio Munim. No **Capítulo III** foi estimada a Relação Peso- Comprimento para a ictiofauna da Mata de Itamacaoca. No **Capítulo IV** foi testado se a população de *Nannostomus beckfordi* Günther 1872 exibe plasticidade fenotípica em resposta à sazonalidade (ou seja, estações seca e chuvosa). O **Capítulo V** teve como objetivo avaliar as relações entre morfologia, ecologia trófica e uso de recursos alimentares da ictiofauna da Mata de Itamacaoca. Por fim, apresentamos o **Capítulo VI** como uma conclusão da dissertação, na qual, reúne e resume todos os resultados da dissertação e as perspectivas futuras.

**RESUMO**

A ictiofauna de água doce da região Neotropical é a mais rica em espécies do mundo, compreendendo mais de 6.000 espécies já descritas, e estima-se que esse número possa chegar a mais de 9.000 espécies descritas nos próximos anos. Dentro dessa mega diversidade, os peixes de pequeno porte apresentam uma abundância expressiva, correspondendo a 70% de toda a ictiofauna Neotropical. A Mata de Itamacaoca é uma das poucas áreas urbanas protegidas da Bacia do Alto Rio Munim, Estado do Maranhão, Brasil. Embora, a Bacia do Alto Rio Munim seja uma das principais drenagens da Unidade Hidrológica do Maranhão, existem poucos estudos publicados que enfoquem levantamentos ictiológicos e trabalhos taxonômicos desta bacia. Em face dessa lacuna, este estudo propôs fornecer um inventário e analisar os padrões morfológicos e tróficos da comunidade de peixes da Mata de Itamacaoca, Bacia do Alto Rio Munim. A amostragem foi conduzida em cinco locais de coleta distribuídos dentro dos limites da Mata de Itamacaoca, bacia do alto rio Munim. Um total de seis ordens, 13 famílias e 23 espécies de peixes foram encontradas, sendo que a ordem com maior riqueza de espécies, considerando todos os trechos, foi Characiformes seguida por Cichliformes. Não houve espécies exóticas invasoras coletadas na área de estudo. Dez espécies apresentaram crescimento alométrico negativo. A população de *Nannostomus beckfordi*, espécie mais abundante da área, apresenta algumas evidências de estratégias ecológicas estáveis em termos de ecomorfologia relacionadas com as estações e regime de vazão. Variações morfológicas em conjunto com a disponibilidade de recursos determinaram as diferenças dietéticas, bem como o perfil trófico da comunidade de peixes da Mata de Itamacaoca. Por fim, os resultados apresentados evidenciam a importância da integridade dos ecossistemas aquáticos para a estruturação trófica e ecomorfológica da ictiofauna e fornece informações que podem contribuir para a comparação com outros ambientes intocados e para a avaliação de riachos perturbados.

**Palavras-chave:** Biodiversidade, Conservação, Diversidade de Espécies, Ecologia de Peixes.

**ABSTRACT**

The freshwater ichthyofauna of the Neotropical region is the most species-rich of the world, comprising more than 6.000 described species, and it is estimated that this number could reach more than 9.000 described species in the next years. Within this megadiversity, small fish have an expressive abundance, correspondente a 70% ichthyofauna of Neotropical region. The Mata de Itamacaoca is one of the few protected urban areas in the Upper Munim River Basin, State of Maranhão, Brazil. Although, the Upper Munim River Basin is one of the main drainages of Hydrological Unit of Maranhão, there are few published studies focusing on ichthyological surveys and taxonomic works of this basin. In the face of this gap, this study proposed to provide an inventory and analyze morphological and trophic patterns of the fish community of Mata de Itamacaoca, Upper Munim River Basin. The sampling was conducted in five collection sites distributed within the limits of Mata de Itamacaoca, Upper Munim River Basin. A total of six orders, 13 families and 23 species of fish have been found, the order with the greatest species richness, considering all the excerpts, was Characiformes followed by Cichliformes. There were no invasive alien species collected in the study area. 10 species showed negative allometric growth. The population of *Nannostomus beckfordi*, the most abundant species in the area, presents some evidence of stable ecological strategies in terms of ecomorphology related to stations and flow rate. Morphological variations in conjunction with the availability of resources determined dietary differences, as well as the trophic profile of the fish community of Mata de Itamacaoca. Finally, the results show the importance of the integrity of aquatic ecosystems for trophic and ecomorphological structuring of ichthyofauna and provides information that can contribute for comparison with other untouched environments and for evaluation of disturbed streams.

**Keywords:** Biodiversity, Conservation, Ecology of Fish, Species Diversity.

**CAPÍTULO I**

**INTRODUÇÃO GERAL**

A Ecomorfologia é o ramo da ecologia que visa relacionar a morfologia das espécies com os aspectos ecológicos a partir de dados quantitativos (NEVES et al. 2015; PORTELLA et al. 2016; BALDASSO et al. 2019). Estudos de natureza ecomorfológica podem, ainda, refletir padrões importantes em espécies filogeneticamente distantes, e estabelecer diferenças ecomorfológicas entre espécies relacionadas (NORTON; BRAINERD, 1993, GARCIA et al. 2020), sendo uma área que integra vários campos da biologia, como a zoologia, morfologia, ecologia, fisiologia, comportamento animal e biologia evolutiva (EVANS et al. 2019). De modo geral, estudos ecomorfológicos e morfométricos predizem que a morfologia externa é adaptativa, ela evolui e se diversifica no decorrer do tempo e das pressões ambientais, como os fatores antropogênicos, recursos disponíveis no ambiente, predação e competição, ambas utilizam técnicas de análises multivariadas (SANTOS et al. 2011; LEAL et al. 2011; BARR, 2018; GARCIA et al. 2020).

Devido à suas abordagens preditivas, a ecomorfologia e a morfometria são utilizadas em estudos de diversos organismos, como exemplo os peixes que é o grupo de vertebrados que possuem maior variação morfológica quanto ao alimento disponível, condição estrutural do hábitat, competição e predação (NORTON; BRAINERD, 1993; GURGEL, et al. 2005; TEIXEIRA; BENNEMANN, 2007; BALDASSO et al. 2019), e acredita-se que a ecologia trófica é uma ferramenta importante para descrever a diversificação morfológica em peixes, que podem apresentar variações morfológicas em relação ao comportamento e eficiência na captura de presas (BENNEMANN; GALVES; CAPRA, 2011; EVANS et al. 2019; GARCIA et al. 2020). Como resultado, essas diversificações morfológicas em peixes tornam-se modelos essenciais para estudos ecomorfológicos e morfométricos ligando a forma das estruturas morfológicas com o desempenho ecológico (EVANS et., 2019; BALDASSO et al. 2019).

Os estudos morfométricos em peixes predizem os padrões de relação entre o fenótipo e o uso dos recursos (FLORENTINO; SUÁREZ, 2014). Um dos principais focos dos estudos ecomorfológicos é a associação entre as variáveis morfológicas com o comportamento trófico ou o uso do hábitat (TEIXEIRA; BENNEMANN, 2007; OLIVEIRA et al. 2010; FAYE et al. 2012; SILVA-CAMACHO et al. 2014). Um dos primeiros trabalhos ecomorfológicos em peixes foram publicados por Keast e Webb (1966), que a partir de dados quantitativos da morfologia externa de peixes do lago

Opinicon, Canadá, observaram que as especializações das morfologias responsáveis pela alimentação determinaram as preferências de hábitat entre diferentes espécies de peixes da área estudada, diminuindo a competição interespecífica.

A coexistência de várias espécies em comunidades de peixes podem ser facilitadas pela segregação morfológica que permite o particionamento do hábitat e dos recursos alimentares (WIKRAMANAYAKE, 1990; SILVA-CAMACHO et al. 2014; GARCIA et al. 2020). Desta forma, estudos acerca da sua alimentação são de extrema importância para o esclarecimento do desenvolvimento, crescimento, reprodução, manutenção e exploração de recursos desses organismos (TEIXEIRA; BENNEMANN, 2007; BALDASSO et al. 2019). O estudo dos hábitos alimentares de peixes possibilita o entendimento do ciclo de vida das espécies e identificação das espécies predadoras de topo (FAYE et al. 2012; SILVA-CAMACHO et al. 2014). Além disso, esse objeto de estudo fornece o entendimento do mecanismo de coexistência e da exploração de recursos de várias espécies que habitam um mesmo sistema (BALDASSO et al. 2019; GARCIA et al. 2020). A dinâmica de comunidades aquáticas também pode ser explicada pelo entendimento da estrutura trófica (BENNEMANN; GALVES; CAPRA, 2011; SANTOS et al. 2011). Contudo, o hábito alimentar não é apenas uma sombra das interações ecológicas, mas no ponto de vista ecológico é um reflexo de múltiplos eventos anteriores que preparam as espécies para a estação seguinte (GURGEL et al. 2005; KRIVAN; DIEHL, 2005; BALDASSO et al. 2019; GARCIA et al. 2020).

A condição do ambiente aquático pode influenciar as interações ecológicas em diversas comunidades ictiofaunísticas (LEAL et al. 2011), sendo que a ictiofauna de um riacho é correlata às diferentes condições estruturais, como a presença de substratos, profundidade e corredeiras (LEAL et al. 2011; SILVA-CAMACHO et al. 2014). A ictiofauna de riachos de cabeceiras deve ser considerada uma prioridade como objeto de estudo, tanto do ponto de vista do ecólogo, como do ictiólogo, uma vez que estes ambientes apresentam uma grande variedade de espécies e, de alguma maneira, as comunidades naturais "partilham" os recursos disponíveis no ambiente, um padrão bastante comum em riachos brasileiros (ROSS, 1986; WIKRAMANAYAKE, 1990; ESTEVES; LOBÓN-CERVIÁ, 2001; GARCIA et al. 2020). Além disso, riachos de cabeceiras apresentam uma grande diversidade de espécies de peixes, muitas delas com distribuição geográfica restrita e, até mesmo, espécies endêmicas que ainda são desconhecidas pela ciência (ZANINI et al. 2017; FROTA et al. 2019). Contudo, faz-se necessário e de extrema urgência trabalhos científicos voltados para essas áreas que são

fortemente influenciados pelo meio terrestre para que iniciativas de conservação e manejo venham ser implementadas (FROTA et al. 2019).

## REVISÃO BIBLIOGRÁFICA

### Riachos de cabeceiras

Riachos de cabeceiras são conhecidos mundialmente como uma das redes hídricas mais densas do planeta (HUMPHRIES; KECKEIS; FINLAYSON, 2014). Isso porque, eles são fortemente submetidos a um grande regime de inundação em um curto período de tempo influenciados pelas chuvas locais (MCCLUNEY et al. 2014; HUMPHRIES; KECKEIS; FINLAYSON, 2014). O volume de água do riacho se eleva rapidamente, aumentando a turbidez da água, contudo, o volume de água tende a diminuir após o término da chuva (PAZIN et al. 2006; ZANINI et al. 2017; AZEVEDO-SANTOS et al. 2018). Entretanto, riachos e pequenos córregos são alvo frequentes da ação antrópica (AZEVEDO-SANTOS et al. 2018; FROTA et al. 2020; CAVALHEIRO; FIALHO, 2020), sobretudo da poluição (FELIPE; SÚAREZ, 2010), urbanização (ALEXANDRE; ESTEVES; MELO, 2009; BELTRÃO et al. 2018), agricultura (OLIVEIRA; TEJERINA-GARRO, 2010; TÓTH et al. 2019); e invasão de espécies não-nativas (PELICICE et al. 2017; GUBIANI et al. 2018).

Estes ecossistemas atuam como locais de desova, corredores de migração e refúgio durante as inundações e escassez de alimentos (MEYER et al. 2007; ALEXANDRE; ESTEVES; MELO, 2009; BELTRÃO et al. 2018; FROTA et al. 2020). Em riachos de cabeceiras habitam várias espécies, principalmente peixes de pequeno e médio porte, peixes oportunistas ou que possuem reduzidas especializações tróficas (AZEVEDO-SANTOS et al. 2019; FROTA et al. 2020), com destaque para espécies raras e ameaçadas (FAGUNDES et al. 2015; CETRA et al. 2016; AZEVEDO-SANTOS et al. 2019; FROTA et al. 2020). Entretanto, a diversidade de regiões de cabeceiras tende a aumentar com o nível de complexidade e dimensões do hábitat (AZEVEDO-SANTOS et al. 2019; FROTA et al. 2019; FROTA et al. 2020).

Regiões de cabeceiras abrigam uma rica diversidade ictiofaunística (CETRA et al. 2016; PELICICE et al. 2017; FROTA et al. 2020; CAVALHEIRO; FIALHO, 2020). Além disso, por serem sensíveis a distúrbios e alterações climáticas (ZANINI et al. 2017), peixes de riachos de cabeceiras possuem uma grande importância ecológica como indicadores da qualidade do hábitat (CETRA et al. 2016; AZEVEDO-SANTOS et al. 2019). No entanto, essas regiões recebem pouca atenção dos pesquisadores devido a

acessibilidade desses locais, dificuldade de captura e complexidade das espécies (FAGUNDES et al. 2015; FROTA et al. 2019; FROTA et al. 2020). Novas espécies são frequentemente descobertas à medida que mais estudos são realizados nessas áreas (FAGUNDES et al. 2015; AZEVEDO-SANTOS et al. 2019; FROTA et al. 2020). Desta forma, os ambientes de cabeceiras são fontes importantes de estudos ictiológicos devido ao seu grande potencial ictiofaunístico (PAZIN et al. 2006; MEYER et al. 2007; FAGUNDES et al. 2015; CAVALHEIRO; FIALHO 2020).

**Adaptação morfológica em peixes**

Atualmente, os organismos de água doce são um dos principais alvos da ação antrópica, que incluem poluição, desmatamento, obstrução e regulação dos fluxos de água por barragens e açudes (PELICICE et al. 2017). Além disso, períodos de chuvas torrenciais, sob o cenário das alterações climáticas em andamento, causam inundações bruscas nos ambientes aquáticos e podem comprometer a biota aquática (RUBIO-GRACIA et al. 2020). A soma de todos esses fatores são prejudiciais para várias espécies de peixes, principalmente em estágios larvais (PELICICE et al. 2017).

Neste cenário, as características fenotípicas dos peixes precisam ser maximizadas, uma vez que, dependendo do estágio do seu ciclo de vida, os peixes precisam migrar para novas áreas em busca de um hábitat adequado e para o seu desenvolvimento (PORTELLA et al. 2016; BALDASSO et al. 2019). Contudo, os peixes ao longo do seu desenvolvimento apresentaram uma série de características ecológicas, comportamentais e morfológicas responsáveis pelo sucesso adaptativo do grupo (e.g. corpo hidrodinâmico, configurações das barbatanas, alterações fisiológicas, como a musculatura branca e vermelha) que permitiram-lhes habitar diferentes ambientes com velocidade da água mais elevada, auxílio na captura de diferentes alimentos - elevando a diversificação de nicho e atenuando a competição e na capacidade de migrar entre diferentes habitats (FRANSSEN, 2011; ALEXANDRE et al. 2014; RUBIO-GRACIA et al. 2020)

A natação dos peixes exige um grande gasto energético e é ecologicamente relevante para a determinação da taxa de sobrevivência de peixes em ambientes aquáticos (RUBIO-GRACIA et al. 2020; SILVA et al. 2020). Este comportamento é dependente das características morfológicas e da capacidade que os peixes possuem de interagir com o ambiente, como interações predador-presa, captura de alimentos, competição e fuga de eventuais predadores (RUBIO-GRACIA et al. 2020). Nesta situação, a adaptação da forma do corpo ao ambiente torna-se fundamental para a redução do gasto energético e para um bom funcionamento do nicho ecológico (FRANSSEN, 2011; ALEXANDRE et

al. 2014; RUBIO-GRACIA et al. 2020). No meio natural, a adaptação das nadadeiras dos peixes é constantemente utilizada para o nado, reprodução, busca por alimentos e exploração de novos ambientes (RUBRIO-GRACIA et al. 2020; SILVA et al. 2020).

A seleção natural divergente é responsável pela adaptação morfológica em peixes e esta, por sua vez, em ambientes aquáticos surge pela combinação de dois fatores: I) Troca funcional: responsável pela otimização do desempenho morfológico; II) Heterogeneidade ambiental: responsável pela adaptação morfológica em função do ambiente. Tanto as trocas funcionais como a heterogeneidade ambiental são responsáveis pela grande diversidade fenotípica em peixes (LANGERHANS, 2010; ALEXANDRE et al. 2014; SILVA et al. 2020). Desta forma, a morfologia e o desempenho da natação dos peixes refletem interações correlatas com o meio ambiente (FRANSSEN, 2011; ALEXANDRE et al. 2014), e estão frequentemente associadas ao uso do hábitat e alimentação (EVANS et., 2019; BALDASSO et al. 2019; GARCIA et al. 2020).

**Estudos morfológicos refletindo adaptações em peixes**

A diferenciação de nicho pode ser caracterizada por meio de padrões de tamanhos combinados com os diferentes atributos morfológicos de um indivíduo (SILVA-CAMACHO et al. 2014; NEVES et al. 2015; PORTELLA et al. 2016; BALDASSO et al. 2019; GARCIA et al. 2020). Além disso, os aspectos do nicho ecológico em peixes podem ser inferidos por meio da relação entre o formato do corpo e as características do hábitat, por meio da relação entre peso e comprimento, ou a combinação entre as características morfológicas, altura e largura da boca e tipos de dentição com a alimentação (TEIXEIRA; BENNEMANN, 2007; SANTOS et al. 2011; BALDASSO et al. 2019; GARCIA et al. 2020). O campo da ecologia que se preocupa em explicar as relações entre os atributos morfológicos com os aspectos ambientais é a Ecomorfologia (NORTON; BRAINERD, 1993; NEVES et al. 2015; PORTELLA et al. 2016; BALDASSO et al. 2019).

Em um trabalho pioneiro, GATZ (1979), estudando a morfologia ecológica de peixes em um córrego de água doce, classificou 56 medidas morfológicas que podem apresentar associação ao uso do hábitat e dieta em peixes. Entre as medidas morfológicas associadas ao uso do hábitat destacam-se o comprimento padrão, largura e altura do corpo, comprimento da nadadeira dorsal, peitoral e caudal, largura do pedúnculo caudal e orientação da boca (GATZ, 1979; WIKRAMANAYAKE, 1990; NEVES et al. 2015; BARR, 2018; BALDASSO et al. 2019; EVANS et al. 2019; GARCIA et al. 2020). Já em estudos ecomorfológicos que refletem a dieta em peixes destacam-se o comprimento

padrão, comprimento da cabeça, altura da linha dos olhos, largura da boca, comprimento do dentário, altura máxima do corpo, comprimento e altura do pedúnculo caudal (GATZ, 1979; WIKRAMANAYAKE, 1990; TEIXEIRA; BENNEMANN, 2007; OLIVEIRA et al. 2010; FAYE et al. 2012; SILVA-CAMACHO et al. 2014; BALDASSO et al. 2019; EVANS et al. 2019; GARCIA et al. 2020)

A posição que os peixes ocupam na coluna d'água durante o forrageamento podem levá-los à respostas adaptativas em sua morfologia e comportamento alimentar (FRANSSEN, 2011; ALEXANDRE et al. 2014; RUBIO-GRACIA et al. 2020). Um exemplo é a relação entre o tamanho da boca com o tamanho da cabeça, esse padrão pode explicar adaptações morfológicas para a captura de presas em potencial (GATZ, 1979; BENNEMANN; GALVES; CAPRA, 2011; SILVA-CAMACHO et al. 2014; EVANS et al. 2019; BALDASSO et al. 2019; GARCIA et al. 2020). Da mesma forma, o tamanho da boca pode fornecer subsídios importantes para explicar a variação interespecífica da dieta em peixes (TEIXEIRA; BENNEMANN, 2007; FRANSSEN, 2011; ALEXANDRE et al. 2014; BALDASSO et al. 2019). Porém, apenas o estudo da morfologia externa não consegue explicar sozinho a preferência alimentar em peixes, estudos acerca do conteúdo estomacal se fazem importantes no intuito de complementar os dados e determinar as variações interespecíficas na dieta em peixes (GATZ, 1979; HYSLOP, 1980; GIBRAN, 2007; TEIXEIRA; BENNEMANN, 2007; ALEXANDRE et al. 2014; GARCIA et al, 2020).

No Brasil, alguns estudos ictiológicos descrevem a ocorrência, distribuição e abundância de peixes em regiões de cabeceiras (e.g. MIRANDA; MAZZONI, 2003; BAGINSKI et al. 2007; MIRANDA; MAZZONI, 2009; ALEXANDRE; ESTEVES; MELO, 2009; BERTACO; CARVALHO, 2010; BELTRÃO et al. 2018; FROTA et al. 2020), a dieta (e.g. OLIVEIRA; BENNEMANN, 2004; XIMENES et al. 2011), e outros trabalhos fazem a relação entre a alimentação e padrões ecomorfológicos (e.g. MARINELLI et al. 2001; TEIXEIRA; BENNEMANN, 2007; MAZZONI et al. 2010; BENNEMANN; GALVES; CAPRA, 2011; SILVA-CAMACHO et al. 2014; ; EVANS et al. 2019; BALDASSO et al. 2019; GARCIA et al. 2020).

Cardoso et al. (2015) realizando uma revisão bibliográfica sobre a ecomorfologia e sua utilização em estudos da dieta e uso do hábitat em peixes, perceberam que os trabalhos pioneiros publicados na década de 70 descreviam apenas os aspectos de uso de hábitat e dieta de forma conjugada, já a partir dos anos 2000, os trabalhos começaram a

diversificar-se em estudos sobre dieta, uso do hábitat ou a conjugação entre essas duas temáticas (Figura 1).

**Figura 1.** Número de Trabalhos com ecomorfologia descrevendo o habitat, dieta e habitat e dieta.
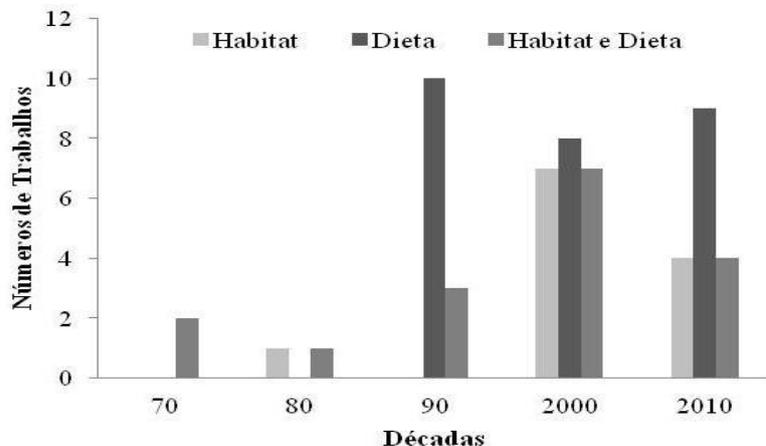


**Fonte:** CARDOSO et al. (2015).

Evans et al. (2019) defendendo a hipótese de que a função e a forma das características morfológicas são correlatas e ambas desempenham um papel integral na formação de padrões morfológicos, evolução morfológica em larga-escala e utilização de recursos, encontraram relações significativas entre medidas de ecologia trófica e a forma do crânio em peixes elétricos da bacia Amazônica. Baldasso et al. 2019 testando a teoria de que os padrões tróficos são explicados por variações ecomorfológicas em peixes, concluíram que as adaptações morfológicas e disponibilidades de recursos no ambiente são os principais mecanismos de segregação trófica e coexistência entre espécies de peixes. Garcia et al. 2020 mostraram diferenças ecomorfológicas entre espécies simpátricas, e tais variações explicam as preferências alimentares em diferentes espécies.

## HIPÓTESES TESTADAS

O conhecimento dos padrões morfológicos e da alimentação de peixes são fundamentais para a compreensão das interações ecológicas e para a conservação desses organismos (SILVA-CAMACHO et al., 2014; GARCIA et al., 2020). Grande parte dos peixes que habitam regiões neotropicais podem alterar sua dieta ao longo do seu desenvolvimento, isso devido às flutuações estacionais, disponibilidade de alimento ou devido às inúmeras mudanças de biótopo, tal fator propicia mudanças tanto nos padrões morfológicos como na função e estrutura das comunidades ictiofaunísticas e, ainda

acredita-se que, em riachos neotropicais há a partilha de recursos, tal padrão facilita a coexistência das espécies (ROSS, 1986; WIKRAMANAYAKE, 1990; ESTEVES; LOBÓN-CERVIÁ, 2001; SILVA-CAMACHO et al., 2014; GARCIA et al., 2020). Contudo, as hipóteses testadas foram:

**H₁1:** A abundância e representatividade das espécies de peixes que ocorrem na Reserva de Itamacaoca seguem os mesmos padrões dos principais inventários da América do Sul;

**H₁2:** As características biológicas das espécies estão associadas à relação Peso Total – Comprimento Total;

**H₁3:** *Nannostomus beckfordi* exibe variação morfológica em resposta à estação (seca e chuvosa) usada como adaptação para o regime de vazão.

**H₁4:** É provável que haja uma similaridade ecomorfológica e trófica nas variações estacionais (períodos seco e chuvoso) em diferentes condições ambientais (riachos de cabeceira X Represa) na comunidade de peixes da Mata de Itamacaoca.

## 4. OBJETIVOS

### 4.1 Geral

Fornecer um inventário de espécies de peixes da Mata da Itamacaoca e realizar um estudo ecomorfológico e da alimentação das espécies de peixes que ocorrem na Reserva de Itamacaocoa, examinando dois tipos diferentes de ambientes (riachos de cabeceira X reservatório), em duas épocas sazonais diferentes (chuva X seca), localizada no Município de Chapadinha, leste do Maranhão.

### 4.2 Específicos

- Inventariar e verificar as abundancias das espécies e representatividade dos grupos de peixes da área em questão, comparando com estudos de outros rios da América do Sul;

- Levantar dados sobre Peso-Total e Comprimento-Total, e verificar se essas características são correlatas;

- Verificar se as características morfológicas estão associadas ao uso do hábitat e alimentação em diferentes variações estacionais (período seco X chuvoso) e em condições ambientais diferentes (riachos de cabeceira X reservatório);

- Verificar se há similaridade ecomorfológica e trófica entre as espécies estudadas.

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## CAPÍTULO II

## ICHTHYOFAUNA OF THE MATA DE ITAMACAOCA, AN URBAN PROTECTED AREA FROM THE UPPER MUNIM RIVER BASIN, NORTHERN BRAZILIAN CERRADO[1]

ICTIOFAUNA DA MATA DE ITAMACAOCA, UMA ÁREA DE PROTEÇÃO URBANA DA BACIA DO ALTO RIO MUNIM, CERRADO NORTE BRASILEIRO

# Ichthyofauna of the Mata de Itamacaoca, an urban protected area from the upper Munim River basin, Northern Brazilian Cerrado

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**Abstract:** The Munim River basin is one of the main river drainages of the Hydrological unit Maranhão, but there are few published studies which focus on ichthyological surveys and taxonomic work within this basin. The present study aims to provide a fish species inventory of the Mata da Itamacaoca, one of the few urban protected areas from the upper Munim River basin, comparing the ichthyofauna with other lists by conducted at the upper Munim River basin. A total of 42 collection expeditions were conducted, the sampling was conducted at five collecting sites distributed within the boundaries of Mata de Itamacaoca, upper Munim River basin. Diversity indices were calculated and generalised linear models (GLMs) were employed to assess differences in species richness, diversity and evenness depending on season and location in relation to the reservoir dam wall. In order to visualize fish community differences, non-metric multidimensional scaling (nMDS) and a one-way PERMANOVA was used to understand whether factors of site, season and location to the dam wall had an effect on fish community compositions. A total of six orders, 13 families, and 23 fish species were found, and the order with the highest species richness, considering all reaches, was Characiformes followed by Cichliformes. The most abundant species was Nannostomus beckfordi, while Pimelodella parnahybae and Hoplerythrinus unitaeniatus were the rarer species sampled. There were no alien invasive species collected within the study area. Species richness was significantly higher below the dam wall, but there were no other

significant differences in diversity indices with regards to season or location. Fish community composition was significantly different above and below the dam wall and was significantly affected by sampling site. Season did not have an effect on fish community. This study corroborates other studies conducted in the Unidade Hidrológica Maranhão sensu Hubbert and Renno (2006), that the ichthyofaunal composition and taxonomy of species within this region face major data deficits, anthropogenic impacts, this study may be a baseline for comparing similar environments throughout the region.

**Keywords:** Biodiversity, Conservation, fish inventory, Maranhão, Neotropical, Species richness

## Ictiofauna da Mata de Itamacaoca, uma área de proteção urbana da bacia do alto Rio Munim, Cerrado norte brasileiro

**Resumo:** A bacia do rio Munim é uma das principais drenagens da unidade Hidrológica do Maranhão, mas existem poucos estudos publicados que enfoquem levantamentos ictiológicos e trabalhos taxonômicos dentro desta bacia. O presente estudo tem como objetivo fornecer um inventário de espécies de peixes da Mata da Itamacaoca, uma das poucas áreas protegidas urbanas da bacia do alto rio Munim, comparando a ictiofauna com outras listas realizadas na bacia do alto rio Munim. Foram realizadas 42 expedições de coleta, a amostragem foi conduzida em cinco locais de coleta distribuídos dentro dos limites da Mata de Itamacaoca, bacia do alto rio Munim. Índices de diversidade foram calculados e modelos lineares generalizados (GLMs) foram empregados para avaliar diferenças na riqueza de espécies, diversidade e equitabilidade dependendo da estação e localização em relação à parede da barragem do reservatório. A fim de visualizar as diferenças da comunidade de peixes, escalonamento multidimensional não métrico (nMDS) e um PERMANOVA unilateral foi usado para entender se os fatores de local, estação e localização da parede da barragem afetavam a composição da comunidade de peixes. Um total de seis ordens, 13 famílias e 23 espécies de peixes foram encontradas, sendo que a ordem com maior riqueza de espécies, considerando todos os trechos, foi Characiformes seguida por Cichliformes. A espécie mais abundante foi *Nannostomus beckfordi,* enquanto *Pimelodella parnahybae Hoplerythrinus unitaeniatus* foram as espécies mais raras amostradas. Não houve espécies exóticas invasoras coletadas na área de estudo. A riqueza de espécies foi significativamente maior abaixo da parede da barragem, mas não houve outras diferenças significativas nos índices de diversidade em relação à estação do ano ou localização. A composição da comunidade de peixes foi significativamente diferente acima e abaixo da parede da barragem, e foi significativamente afetada pelo local de amostragem. A estação do ano não afetou a comunidade de peixes. Este estudo corrobora outros estudos realizados na Unidade Hidrológica Maranhão sensu Hubbert & Renno (2006), que a composição ictiofaunística e taxonomia das espécies desta região enfrentam grandes déficits de dados. Impactos antropogênicos, este estudo pode ser uma linha de base para comparar ambientes semelhantes em toda a região.

**Palavras-chave:** Biodiversidade, Conservação, Inventário de peixes, Neotropical, Riqueza de espécies.

## Introduction

The Neotropical freshwater ichthyofauna is the most species-rich of the world,

comprising more than 6,000 described species, with estimates of over 9,000 species (Reis et al. 2016, Birindelli & Sidlauskas 2018, Castro & Polaz 2020). Within this huge species assemblage, most (about 70%) are small-sized fishes, with adults around 15 cm or less standard length (SL), which can inhabit a variety of aquatic environments, such as streams, small and large rivers, lagoons, pools, temporary pools, swamps, amongst others (Reis et al. 2003, Castro & Polaz 2020). Literature concerning the diversity and evolution of the Neotropical ichthyofauna has improved in recent years; however, studies are still few and underestimate their real biodiversity (Buckup et al. 2007, Reis et al. 2016, Birindelli & Sidlauskas 2018, Malabarba & Malabarba 2020). In addition to the lack of taxonomic and ecological knowledge, the rapid loss and degradation of natural environments because of anthropogenic drivers (i.e. invasive species, climate change, abstraction, pollution etc) has affected many fish species (Agostinho et al. 2008, Nogueira et al. 2010, Azevedo-Santos et al. 2019, Castro & Polaz 2020). Fish species which are particularly at risk are either charismatic megafauna or small sized fish species, the latter of which unfortunately receive rather less conservation attention (He et al. 2019, Castro & Polaz 2020).

Due to the alarming rate of biodiversity loss, combined with multiple and interacting anthropogenic stressors, freshwater ecosystems are facing a "biodiversity crisis" (Darwall et al. 2018, Harrison et al. 2018). In the last two decades, the rate of species extinctions worldwide has been much higher than natural extinction rates, with the subsequent extinction of thousands of species and loss populations, several of them still unknown to the science. This is of great concern within freshwater environments as the biotic communities represent around 6% of currently described species and yet are vastly understudied commensurate to the species diversity and ecosystem services that they represent (Dudgeon et al. 2006, Lynch et al. 2020). The mis-match in data availability and research output compared to intrinsic value is notable in Brazilian freshwater systems, whereupon species are threatened before they even are described and their ecology characterised (Wilson 1985, 1992, Brooks et al. 2002, Olson et al. 2002, Singh 2002, Brook et al. 2006, Laurance 2007, Wheeler 2008, Costa et al. 2012, Kalinkat et al. 2017, Azevedo-Santos et al. 2019).

Freshwater environments and the biota within are more vulnerable to global change than marine and terrestrial ecosystems, as such they warrant the need for urgent and special attention regarding diversity estimations and conservation actions (Arthington et al. 2016, Darwall et al. 2018, Harrison et al. 2018, Azevedo-Santos et al.

2019, Castro & Polaz 2020). Unfortunately, designation of protected areas combined with current conservation policies, especially in Brazil, have limited efficacy in protecting freshwater biodiversity (Azevedo-Santos et al. 2019, Castro & Polaz 2020). Due to the continued high rate of habitat destruction, the identification of new species and comprehensive compilations of regional inventories as well as improving the taxonomic resolution of under-studied and taxonomically confusing is a research priority. It is imperative that this occurs before these species and populations are extinct, so that appropriate interventions can be actioned, especially in areas of high risk of anthropogenically driven change (Brook et al. 2006, Laurence 2007, Wheeler 2008, Costa et al. 2012, Pimm et al. 2014, Darwall et al. 2018, Oliveira-Silva et al. 2018, Harrison et al. 2018, Frota et al. 2019).

The Munim River basin (~16.000 km2) is a coastal river basin located at the northeastern portion of the State of Maranhão (Ribeiro et al. 2014). It represents one of the main river drainages of the Hydrological unit Maranhão sensu Hubbert and Renno (2006) (Hereafter Mrn). This eastern portion of the Hydrological unit has conspicuously few published fish surveys and ichthyological taxonomic studies. Ribeiro et al. (2014) published a study on an artisanal fishing technique (known as Moita) conducted at the upper Munim River basin, listing 20 fish species, predominantly middle to large-sized species. Matavelli et al. (2015) conducted an inventory of fishes associated with tadpole community in lentic and lotic environments in northeastern Maranhão (some of the sampled areas belonging to the lower Munim River basin) listing 13 fish species. Guimarães et al. (2018a,b) described two new species of Characidae occurring at the upper Munim River basin; and Nunes et al. (2019) carried out a work on length-weight relationship of 15 species from the upper Munim River basin. This gap in knowledge, specifically relating to fish taxonomy and species assemblages is a shared trait throughout the Munim River Basin as well as other river drainages and basins from Mrn (Piorski, 2010, Guimarães et al. 2018a). Therein there is a massive lack of information related to the taxonomy and systematics of the species and groups, species composition, geographical distribution and biogeography of the ichthyofauna from this region.

The fluvial channels in the State Maranhão are constantly threatened by degradation. This includes: removal of riparian forests; pollution and contamination of rivers; occurrences of erosion processes intensified by human activities; and abstraction and fragmentation of watercourses. The Munim River basin is no exception to this trend

(Ribeiro et al. 2006,Silva et al. 2008, Lima et al. 2009), and there is a high likelihood that this will significantly impact the regional biodiversity (Pelice et al. 2017). Pervasive and damaging stressor effects upon a data deficit system means that subjects such as fish biodiversity and taxonomy within the Mrn should be urgently addressed in order to combat further losses within the region.

The present study aims to provide a fish species inventory of the Mata da Itamacaoca and compare the species listed by our survey with the species listed by the other published works conducted at the upper Munim River basin. The study area is an urban protected area from the upper Munim River basin, Northern Brazilian Cerrado, a biome considered as one of the world biodiversity hotspots according to Myers et al. (2000). This study is especially important, being considered a baseline for the region, because it was conducted within an urban protection area, which is more exposed to human impacts than other protected areas. In addition, due to fact the Mata de Itamacaoca is an urban protected area, we believe that it has an ichthyofauna representation closer to the original of the region than the other degraded areas. This thus allowed for us to estimate the ichthyofauna diversity for the upper Munim River basin, especially regarding the small-sized, rare, and more ecologically demanding species. We also assessed fish diversity with regards to season and position above or below the reservoir in order to assess for possible effects of fragmentation.

**Materials and Methods**

This study was conducted in the Mata de Itamacaoca, an urban protected area belonging to CAEMA (Companhia de Saneamento Ambiental do Maranhão). It is located within the Municipality of Chapadinha, State of Maranhão (24°25'47" S, 58°44'05" W), and is approximately 90 meters above sea level. The predominant biome in the region is the Brazilian Cerrado (Figure 1).

**Figure 1.** Map of the studied area. Collecting sites (C1-C5) listed in Table 1.

The study area covers about 460 hectares consisting of a mosaic of plant formations including, along with the watercourses, riparian and gallery forests, as well as some stream springs; and formation of closed forest, with trees reaching more than 10 meters in height. The protected area was created to maintain water supply to the city, thus the need to preserve the integrity of vegetation around the springs, water bodies and reservoir (Silva et al. 2008). It is also important to emphasize that this area has been recognized as an Area of Relevant Ecological Interest for the conservation of fauna and flora by the Decreto Municipal Nº 05/2018.

The collection of samples was conducted at five sample sites (C1-C5) distributed within the boundaries of Mata de Itamacaoca, upper Munim River basin, comprising springs, streams, pools, and a reservoir (Table 1, Figures 1 and 2). A total of 42 collection expeditions were conducted. The collections occurred from August 2014 to February 2020, during both dry and wet seasons. All the collection expeditions were conducted during daylight, except for a one off night collection expedition conducted in October 2019 at C4.

**Table 1.** Samples localities at the Mata de Itamacaoca, upper Munim River basin, State of Maranhão, Brazil.

| Collecting site | Coordinates | Altitude (meters) | water temperature (°C) | water ph | Remarks |
|---|---|---|---|---|---|
| C1 | 3°44'45.20"S 43°19'15.10"W | ~80 | ~ 28.1 | ~ 6.2 | Stream near spring, with gallery and riparian forest, at Mata de Itamacaoca, Municipality of Chapadinha, State of Maranhão. Obs.: collections on this site were conducted through about 200 meters along the water course. |
| C2 | 3°44'58.24"S 43°20'23.91"W | ~90 | ~26.2 | ~ 6.6 | Stream in the locality Repouso do Guerreiro, at Mata de Itamacaoca, Municipality of Chapadinha, State of Maranhão. |
| C3 | 3°44'27.1"S 43°19'36.4"W | ~80 | ~ 26.8 | ~ 6.4 | Stream near spring, with gallery and riparian forest, at Mata de Itamacaoca, Municipality of Chapadinha, State of Maranhão. |
| C4 | 3°44'55.16"S 43°19'57.10"W | ~80 | ~ 30.7 | ~6.6 | Itamacaoca dam, Municipality of Chapadinha, State of Maranhão. |
| C5 | 3°45'8.20"S 43°20'4.13"W | ~75 | ~28.5 | ~6.6 | Stream, after the dam at Mata de Itamacaoca, Municipality of Chapadinha, State of Maranhão. Obs.: collections on this site were conducted along a trail of about 500 along the water course. |

**Figure 2.** Collecting sites (C1-C5) listed in table 1. A and B - C1, C - C2, D and E - C3, F and G - C4, and H and I - C5. Photographed by Felipe Ottoni.



Fishes were collected using manual trail-net (2 m long × 1.8 m high; mesh size, 2 mm), cast nets (2 m height, mesh size 15 mm), gillnets of various mesh sizes (15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 100 mm), and dip nets (mesh size 5 and 10 mm). We tried to collect fishes using traps, such as "fish trap", but we were not successful. The ichthyological material obtained in the samples was euthanized in a buffered solution of ethyl-3-amino-benzoate-methanesulfonate (MS-222) at a concentration of 250 mg/l until completely ceasing opercular movements, according to animal welfare laws and guidelines (Close et al. 1996, 1997, Leary et al. 2013). Specimens selected for morphological analysis were fixed in formalin and left for 15 days, after which they were preserved in 70% ethanol. Molecular data also obtained from specimens, and preserved in absolute ethanol. Sorting and identification of specimens were carried out at the Laboratório de Sistemática e Ecologia de Organismos Aquáticos of the Universidade Federal do Maranhão using specialized bibliography for each taxonomic group and consulting experts. The ichthyological material is deposited in the Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais of the Universidade Federal do Maranhão (CICCAA). A list of all the examined material is presented in Appendix 1. The taxonomic classification, the names of species considered as

valid, authors and years of species descriptions, and geographic distribution, were based on the compilations proposed by Fricke et al. (2020a,b), where the authors gather all the most recent classifications for each group of fish.

In order to assess whether this baseline fish community assessment differed between season and location above or below the dam wall, diversity indices were calculated for each discrete sampling events per year. Whereupon, sampling in the dry season above the dam wall was n=27, rainy season above the dam wall n=24, dry season below the dam n=5 and rainy season below the dam wall was n=7.

As the data was unbalanced, a generalised linear model (GLM) was used to determine whether season (dry or rainy) and location with regards to the dam wall (above or below), including an interaction term, affected the species richness (Sprich).

Shannon-Weiner Index of Diversity (Shannon 1948) was calculated for each sampling date at site, season and locality above and below the dam using the package 'vegan' version 2.5-5 (Oksanen et al. 2019). This index describes the entropy of a given community:

$$(H) = \sum_{i=1}^{s} p_i \ln p_i$$

Where H is the Shannon diversity index, which has no bounded upper value, S is the total number of species in the community, $P_i$ is the proportion of S made up of the ith species. Shannon's evenness (eqn 2) was calculated from the results of eqn 1:

$$E_H = H/H_{max} = lnS$$

Where $E_H$ is Shannon's evenness, H is Shannon's diversity index, and $H_{max}$ is the natural log of total species detected in the basin. $E_H$ is bounded between zero and one where one represents complete evenness. In some cases the $E_H$ could not be calculated due to zeros in the dataset. Separate GLMs were completed on the H and $E_H$ values using the same terms as above.

Fish community assemblages and associations regarding season and the dam wall were visualized using non-metric multidimensional scaling (nMDS) ordination via 'vegan' (Okasanen et al. 2019). The community data matrix was compiled using the species counts from the survey and environmental data included was site, season and location to the dam wall, as in the analyses above. A one-way PERMANOVA using Bray–Curtis non-metric similarity and 999 permutations was then used to test for significant effects of environmental factors on fish species abundance. Community data was square-root transformed and Wisconsin double standardization was applied

(*vegan::metaMDS*). Ordination stress was used to assess whether a two-dimensional ordination biplot was suitable to represent community data variation. Stress values < 0.15 were considered appropriate (Quinn & Keough 2002; Cousins et al. 2017). All statistical analyses were performed within the R software environment version 4.0.2 (R Core Team 2020).

**Results**

We sampled 18,289 specimens representing six orders, 13 families, and 23 fish species (Tables 2 and 3, Figures 3, 4 and 5). The order and family with the highest species richness, considering all reaches, were Characiformes and Characidae, respectively, followed by Cichliformes and Cichlidae, respectively (Figures 4 and 5). *Nannostomus beckfordi* Günther, 1872 was the most abundant species, with about 8,000 specimens sampled. *Pimelodella parnahybae* Fowler, 1941 and *Hoplerythrinus unitaeniatus* (Spix & Agassiz 1829) were the rarest species represented, with only two specimens collected for each species (see appendix 1). We found the highest species richness at sample site (C2), with a total of 20 species, followed by C1 with 13, C5 with 10, C4 with 9, and C3 with only 2 (Table 3). Three putatively undescribed species were collected: *Hemigrammus* sp.1, *Hemigrammus* sp. 2, and *Curimatopsis* aff. *cryptica*. All of the collected species were small/ middle-sized native species as there were no exotic nor large-sized species collected in the studied area.

There were three categories of fishs based on their occurence: 1) Only found above the dam wall: *Copella arnoldi* (Regan, 1912), and *Hemigrammus* sp. 2; 2) only found below the dam: *Astyanax* cf. *bimaculatus*, *Brachyhypopomus* sp., *Characidium* sp., *Hoplerythrinus unitaeniatus*, *Knodus victoriae* (Steindachner, 1907), *Moenkhausia oligolepis* (Günther, 1864), *Pimelodella parnahybae*, *Poecilia sarrafae* Bragança & Costa, 2011 and *Steindachnerina notonota* (Miranda Ribeiro, 1937); and 3) Above and below the dam: *Anablepsoides vieirai* Nelson, 2016, *Apistogramma piauiensis* Kullander, 1980, *Cichlasoma* cf. *zarskei*, *Crenicichla brasiliensis* (Bloch, 1792)*, Curimatopsis* aff. *cryptica*, *Gymnotus carapo* Linnaeus, 1758*, Hemigrammus* sp. 1, *Hoplias malabaricus* (Bloch, 1794)*, Hyphessobrycon piorskii* Guimarães, Brito, Feitosa & Ottoni, 2018, *Megalechis thoracata* (Valenciennes, 1840), *Nannostomus beckfordi* and *Synbranchus marmoratus* Bloch, 1795 (see Table 3).

There was no interaction effect on Sprich by season and location to the dam wall, nor a main effect of season (Table 4; Fig. 6). Location to the dam wall did have a

significant main effect on Sprich, whereupon sites below the dam wall had higher Sprich than those above (z=1.90, $p$=0.05; Table 4; Fig. 6). There were no significant effects of any factors or interactions on Shannon diversity index (H) or on Shannon evenness ($E_H$) (Table 4; Fig. 6).

The nMDs showed obvious distinctions in fish communities above and below the dam wall (Figure 7). The ordination stress was 0.05 and therefore appropriate to display on a two-dimensional scale and and acceptable representation of the community data. The PERMANOVA showed that the sample sites themselves contributed to 70% of the variance in fish community ($R^2 = 0.70$, $F_{3,8} = 11.00$, $p < 0.001$), and position to the dam wall was responsible for 18% of the variance ($R^2 = 0.18$, $F_{3,8} = 8.54$, $p < 0.01$). Season did not have an effect on fish community ($R^2 = 0.04$, $F_{3,8} = 2.26$, $p = 0.13$).

**Table 2.** List of species recorded for the Mata de Itamacaoca, upper Munim River basin, State of Maranhão, Brazil. The categories are defined as: native (N), endemic to the Hydrological units Maranhão and Parnaíba *sensu* Hubbert and Renno (2006) (E), widely distributed along Northeastern Brazil river basins (NE), probably undescribed species (U), with known distribution to the Amazon River basin (A), and widely distributed along several river basins of the Neotropical Region, including river basins located south to the Amazon River basin (W). Information obtained from Fricke et al. (2020b).

| CLASS/ORDER/FAMILY/GENUS/SPECIES | Category |
|---|---|
| **CLASS ACTINOPTERYGII** | |
| **Order Characifomes** | |
| **Family Characidae** | |
| *Astyanax* cf. *bimaculatus* | N |
| *Hemigrammus* sp.1. | N, U |
| *Hemigrammus* sp.2. | N, U |
| *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa and Ottoni, 2018 | N, E |
| *Knodus victoriae* (Steindachner, 1907) | N, E |
| *Moenkhausia oligolepis* (Günther, 1864) | N, A |
| **Family Crenuchidae** | |
| *Characidium* sp. | N |
| **Family Curimatidae** | |
| *Curimatopsis* aff. *cryptica* | N, U |
| *Steindachnerina notonota* (Miranda Ribeiro, 1937) | N, NE |

| | |
|---|---|
| **Family Erythrinidae** | |
| *Hoplerythrinus unitaeniatus* (Spix and Agassiz, 1829) | N, W |
| *Hoplias malabaricus* (Bloch, 1794) | N, W |
| **Family Lebiasinidae** | |
| *Copella arnoldi* (Regan, 1912) | N, A |
| *Nannostomus beckfordi* Günther, 1872 | N, A |
| **Order Cichliformes** | |
| **Family Cichlidae** | |
| *Apistogramma piauiensis* Kullander, 1980 | N, E |
| *Cichlasoma* cf. *zarskei* Ottoni, 2011 | N |
| *Crenicichla brasiliensis* (Bloch, 1792) | N, NE |
| **Order Cyprinodontiformes** | |
| **Family Poeciliidae** | |
| *Poecilia sarrafae* Bragança and Costa, 2011 | N, E |
| **Family Rivulidae** | |
| *Anablepsoides vieirai* Nelson, 2016 | N, E |
| **Order Gymnotiformes** | |
| **Family Gymnotidae** | |
| *Gymnotus carapo* Linnaeus, 1758 | N, W |
| **Family Hypopomidae** | |
| *Brachyhypopomus* sp. | N |
| **Order Siluriformes** | |
| **Family Callichthyidae** | |
| *Megalechis thoracata* (Valenciennes, 1840) | N, W |
| **Family Heptapteridae** | |
| *Pimelodella parnahybae* Fowler, 1941 | N, E |
| **Order Synbranchiformes** | |
| **Family Synbranchidae** | |
| *Synbranchus marmoratus* Bloch, 1795 | N, W |

**Table 3.** Species collected (X) in each collecting site (C1-C5) according to the Table 1 at Mata de Itamacaoca, upper Munim River basin, State of Maranhão, Brazil.

| Species | C1 | C2 | C3 | C4 | C5 |
|---|---|---|---|---|---|
| *Astyanax* cf. *bimaculatus* | | X | | | X |
| *Hemigrammus* sp.1. | X | X | | X | X |
| *Hemigrammus* sp.2. | | | | X | |
| *Hyphessobrycon piorskii* | X | X | | X | |
| *Knodus victoriae* | | X | | | |
| *Moenkhausia oligolepis* | | X | | | |
| *Characidium* sp. | | X | | | |
| *Curimatopsis* aff. *cryptica* | X | X | | X | |
| *Steindachnerina notonota* | | X | | | X |
| *Hoplerythrinus unitaeniatus* | | X | | | |
| *Hoplias malabaricus* | X | X | | X | |
| *Copella arnoldi* | X | | | | |
| *Nannostomus beckfordi* | X | X | X | X | X |
| *Apistogramma piauiensis* | X | X | | X | X |
| *Cichlasoma* cf. *zarskei* | X | X | | X | X |
| *Crenicichla brasiliensis* | X | X | | X | |
| *Poecilia sarrafae* | | X | | | X |
| *Anablepsoides vieirai* | X | X | X | | X |
| *Gymnotus carapo* | X | | | | X |
| *Brachyhypopomus* sp. | | X | | | |
| *Megalechis thoracata* | X | X | | | X |
| *Pimelodella parnahybae* | | X | | | |
| *Synbranchus marmoratus* | X | X | | | |
| **Species richness** | **13** | **20** | **2** | **9** | **10** |

**Table 4.** Model terms for all factors and interactions from GLMs used to determine differences in a) species richness, b) Shannon-Weiner diversity index (H) and Shannon's evenness (EH). Significant p-values are highlighted in bold.

| Model term | Est | SE | *t-*value | *p-*value |
|---|---|---|---|---|
| **a) Species richness** | | | | |
| Season | -0.14 | 0.77 | -0.12 | 0.90 |
| Location to dam | 4.71 | 1.95 | 2.41 | **0.02** |
| Season*Location to dam | -4.46 | 2.60 | -1.71 | 0.09 |
| **b) Shannon-Weiner (H)** | | | | |
| Season | -0.00 | 0.28 | -0.01 | 0.99 |
| Location to dam | 0.77 | 0.49 | 1.57 | 0.12 |
| Season*Location to dam | -0.77 | 0.66 | -1.17 | 0.25 |
| **c) Shannon's Evenness (EH)** | | | | |
| Season | -0.01 | 0.01 | -0.92 | 0.36 |
| Location to dam | -0.00 | 0.02 | 0.20 | 0.84 |
| Season*Location to dam | 0.01 | 0.02 | 0.55 | 0.58 |

**Figure 3.** Fish species collected at Mata de Itamacaoca: 1- *Cichlasoma* cf. *zarskei* (CICCAA 03877, 97.3 mm SL), 2 - *Anablepsoides vieirai* (CICCAA 03729, male 29.9 and female 39.4 mm SL), 3- *Nannostomus beckfordi* (CICCAA 03732, 28.9 mm SL), 4- *Hoplias malabaricus* (CICCAA 03880, 96.2 mm SL), 5- *Hoplerythrinus unitaeniatus* (CICCAA 02512, 116.5 mm SL), 6- *Astyanax* cf. *bimaculatus* (CICCAA 03754, 54.2 mm SL), 7 - *Apistogramma piauiensis* (CICCAA 04585, 39.9 mm SL), 8- *Curimatopsis* aff. *cryptica* (CICCAA 02014, 33.6 mm SL), 9 - *Hemigrammus* sp.1. (CICCAA 04593, 26.0 mm SL), 10 – *Knodus victoriae* (CICCAA 02466, 32.5 mm SL), 11- *Moenkhausia oligolepis* (CICCAA 04731, 53.1 mm SL), 12 - *Brachyhypopomus* sp. (CICCAA 02457, 95.1 mm TL), 13- *Steindachnerina notonota* (CICCAA 04729, 67.15 mm SL), 14 - *Megalechis thoracata* (CICCAA 03447, 47.5 mm SL), 15 - *Synbranchus marmoratus* (CICCAA 03400, 137.8 mm TL), 16 - *Hemigrammus* sp.2 (CICCAA 02555, 22.9 mm SL), 17 - *Crenicichla brasiliensis* (CICCAA 03402, 104.3 mm SL), 18 - *Pimelodella parnahybae* (CICCAA 03753, 60.1 mm SL), 19 – *Copella arnoldi* (CICCAA 00081, 26.2 mm SL), 20 - *Hyphessobrycon piorskii* (CICCAA 02421, 22.7 mm SL), 21 - *Poecilia sarrafae* (CICCAA 02506, male 20.6 and female 24.5 mm SL), 22 - *Gymnotus carapo* (CICCAA 00879, 96.8 mm TL), and 23 - *Characidium* sp. (CICCAA 03751, 26.1 mm SL). Photographed by Felipe Ottoni.

**Figure 4.** Ranking of richness by orders observed in the studied area. The numbers in the left column correspond to the number of species.



**Figure 5.** Ranking of richness by families observed in the studied area. The numbers below the graphic correspond to the number of species. The grey bar evidences the most species-rich family of this study.

**Figure 6.** Species richness (Sprich), Shannon-Weiner diversity (H), and Shannon's Evenness (EH) of sampling sites in the dry and rainy seasons, above and below the dam wall. Boxplot indicates median and inter-quartile ranges while points indicate species richness per site.
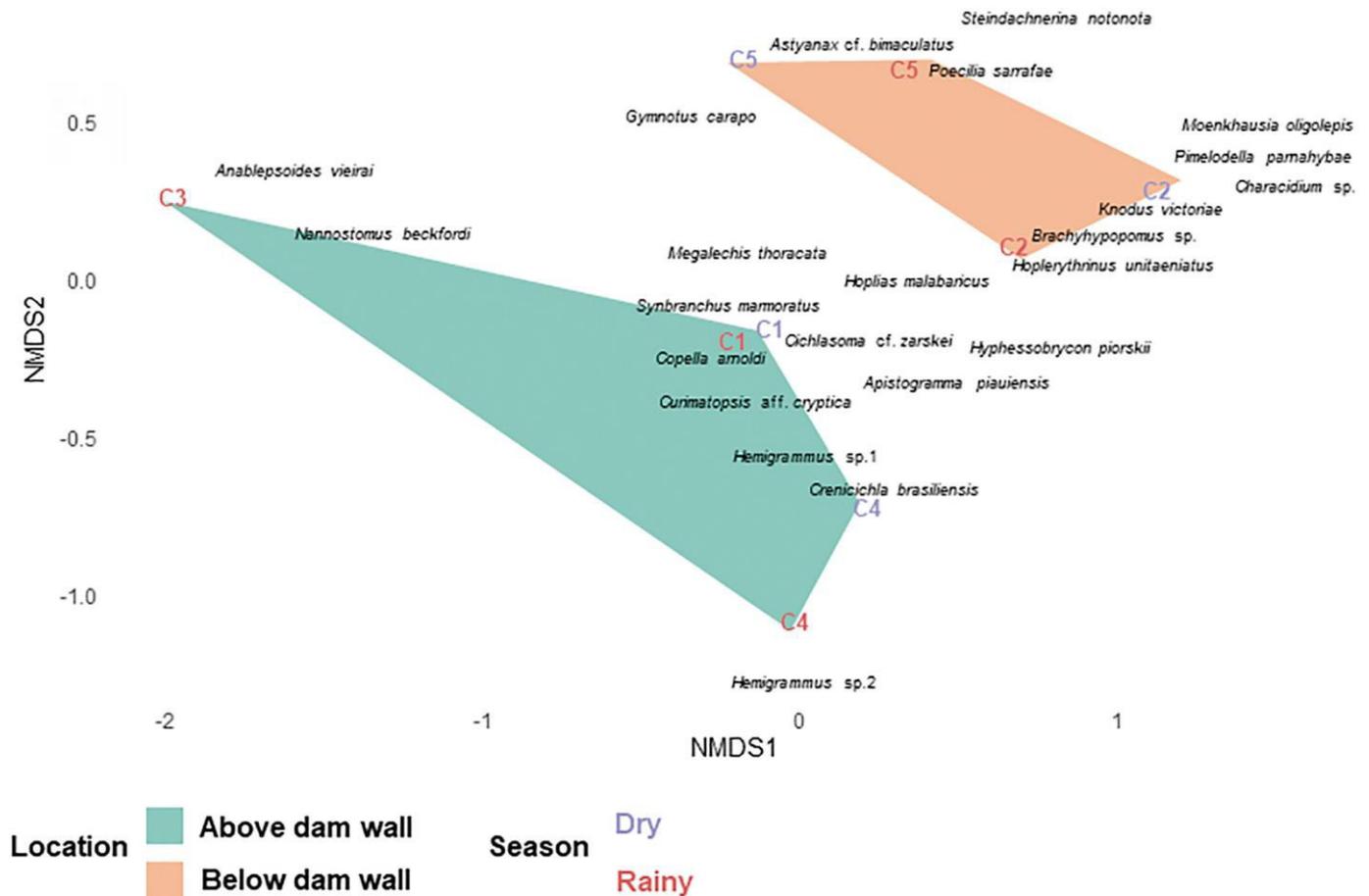
**Figure 7.** Biplot of nMDS ordination of fish communities of the Mrn above and below the dam wall in dry and rainy seasons. Convex hulls denote sites with relation to the dam wall.

**Discussion**

The Mrn urban protected area appears to have efficiently conserved freshwater fish biodiversity, as all of the 23 fish species registered to in the study area are native species (there are no introduced species). Another curious fact is the absence of large-sized species in the studied area. This could be explained by the history of the construction of the dam, which was formed by the damming of small streams, not including originally large rivers. In addition to the high sampling effort, information provided by local fishermen confirms the absence of large-sized fish species in the area. Small-sized fish species are the most threatened among the Neotropical freshwater fish fauna (small- sized fish species comprise around 250 species - about 80% - of the total endangered fish species). This size class of fish represents about 70% of fish species richness of the Neotropical Region, however, they are usually overlooked by the general public as well as conservation agencies and organizations in favour of large charismatic species

(Kalinkat et al. 2017). Further, threats to small fish species are enhanced due to their habitats being far smaller, often fragmented, linear systems that are usually more vulnerable to human impacts (Arthington et al. 2016, Castro & Polaz 2020). Generating robust baseline data of ecological, biological, geographic distributional and taxonomic aspects of these species ought to be a conservation priority, especially in under-studied Neotropical regions.

The Mata da Itamacaoca is an urban protected area from the upper Munim River basin, Northern Brazilian, located at the Municipality of Chapadinha-MA, in the Northern Brazilian Cerrado. This protected area is efficient in protecting freshwater biodiversity, since it includes not only the main tributaries of the area, but also the reservoir itself as well as associated springs (Azevedo-Santos et al. 2018). This has protected the system from a series of urban impacts in this protected area, such as pollution and introduced species, which are present in tributaries, rivers and small streams not included in the protected area. The designated area was specifically designated by the CAEMA (Companhia de Saneamento Ambiental do Maranhão) with the aim to supply the city of Chapadinha- MA with water (including potable water) (Silva et al. 2008). The CAEMA built the reservoir to store water for use during the regions severe dry season, thus providing the city with access to water throughout the year. The region where the dam was created was composed of several streams and spring, the main one being the Itamacaoca stream (Silva et al. 2008) and includes streams and fountains in the vicinity to prevent the water pollution of the reservoir. While the study area is currently in pristine condition with regards to non-native alien species, the proximity to the city, Brazilian legislation changes and the nature of the reservoir puts the protected area at risk of invasion in the future (Garcia et al. 2017, Pelicice et al. 2018, Geller et al. 2020). Dams can act as invasion hubs as well as modifying environmental conditions which allow non-native species to proliferate, all of which can contribute towards biotic homogenisation (Bunn & Arthington 2002, Daga et al. 2020), as well as acting as a barrier for natural species, which are restricted to parts above or below the dam. Indeed, the clear different fish community composition above and below the dam wall indicates that despite the comparable species diversity and richness, there is evidence of alteration in the communities. Future work should identify whether these communities are also separated in terms of functional trait based metrics, as this can provide information on community resilience and biotic resistance (Moyle & Light 1996, Olden et al. 2010, Brito et al. 2020).

None of the species inventoried in the present study have the status of threat of extinction (ICMBIO, 2018). Some species could not be categorized because we were unable to reach species-level identification, or were described after the publication of the red book. However, the species *Hyphessobrycon piorskii* recorded by the present study is probably not threatened with extinction, since it does not have a restricted distribution and also occurs in another federal protected area, the Lençóis Maranhenses National Park (Guimarães et al. 2018b, 2019, Brito et al. 2019, 2020).

The order and family with the highest species richness in the Mata de Itamacaoca, was Characiformes and Characidae, respectively, followed by Cichliformes and Cichlidae, respectively. Characiformes and Characidae are usually the most species-rich groups (Order and Family, respectively) found in any freshwater fish survey of the Neotropical Region. Usually, the second most representative Order is Silurifomes (e.g. Lucinda et al. 2007, Ferreira et al. 2011, Claro-García & Shibatta 2013, Ramos et al. 2014). This trend diverges in the Mata de Itamacaoca, where Cichliformes and Cichlidae (Order and Family, respectively) are the second most diverse groups. This difference found here in the composition pattern of the fish community is probably due to the absence of the Family Loricariidae in the studied area, which is usually one of the most representative freshwater fish families in Neotropical region inventories (e.g. Lucinda et al. 2007, Ferreira et al. 2011, Claro-García & Shibatta 2013, Ramos et al. 2014).

Published studies on fish inventories and taxonomic studies are very scarce in the upper Munim River basin. Recently, two new species were described which occur in the river portion (Guimarães et al. 2018a,b). However, only *Hyphessobrycon piorskii* occurs at Mata the Itamacaoca, including type material (see Appendix 1, Tables 2 and 3, Figure 5, Guimarães et al. 2018b). The dataset from the present study documented more species than previous work by Ribeiro et al. (2014) and Nunes et al. (2019) which list between 15-20 middle to large sized species present in the upper Munim River basin, of which, the majority of the species were not the same. Comparing the present study to Ribeiro et al. (2014) only four species were the same: *Crenicichla brasiliensis* [*Crenicichla menezesi* Ploeg, 1991 in Ribeiro et al. (2014)], *Hoplias malabaricus*, and *Synbranchus marmoratus*. Whereas, compared to Nunes et al. (2019), only three species were the same: *Astyanax* cf. *bimaculatus*, *Crenicichla brasiliensis*, and *Hoplias malabaricus*. This study represents the first time that an ictythological survey conducted at the upper Munim River basin has presented photographs of all of the recorded species and provided

specific voucher number for each examined fish lot. By including this type of information it promotes transparency by allowing other researchers to confirm the identifications, as well as facilitating further research by providing a much needed resource for fish identification. Currently, besides the present study, any records of fish inventories for the Mrn, which include illustrations, let alone photographs of the species collected are rare (e.g. Soares, 2013, Matavelli et al. 2015, Piorski et al. 2017).

We found the highest species richness at collecting site (C2), with a total of 20 species, followed by C1 with 13, C5 with 10, C4 with 9, and C3 with only 2 (see Appendix 1). The collecting sites C1, C2, C3 and C5 have the most preserved ecological integrity, while C4 (the reservoir) is the most modified area in relation to the original conditions. The reservoir dam inhibits dispersion of fishes occurring in C2, which is located below the reservoir dam. It is likely that this is driving the greater species richness in C2 compared to C1, C3 and C4. One other site, C5, is also located below the reservoir dam, however, its lower species richness compared to C2 could be explained by two reasons. Firstly, this collecting site was less sampled than C2, and secondly it dries almost completely during the dry season, while C2 is permanently inundated. The low number of species in C3 is likely to be due to the sample being collected in one collection expedition due to issues in access to the site.

There were three categories of fishes based on their occurrence: 1) only found above the dam wall, 2) only found below the dam, and 3) above and below the dam. Despite these three distinct categories, it was not possible to observe any ecological pattern that correlates these species with these three distribution patterns. Similarly, despite the different fish community composition above and below the dam wall there is no concrete evidence nor immediate explanation for this. This is compounded by the regional data deficit and lack of historical data prior to the dam construction. Nonetheless, it is important to emphasize that the absence of *Anablepsoides vieirai* and *Copella arnoldi* at locality C4 was already expected, due to the ecological requirements of these species, which do not occur in this type of environment. In addition, the absence of *Pimelodella parnahybae* in the rainy season could be explained because catfishes usually inhabit caves or burrows and are usually associated with the benthos. As in the dry season the water level is lower, it is easier to collect catfishes, since we can access the bottom of the river more easily. Possible reasons for this differentiation, which ought to be further explored, are the effects of habitat filtering,

river continuum concept and functional traits of the fish community. This research would further both the conservation of Neotropical freshwaters but also challenge or confirm whether these concepts subscribe to theories that have generally been developed in temperate systems and have data gaps in the tropics (Boulton et al. 2008, Dudgeon 2008, Sternberg & Kennard, 2013).

From the 23 species herein recorded, we were not able to accurately identify seven species to the species level. Three of them correspond to new species (*Curimatopsis* aff. *cryptica*, *Hemigrammus* sp.1, and *Hemigrammus* sp.2), and are in the process of being described. The other four species need a more comprehensive taxonomic investigation, especially comparing with specimens from other populations from other river basins and regions, as already proposed for other fish groups occurring along the coastal river basins of the State of Maranhão by Guimarães et al. (2020). The State of Maranhão (northeastern Brazil), compared to other Brazilian regions, has distinctly few studies related to its freshwater ichthyofauna, especially in taxonomic studies (Piorski, 2010, Guimarães et al. 2018a, 2020). Thus, at the outset it was anticipated that some species would remain taxonomically indeterminate in this study and therefore was a motivation for carrying out this inventory. Of the other 16 species we were able to identify accurately at the species level, six of them are endemic to the Hydrological units Maranhão and Parnaíba sensu Hubbert and Renno (2006) (hereafter Mrn and Prn, respectively). Two are widely distributed along Northeastern Brazil river basin. Five are widely distributed along several river basins of the Neotropical Region, including river basins located south to the Amazon River basin, and three also have their known distribution to the Amazon River basin. Therefore, we would like to emphasize that a significant number of the species registered here are endemic to the Mrn and Prn; and in addition, the composition of the ichthyofauna in the studied area has little influence from the Amazon basin. Thus, the present study contributes considerable addition to the knowledge around endemic small sized fishes. This information is integral for future planning and conservation endeavours as these species are disproportionately at risk (Arthrington et al. 2016). Further, our data mirrors trends seen in other tropical protected areas, wherein the fish species diversity is as yet undescribed but under high potential risk (Rico-Sánchez et al. 2020).

Considering the small size and similarity oh habitats within the sampled area, the diversity of fish species found in the Mata de Itamacaoca was surprisingly high. Comparing the number of species found by our study (23 species) with other studies that

carried out inventories of entire river basins or much larger areas of the region. For example, Barros et al. (2011) (69 species) and Nascimento et al. (2016)(64 species) both inventoried the fish fauna of the entire Itapecuru River basin, a of the significant coastal river basins of the Mrn and Prn. Further, Brito et al. (2019, 2020) inventoried 56 species of freshwater fish from the Parque Nacional dos Lençóis Maranhenses and the adjacent areas. Therein, the Mata de Itamacaoca holds around a third of the number of the species reported in far larger systems, thus demonstrating the importance of the studied area in the protection of the fish fauna inhabiting it.

**Conclusions and Future Perspectives**

This study corroborates other studies carried out in the Mrn that the ichthyofauna of this region still has many knowledge gaps, especially concerning the composition and taxonomy of the fish groups occurring in the basins. Especially regarding the diversity of small-sized fishes (Piorskii 2010, Guimarães et al. 2018a). The present study reveals three species not yet described in this study area, and one species recently described (in 2018). This shows the potential of the region in still having freshwater fish species which need a formal description. In addition, the fact that small streams can host a comparatively high species richness combined with the possibility of finding endemic and undescribed fish species emphasises need for these environments to be treated as priority in conservation policies. Further work should focus on the diversity of neglected small sized fish species with particular focus on taxonomy and community ecology in vulnerable Mrn stream environments. All species listed here are small and medium sized and some attention is needed for these species (Castro & Polaz 2020). Problems like the lack of knowledge about them, as well as the reduction of the original riparian vegetation cover lead to changes that affect the existence of these smaller species (Castro & Polaz 2020). An important facet to note is the success in the protected area designation, which is currently free from harmful non-native species. With this respect, this area appears to be a valuable refuge for small fish species. Moreover, due to the constant anthropogenic change, this study may be a baseline for similar environments in the region as it presents a native fish assemblage unaffected by biotic drivers yet exposed to other abiotic drivers.

**Supplementary Material**

The following online material is available for this article: Appendix 1 - Examined material.

## Acknowledgements

## Author Contributions

Elioenai da Silva Oliveira: Specimen Collection, expedition organization, data analysis, statistical analysis, manuscript writing. Erick Cristofore Guimarães: Specimen Collection, expedition organization, data analysis, specimens identification, manuscript writing. Pâmella Silva de Brito: Specimen Collection, expedition organization, data analysis, specimens identification, manuscript writing. Lucas de Oliveira Vieira: Specimen Collection, expedition organization, data analysis, specimens identification. Rafael Ferreira de Oliveira: Specimen Collection, expedition organization, data analysis, specimens identification. Diego Sousa Campos: Specimen Collection, expedition organization, data analysis, specimens identification, map preparation. Axel Makay Katz: Specimens identification, data analysis, image preparation, manuscript writing. Josie South: Data analysis, Statistical analysis, manuscript writing, language revision. Jorge Luiz Silva Nunes: Specimen Collection, expedition organization, data analysis, manuscript writing. Felipe Polivanov Ottoni: Specimen Collection, expedition organization, data analysis, specimens identification, manuscript writing, project financing.

## Conflicts of Interest

The authors declare that they have no conflict of interest.

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# CAPÍTULO III

# LENGTH–WEIGHT RELATIONSHIPS FOR ICHTHYOFAUNA OF THE MATA DE ITAMACAOCA, MUNIM RIVER BASIN, NORTHERN BRAZILIAN CERRADO

RELAÇÕES PESO-COMPRIMENTO PARA A ICTIOFAUNA DA MATA DE ITAMACAOCA, BACIA DO RIO MUNIM, CERRADO NORTE DO BRASIL

# Length–weight relationships for ichthyofauna of the Mata de Itamacaoca, Munim River basin, Northern Brazilian Cerrado

**Abstract.** The present study described the length/weight ratio (LWR) of 14 species fish caught at five different points in the Mata de Itamacaoca, upper Munim River basin. Fish were sampled using manual trail-net (2 m long × 1.8 m high; mesh size, 2 mm), cast nets (2 m height, mesh size 15 mm), gillnets of various mesh sizes (15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 100 mm), and dip nets (mesh size 5 and 10 mm). Negative allometric growth ($b<3$) was predominant, occurring in 71% species, 29% presented positive allometry ($b>3$). Results may be influenced by different environmental factors, ontogenetic changes and sexual dimorphism that were unable to be extricated from the data at this point.
**Keywords:** Allometric coefficient, Biodiversity, Growth pattern, Munim basin.

## Introduction

Climate changes has a significant effect on terrestrial ecosystems, causing changes in the composition of communities, geographical distribution and abundance of species of fishes (SIMPSON; BLANCHARD; GENNER, 2013). Aquatic habitats are the most vulnerable to environmental degradation and to climate change, this poses a major threat to the continued existence of many freshwater fish species (ALBERT; REIS, 2011). The Neotropical region encompasses the world's largest freshwater fish diversity, with more than 7.000 described species (REIS et al. 2016). This megadiversity of ichthyofauna results from the complex diversity of conditions and geological, biological and evolutionary histories of the Neotropical tributaries (ALBERT; REIS, 2011; REIS et al. 2016). Due to high environmental heterogeneity, Neotropical fish species become biologically complex (REIS et al. 2016), such characteristics may reflect variations biological parameters such as those obtained from LWR studies.

Species responses to ecological perturbations can be detected in a low intensity manner through deriving length weight relationships and how these change over time. Descriptions of the length-weight (LWR) parameter have been employed since the end of the 19th century in ichthyology studies (LE CREN, 1951; FROESE et al. 2006). Determining what mass a fish should be at a certain length allows comparisons and inferences to be made regarding fisheries population shifts and deviations from a baseline in response to perturbation. For example, this relationship is able to determine the welfare of fish in their habitat and compare types of growth in different fish populations (FROESE et al. 2006; RODRÍGUEZ-OLARTE; TAPHORN; AGUDELO-ZAMORA, 2018), estimate the growth rate and age structures in fish groups (FROESE et al. 2006), evaluate

possible contamination of fish by heavy metals (MATOS et al. 2018), to analyse the effects of seasonality in freshwater environments and can be used as an environmental monitoring tool in disturbed areas (GIARRIZZO et al. 2015; TRIBUZY-NETO et al. 2018, NUNES et al. 2019).

The curve of the LWR of a species is determined by the parameter "$a$" which is a constant of that relation (or the intercept of linear regression), while "$b$" is the allometric coefficient of the relation (or the slope of linear regression). Thus it is possible to quantitative expressing the degree of dependence of length (x-axis) on mass (y-axis) in a sample population (GIARRIZZO et al. 2015). The relationship between two variables in linear regressions can be explained by the pattern of functional dependence exerted between the variables. That is, the magnitude of the dependent variable is often determined by the magnitude of the independent variable, although the reverse situation may happen (CELLA-RIBEIRO, et al. 2015). The value of the b (slope) indicates isometric or allometric growth, whereupon $b=3$ describes isometric growth (i.e. linear growth). Values of b<3 infer negative allometry, as such the animal does not grow in body depth as it grows longer and thinner, whereas values of b>3 infer positive allometry, in which, body depth increases with increases in length (RIEDEL; CASKEY; HURLBERT, 2007).

A variety of factors, both biotic and abiotic may cause changes in the LWR paramters. For example, the strength of inter-specific interactions, availability of resources in the environment, sex and life history of individuals (e.g. LE CREN, 1951; FROESE, 2006; CELLA-RIBEIRO, 2015; FREITAS et al. 2017; SAMPAIO et al. 2019). A solid baseline of fundamental ecological knowledge is an imperative first step in assessing species resilience and responses to change. In order to robustly assess fish stock structure and estimate biomass, practitioners need to consider that the coefficients for estimating the LWR of the same species may vary from place to place, because each locality has its environmental and physiological peculiarities and the fauna tends to match them (POSSAMAI et al. 2019). Based on these statements, we estimate the LWR for the ichthyofauna of the Mata de Itamacaoca, an urban protected area from the upper Munim River basin (Hereafter Mrn). Previous studies have shown that the Mata de Itamacaoca is currently pristine and has an undisturbed fish community despite the construction of a reservoir (OLIVEIRA et al. 2020). Thus, baseline data on LWR of the fish community specific to the area in question is essential for future planning for the conservation and integrity of fish populations, especially in light of current global change dynamics.

**Material and Methods**

This study was conducted in five sample site (C1-C5) of the Mata de Itamacaoca (Figure 1), an urban area protected for CAEMA (Companhia de Saneamento Ambiental do Maranhão) upper Munim River basin, comprising springs, streams, pools, and a reservoir, localited in to Municipality of Chapadinha, State of Maranhão (24°25'47" S, 58°44'05" W). All collection points are described in Oliveira et al. (2020). The study area covers about 460 hectares and recognized as an Area of Relevant Ecological Interest for the conservation of fauna and flora by the Decreto Municipal Nº 05/2018. The Mata de Itamacaoca consists of small and medium sized plants of up to 10 meters high, forming a large vegetable mosaic and closed forest associated with streams and small watercourses and which empty into an artificial reservoir (SILVA et al. 2008). This paper compares use of pooled samples because some species were few sampled throughout each year, not for lack of sampling effort, but because they present a certain rarity. In addition, there are no significant differences in the indices of diversity in relation to the season and sampling sites and the composition of the fish community is significantly different above and below the dam, as pointed out Oliveira et al. (2020). So, arguably, the data can be collated for a representative sample of each species.

Trimonthly samplings were performed between August 2014 to February 2020, during both dry and wet seasons. Sampling methods used were a manual trail-net (2 m long × 1.8 m high; mesh size, 2 mm), cast nets (2 m height, mesh size 15 mm), gillnets of various mesh sizes (15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 100 mm), and dip nets (mesh size 5 and 10 mm). All specimens were taken to the Laboratório de Sistemática e Ecologia de Organismos Aquáticos of the Universidade Federal do Maranhão for identification according to Oliveira et al. (2020). Afterwards the specimens were measured for standard length (Lt, in mm) using vernier calipers 0.1 mm accuracy and weighed (Wt, in g) with a digital balance of 0,01 g accuracy. Fish from each species were fixed and kept in the Coleção Ictiológica of the Centro de Ciências Agrárias e Ambientais of the Universidade Federal do Maranhão (CICCAA).

Parameter values for "a" and "b" were estimated via least squares linear regression on the log transformed data, following log W = log $a$ + $b$ (logL), where W is total body weight (g), "$a$" is the intercept of linear regression and "$b$" indicates the isometric-allometric growth coefficient (RICKER 1975). The coefficient of determination ($r^2$ value) was used to measure the quality of the adjustment of the data to the allometric model described.

**Results**

Overall, 1557 individuals from 14 species belonging to five orders and nine families were sampled (Table 1). The allometric coefficient ranged from 1.06 to 4.75. The two different types of growth were identified, 10 species (71%) showed negative allometric growth (b<3) and four species (29%) showed positive allometric growth (b>3) (Table 2; Figure 2). The familys Erythrinidae, Lebiasinidae, Rivulidae, Callichtyidae and Synbranchidae, showed trends to negative allometric growth. In Characidae, for the four species analyzed, only *H. piorskii* Guimarães, Brito, Feitosa and Ottoni, 2018 showed trends of positive allometric growth. In Cichlidae, *A. piauiensis* Kullander, 1980 showed trends negative allometric growth and *Cichlassoma cff. zarskei* Ottoni, 2011 with positive allometric growth. Familys Crenuchidae and Poeciliidae showed trends positive allometric growth with one specie each. The coefficient of the determination ranged from 0.14 to 0.99 (Table 2; Figure 2).

**Discussion**

The Mata de Itamacaoca it is an area of environmental protection within the urban perimeter in the Upper Munim River Basin, northern Brazilian Cerrado. This area includes not only the dam, but also various freshwater springs and creeks serving as shelters for small and medium-sized fish (AZEVEDO-SANTOS et al. 2018). In order to supply the city of Chapadinha-MA with drinking water, the CAEMA (Companhia de Saneamento Ambiental do Maranhão), built the dam in an area composed of several streams and springs, with the presence of trees that can reach up to 10 meters, forming a large vegetable mosaic (SILVA et al. 2008). Although not suffer great environmental impacts, the proximity to the city, the Brazilian environmental legislation and the dam (with risk of invasion of alien species in the future), seem to be the main threats in this area (GARCIA et al. 2018, PELICICE et al. 2018, GELLER et al. 2020). Data on the LWR of fish species present in the Mata de Itamacaoca, Munim river basin can benefit scientists and conservationists regarding the ecology of the community in this understudied location, in addition to serving as a basis for the same species occurring elsewhere.

The LWR parameters are available for most species analysed in FishBase (www.fishbase.org), except to *H. piorskii* which is a recently described species, this being the first record of LWR for *H. piorskii.* We found contrasting values compared to available values in FishBase, because the LWR parameters available in the database were

estimated on the basis of an innovative methodology proposed by Froese and Pauly (2021) that using compilations of different genera or species belonging to the same family, but who share the same bodily form. Thus, we provide empirical data to support and update baseline knowledge regarding fundamental fish ecology. This can be built upon as an evidence base for future monitoring.

In this study, we compile different years of sampling (Table 1), we did not consider categorizing males and female because only two of the 14 species analyzed present with any considerable sexual dimorphism (*Poecilia sarrafae* and *Anablepsoides vieirai*). We only considered adult fish by believing that these fit better to LWRs. In addition, small fish species are very abundant in the area, while large species are absent. This can be explained by the history of damming construction, which included only small streams, not including originally large rivers. Small fishes account for approximately 70% of the megadiversity of the Neotropical region, but it is still little known, becoming the most endangered among freshwater ichthyofauna of the Neotropical region (REIS et al. 2016; KALINKAT et al. 2017). Despite these caveats we consider this approach an necessary improvement on the data currently existing from extrapolated information in the FishBase records. The methods for estimated of LWR available values in FishBase, were estimated on the basis of an innovative methodology (Bayesian method) proposed by Froese and Pauly (2021). This Bayesian method is not fully applicable in all cases, since generalization can obscure future trends, especially if viewed in specific families or trophic guilds. In this study, we manually derived LWRs which covers representative size ranges for the adult specimens considered which increases the reliability of the estimates. These differences in parameters values enter of FishBase and the estimated manually are also reported by Allard et. al. (2015), Cella-Ribeiro et al. (2015), Freitas et. al. (2017) and Sampaio et al. (2019). In this study, In addition to environmental variations, factors such as the sampling method (SANTOS; GRIS, 2016), sampling period (CARVALHO et al. 2017), small sampling and presence of juveniles (RODRÍGUEZ-OLARTE; TAPHORN; AGUDELO-ZAMORA, 2018) may also be influencing the growth patterns observed the present study.

In this study, species with negative allometric growth (b<3) were more common than species with positive allometric growth, with 71% and 29% species respectively (Table 1). This indicates that the individual does not increase body depth as it grows longer and thinner (RIEDEL; CASKEY; HURLBERT, 2007). The main representatives of Allometric negative growth were species of the family Characidae with three species

of fish, followed by the families Lebiasinidae, Erythrinidae, Cichlidae, Rivulidae, Callichthyidae and Synbranchidae. Our results concur with those reported by Nunes et al. (2019) (also in the Munim upper river basin), where three Characidae species were analyzed, and showed negative allometric growth coefficient *b* for *Charax awa* to 2.85, for *Metynnis lippincottianus* to 2.93 and coefficient b for *Astyanax* cf. *bimaculatus* to 2.84, while in our study we find coefficient b to 2.79 for the same specie. The persistence of negative allometry is also indicative of elongated torpedo shapes. Indeed, this is reflected in the different morphotypes of the fish of Mrn. Whereby fish with a longer and fatter body, such as *Hoplias malabaricus* have *b*= 2.39 whereas fish with shorter body but larger body width, such as *Nannostomus beckfordi* have *b*= 1.82, and morphologically constrained, such as *Synbranchus marmoratus b*= 2.88 that are long, but with reduced body depth.

Four out of seven species from the family Characidae collected in a tributary of the Amazon River presented negative allometric growth (*Brycon amazonicus*, *b*= 2.944; *Triportheus albus*, *b*= 2.947; *Triportheus angulatus*, *b*= 2.897; *Triportheus auritus*, *b*= 2.666) (CELLA-RIBEIRO et al. 2015). Freitas et al. (2014) who worked in rivers in the eastern Amazon region reported that all species in the family Characidae analyzed showed negative allometric growth (*Bryconops melanurus*, *b*= 2.65; *Triportheus albus*, *b*= 2.67). In contrast, Mereles, Sant'Anna and Sousa (2017) reported that the estimated LWR for 21 species belonging to seven families of fish collected in the basin of Rio Machado, the family Characidae had disproportionately positive allometric growth compared to others. Whereas Lizama and Ambrósio (1999) reported that nine species of the family Characidae collected in flooded plains of the Paraná River, presented an isometric growth. These different results indicate that the same families/species studied in different regions, may present variations in the values of the coefficients resulting from latitudinal distribution of environmental studies and influences (FROESE, 2006). This further supports the necessity of deriving LWR of similar species across different locations and gradients of disturbance to effectively detect population level changes.

It is likely that resource availability alters growth relationships (TRIBUZY-NETO et al. 2018). Especially as the strong seasonality in the tropics affects a variety of biotic and abiotic factors (ECOUTIN; ALBARET; TRAPE, 2004; MATOS et al. 2019; OLIVEIRA et al. 2020). In particular, with the onset of the rainy season there is an expansion of the aquatic environment volume which improves connectivity between the aquatic terrestrial transition zone and increases the resources available in the

environment. In contrast, in the dry period, there is a decrease in depth of aquatic environments which causes a decline in the availability of resources (TRIBUZY-NETO et al. 2018; CASTRO-CAMPANHA et al. 2019). Despite study area is pristine, Considering the small size and similarity oh habitats within the sampled area, The Mata de Itamacaoca it must serve as the reference for comparison with other locations and rivers, mainly in relation to small fish it presents a native fish assemblage unaffected by biotic drivers yet exposed to other abiotic drivers.

## Conclusion

Finally, this study emphasizes the importance of expanding the biological knowledge of the species to improve management and conservation plans. That way, the Mata de Itamacaoca, has a wide variety of small species, having endemic species, species widely distributed in the basins of the northeastern Brazil, in the Amazon River basin and along several basins of the Neotropical region and our study brought a set of primary data from the fish community of the Mata de Itamacaoca. Although LWR is known to most species in the Mata de Itamacaoca, our study shows more accurate and more reliable data than those already available and these data can be used for future proposals and for comparison with others works.

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**Figure 1.** Map of the studied area. Collecting sites (C1-C5) described in Oliveira et al. (2020, fig. 1).
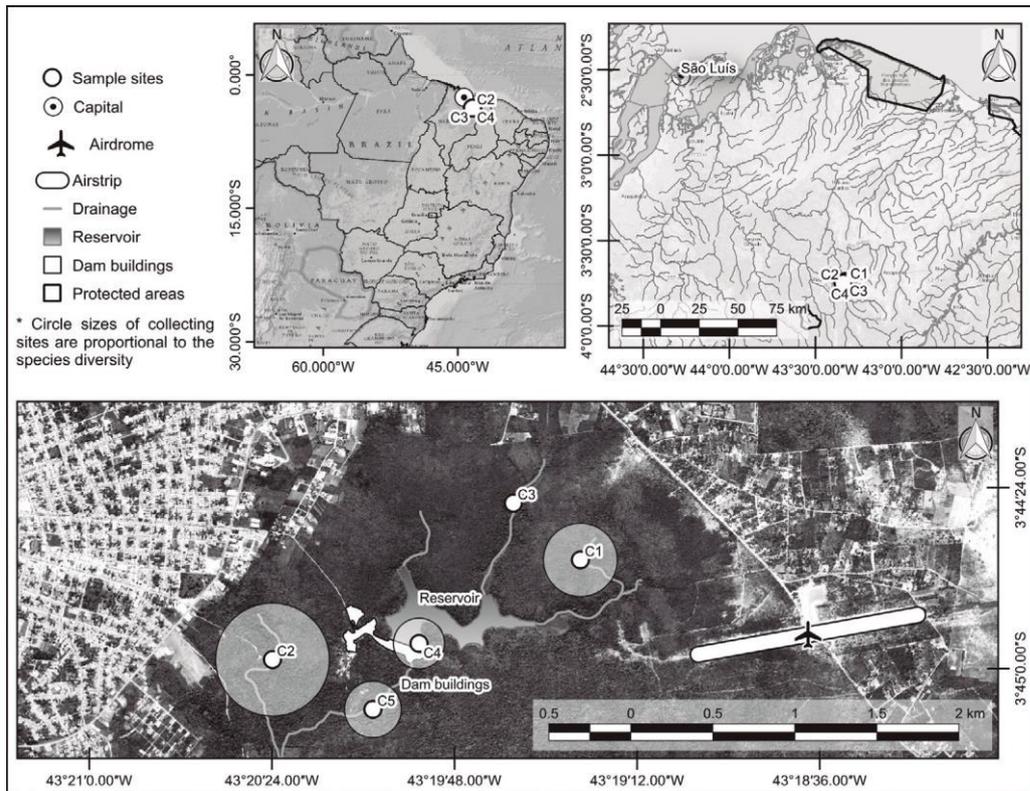


**Figure 2.** Linear regression slopes activated for the 14 species studied.
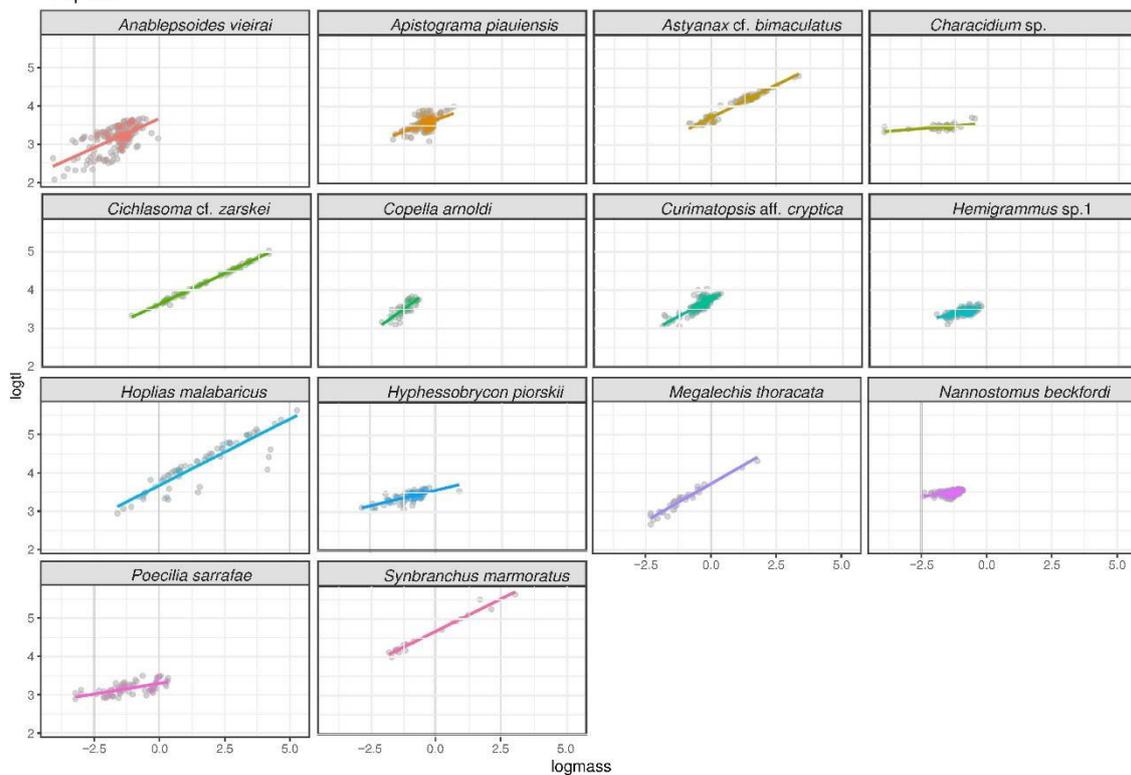
**Table1.** Total sample numbers and sampling year for each species.

| Species | N | Year |
|---|---|---|
| *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa & Ottoni 2018 | 95 | 2014 to 2020 |
| *Astyanax bimaculatus* | 100 | 2019 to 2020 |
| *Hemigrammus* sp.1 | 200 | 2015 to 2020 |
| *Curimatopsis* aff. *cryptica* | 200 | 2015 to 2019 |
| *Hoplias malabaricus* (Bloch, 1794) | 64 | 2016 to 2020 |
| *Nannostomus beckfordi* Günther, 1872 | 200 | 2015 to 2020 |
| *Copella arnoldi* (Regan, 1912) | 49 | 2016 to 2019 |
| *Characidium* sp. | 45 | 2019 |
| *Apistogramma piauiensis* Kullander, 1980 | 200 | 2015 to 2020 |
| *Cichlasoma cf. zarskei* Ottoni, 2011 | 80 | 2016 to 2020 |
| *Anablepsoides vieirai* Nielsen, 2016 | 200 | 2015 to 2020 |
| *Poecilia sarrafae* Bragança & Costa, 2011 | 71 | 2016 to 2020 |
| *Megalechis thoracata* (Valenciennes, 1840) | 33 | 2016 to 2020 |
| *Synbranchus marmoratus* Bloch, 1795 | 20 | 2016 to 2019 |

**Table 2.** Number of specimens (N), total length range (Lt, Min - Max), Mean and Standard Deviation (SD), Allometric coefficient (*b*), coefficient of determination (r$^2$), growth pattern from mean allometric coefficient (a- = negative allometric; a+ = positive allometric) of specimens collected in the Munim River Basin.

| Order/Family/Specie | N | Lt(Min-Max) | Mean Lt-SD | Parameters LWR | | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | *b* | p | r$^2$ | Growth |
| CHARACIFORMES | | | | | | | |
| **Characidae** | | | | | | | |
| *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa & Ottoni 2018 | 95 | 20.77-37.27 | 29.62-3.72 | 3.14-0.31 | 0.001 | 0.50 | a+ |
| *Astyanax bimaculatus* | 100 | 32.88-119.41 | 57.86-18.30 | 2.79-0.06 | 0.001 | 0.94 | a- |
| *Hemigrammus* sp.1 | 200 | 21.75-37.31 | 30.26-2.40 | 2.73-0.23 | 0.001 | 0.39 | a- |
| *Curimatopsis* aff. *cryptica* | 200 | 20.76-55.26 | 36.98-5.98 | 1.81-0.09 | 0.001 | 0.65 | a- |
| **Erythrinidae** | | | | | | | |
| *Hoplias malabaricus* (Bloch, 1794) | 64 | 18.94-278.81 | 80.29-52.69 | 2.39-0.13 | 0.001 | 0.82 | a- |
| **Lebiasinidae** | | | | | | | |
| *Nannostomus beckfordi* Günther, 1872 | 200 | 27.23-36.29 | 32.39-1.80 | 1.82-0.31 | 0.001 | 0.14 | a- |
| *Copella arnoldi* (Regan, 1912) | 49 | 21.55-45.07 | 34.28-6.41 | 1.32-0.14 | 0.001 | 0.63 | a- |
| **Crenuchidae** | | | | | | | |
| *Characidium* sp. | 45 | 27.49-40.20 | 31.62-2.27 | 4.75-1.21 | 0.001 | 0.24 | a+ |
| **CICHLIFORMES** | | | | | | | |
| **Cichlidae** | | | | | | | |
| *Apistogramma piauiensis* Kullander, 1980 | 200 | 21.65-53.46 | 34.94-5.81 | 1.06-0.12 | 0.001 | 0.26 | a- |
| *Cichlasoma cf. zarskei* Ottoni, 2011 | 80 | 27.61-149.93 | 71.53-30.21 | 3.10-0.02 | 0.001 | 0.99 | a+ |
| **CYPRINODONTIFORMES** | | | | | | | |
| **Rivulidae** | | | | | | | |
| *Anablepsoides vieirai* Nielsen, 2016 | 200 | 7.95-39.25 | 24.76-0.50 | 1.31-0.11 | 0.001 | 0.40 | a- |
| **Poeciliidae** | | | | | | | |
| *Poecilia sarrafae* Bragança & Costa, 2011 | 71 | 17.91-32.87 | 24.21-3.93 | 3.66-052 | 0.001 | 0.40 | a+ |
| **SILURIFORMES** | | | | | | | |
| **Callichthyidae** | | | | | | | |
| *Megalechis thoracata* (Valenciennes, 1840) | 33 | 14.18-74.05 | 28.21-12.35 | 2.42-0.10 | 0.001 | 0.94 | a- |
| **SYNBRANCHIFORMES** | | | | | | | |
| **Synbranchidae** | | | | | | | |
| *Synbranchus marmoratus* Bloch, 1795 | 20 | 53.5-274-63 | 109.56-64.17 | 2.88-0.11 | 0.001 | 0.97 | a- |

# CAPÍTULO IV

## MORPHOMETRIC ANALYSIS OF *NANNOSTOMUS BECKFORDI* IN DIFFERENT SEASONAL PERIODS

ANÁLISE MORFOMÉTRICA DE *NANNOSTOMUS BECKFORDI* EM DIFERENTES PERÍODOS SAZONAIS

# Morphometric analysis of *Nannostomus beckfordi* in different seasonal periods

**Abstract.** Form and function of fish species morphological traits are tightly linked. Plasticity and variation in morphology are driven by biotic and abiotic factors. In fish species hydrological regimes are associated with different trait combinations as velocity and turbulence affects swimming ability. We test whether *Nannostomus beckfordi*, exhibits morphological variation in response to season (dry and rainy) used as a proxy for flow regime. We used eight morphometric measurements from 1.261 specimens. GM were used for removes confounding effects of differences in SL of each measured fish. Correlation between raw linear measurements were assessed using Kendalls Tau as data was non - normal (per QQ plots). The non-parametric bootstrap resampling method wasused to calculate confidence intervals. A PCA was completed on the scaled data to determine the ordination of *N. beckfordi* individuals sampled in different seasons. A one-way ANOVA was completed in all the response variables to detect significant differences between seasons. Dry and Rainy Season had a significant effect on all N. beckfordi linear measurements, except for AFL, DFL, and PELVL. The first two axes of the PCA accounted for 47.7% of variation. Overall, individuals sampled in each season broadly overlap in morphology, however, the dry season individuals show a greater variation in morphology. Dry season population is likely comprised of fish of different ontogenies due to the larger variation in size ranges and morphology. There is some evidence of ecologically in *N. beckfordi* stable strategies in terms of ecomorphology related to seasons and flow regime.

**Keywords:** Ecomorphology, Functional Morphology; Flow Regime, Intraspecific Variation, Ontogeny, Season.

## Introduction

Morphological traits are linked to ecological preferences with regards to habitat niche, food preference, and resilience to disturbance (MCGILL et al. 2006, FISHER et al. 2007; RINCÓN et al. 2007; DA SILVA et al. 2019). Functional traits can be used to infer feeding preferences and swimming performance (GATZ, 1979a, WATSON; BALON, 1984, WINEMILLER, 1991; NAGELKERKE et al. 2018). Differences in ecomorphology can be compared to understand community assemblages across macro and microhabitat scales (PEASE et al. 2012; BOWER; WINEMILLER, 2019). Therein comparison of differences in functional traits can be used to elucidate ecological questions such as species co- existence (SIBBING; NAGELKERKE, 2000), invasive species success (LUGER et al. 2020), and the effect of environmental change, predation pressure (SANTI et al. 2020) or susceptibility to parasitism (PEGG et al. 2015).

Much attention has focused on fish functional morphology as aquatic systems are inherently stochastic and generally heterogeneous which promotes huge morphological diversity ( e.g. GROSSMAN; DE SOSTOA, 1994; GROSSMAN et al. 1998; VADAS

JÚNIOR; ORTH, 2000; BLANCK et al. 2007; LEAL et al., 2011). Aquatic environments are generally strongly influenced by seasonal periods and flood pulse dynamics (JUNK et al. 1989; PAZIN et al. 2006). During the rainy season riverine systems are subjected to a flood regime which increases turbidity, resource availability, flow velocity and water height (ALHO; SILVA, 2012). As the flood peak reaches and the system proceeds into dry season, and indeed drought periods are becoming more common, there is a decline in the above- mentioned variables which results in myriad abiotic changes as well as differences in fish community assemblages (WINEMILLER, 1990, 1995; SAINT- PAUL et al. 2000; ESPÍRITO- SANTO et al. 2013). While some fish species experience boom and bust dynamics in line with seasonal reproduction, some persist throughout (FIALHO et al. 2007; ARTHINGTON; BALCOMBE 2011; FITZGERALD et al. 2016). In the case of seasonal persistence, intraspecific morphological variation becomes an important factor for fish survival in stochastic ecosystem s as fish species evolve in line with persistent hydrological regimes (POFF; WARD, 1989; LYTLE; POFF, 2004). Plasticity and diversity in morphology can confer specializations to specific environmental parameters and thus enhances survival across cohorts (LANGERHANS; REZNICK, 2010; COLLIN; FUMAGALLI, 2012).

Hydrological regimes are thus considered highly relevant in determining certain aspects of fish morphology (LANGERHANS, 2008). Flow regimes drive plasticity in aspects of morphology such as body height, caudal peduncle height, and fin placement and shape are related to swimming ability (GATZ, 1979b; CASSATI; CASTRO, 2006). In habitats with higher flow velocity fish should be more streamlined (i.e. fusiform with smaller body height) (VOGEL, 1994; BLAKE, 1983). Whereas low flow is generally associated with deeper caudal peduncles, deeper body height and larger bodies (LANGERHANS et al. 2003; LANGERHANS, 2008; BARROS et al. 2019). Swimming performance determines individual energy expenditure and predator escape ability which translates into individual fitness and persistence in a given environment (BLOB; RIVERA, 2008; LANGERHANS; REZNICK, 2010).

Linear morphometry can be employed to understand the relationships between environmental variation and phenotypic characters. Multivariate statistical analysis techniques can be applied to various morphological measures, these approaches are widely applied in evolutionary biology (SIDLAUSKAS et al. 2011), however, they can also be applied understanding morphological changes with regards to abiotic pressures

(BARROS et al. 2019; BOWER; WINEMILLER, 2019). Morphometric studies can be used to make ecological interpretations around habitat uses and determine selective pressures acting upon fish populations (e.g. LANGERHANS; REZNICK, 2010; HAAS et al. 2010; COLLIN; FUMAGALLI, 2011).

Among the Lebiasinidae, a monophyletic group endemic to the Neotropical region of South America (VARI, 1995), the genus *Nannostomus* Günther 1872 possess the largest diversity of valid species, currently presenting 20 species (FRICKE et al. 2020). The peculiar body shape that this family presents makes them commonly known as pencilfishes. They are distributed along most of the major northern South America hydrological systems like the Amazonas (including the Maranhão watersheds), Guiana and Orinoco rivers basins (NETTO - FERREIRA, 2018; GUIMARÃES et al. 2020; FRICKE et al. 2020), inhabiting the shallow margins of rivers (ZARSKE, 2013). One of the most remarkable species is *Nannostomus beckfordi* Günther 1872, favoured among aquarists due to the presence of vivid and bright colours and as a result has been noted as an emerging invader (MAGALHÃES; JACOBI, 2008; ZARSKE, 2013; MAGALHÃES et al. 2019). This species is characterized by the presence of a longitudinal dark stripe during the day, at night, this pattern disappears and gives way to three round dots on each side of the body (NETTO- FERREIRA, 2018).

Evidence of adaptations to environmental regimes will provide information on ecological mechanisms allowing small - sized fish to persist in stochastic environments, poignant here, as small - sized fish have enormous diversity and are the most threatened of Neotropical fish species (CASTRO; POLAZ, 2020). We use linear morphometry to test whether a population of *N. beckfordi* exhibits phenotypic plasticity in response to seasonality (i.e. dry and rainy seasons) as a proxy for changes in flow regime. This population occurs in the upper Munim River basin, northeastern Brazil (GUIMARÃES et al. 2020), an urban protected area of scientific importance and yet underrepresented in the literature.

**Materials and Methods**

**Study area**

The specimens (GUIMARÃES et al. 2020, fig 3a) were collected in the Mata da Itamacaoca, a urban protected area, located at the Chapadinha municipality, Maranhão state, Northeastern Brazil (Fig. 1) (upper Munim River basin): (P1) stream near spring,

with riparian forest (3°44'45.20"S 43°19'15.10"W; ~80 m above sea level ; water temperature ~ 28.1°C; water PH~ 6.2). In the studied region they are very well - defined seasonal periods: rainy (January –June) and dry (July –December). In the site there was clear water, with moderate water flow (in the rainy season), and very weak water flow or even without flow, forming isolated pools along the river course (in the highest peak of the dry season), and with river bottom composed of sand, leaf, gravel and small rocks).

**Collection, morphometric measurements**

Specimens were collected quarterly during the daylight, in rainy season (January, March, and May) and in dry season (July, September, and November) beginning in the year 2015 to September 2019. Over all 1261 individuals were collected from P1, with 866 individuals collected in the dry season and 395 individuals collected in the rainy season. All the collected samples are deposited in the Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais (CICCAA) of the Universidade Federal do Maranhão. For this study, measurements were taken from 1.261 individuals of *N. beckfordi*, with sizes ranging from 10 to 28 mm (standard length). All individuals were measured with a digital calliper (0.01 mm accuracy). Morphometric characters follow Fink and Weitzman (1974), in which eight measures related to habitat use were analysed (Table 1).

**Data analysis.**

All analyses were performed in an R environment (R CORE TEAM, 20 20). Correlation between raw linear measurements were assessed using Kendalls Tau as data was non - normal (per QQ plots). As all response variables were non - normal, the geometric mean (GM) has been calculated and Mosimans correction for size has been applied to the data, Using the GM removes confounding effects of differences in SL of each measured fish and accounts for differing allometry of measurements (per NAGELKERKE et al. 2018). The non-parametric bootstrap resampling method was used to calculate confidence intervals, from the Hmisc package. A Principal Component Analysis (PCA) was completed on the scaled data using the correlation matrix to determine the ordination of *N. beckfordi* individuals in the morphological space sampled for different seasonal periods (i.e. dry and rainy). The first two axes of the PCA were retained for interpretation (GATZ 1979a, WATSON; BALON, 1984). A one-way ANOVA was completed in all the response variables to detect significant differences between seasons (GOTELLI; ELLISON, 2011).

**Results**

Dry and Rainy Season had a significant effect on all *N. beckfordi* linear measurements, except for AFL ($F_{1261}$= 3.731; p=0.053), DFL ($F_{1261}$=0.441; p=0.506) and PELVL ($F_{1261}$=0.03; p=0.856) (Tabel 2; Figure 2). Most variables correlate with each other significantly correlated and with SL, except BH and DFL, on the other hand, all variables significantly correlated with GM, without exception. This which suggests allometry (Fig 3a, b; Appendix 5). On PC1, there were strong positive loadings for GM, PECTL and DFL, except for CFL, CPH and SL that showed strong negative charges (Table 3). On PC2 had strong positive loadings for DFL and BH, and strong negative loading for AFL (Table 3). The PCA ordination shows that individuals separate along PC1 which is mainly associated with GM, PECTL, CPH and SL (Fig. 3a, b). Overall, individuals sampled in each season broadly overlap in morphology, however, the dry season individuals show a greater variation in morphological features than in the rainy season- likely due to both energy investment and reproduction timing. (Fig. 2, Fig. 3b). This indicates that in the rainy season fin lengths were generally longer than in the dry season but that body height did not contribute significantly to differences in variations in fish morphology between seasons (Table 2, Fig. 2, Fig. 3b).

**Discussion**

*Nannostomus beckfordi* exhibits some degree of phenotypic plasticity in linear morphology with regards to dry and rainy seasons and, by association, flow regime. This indicates that there are population level variations in adaptations to seasonally induced perturbations which allows population persistence in highly stochastic environments. Our results add to the growing literature on intraspecific phenotypic plasticity with regards to hydrological regimes. Specifically, the main findings were: 1) rainy season fish had longer SL; 2) when measurements were corrected with GM, dry season fish had a greater variability in morphology; 3) rainy season fish had longer fin lengths; but 4) there was no difference in DFL, AFL and PELVL between seasons nor correlation of BH with SL, mas all variables were significantly correlated with GM. We discuss these findings in terms of implications for *N. beckfordi* ecomorphology in the Munim River Basin.

Flow regimes can have an effect on fish body size either through driving different growth allometries or habitat selection (BRUCKERHOFF; MAGOULICK, 2017). Many Neotropical fish have spawning seasons associated with flood regimes (KRAMER, 1978; GODINHO et al. 2010). In the rainy season *N. beckfordi* were larger however, in the dry

season it appears that large individuals are still present but that smaller individuals were not present in the rainy season. This greater variation of length in the dry season suggests that *N. beckfordi* also has a spawning season peak coinciding with the rainy season, thus the dry season population is comprised of both juveniles and adults but with population stability throughout the year (NETTO- FERREIRA, 2018). Increased rain and higher water levels increases the coupling of aquatic and terrestrial zones (JUNK et al. 1989). *Nannostomus beckfordi* has a reproduction strategy which combines the Equilibrium Strategy with a Seasonal Strategy (NETTO- FERREIRA, 2018). These strategies are interpreted as an adaptation to different environmental regimes, such as drought, rain, resource availability, and predatory pressure (WINEMILLER, 2005). The equilibrium strategy allows the species to invest in individual offspring and parental care, adapt to resource- constrained environments, despite high predation and competition in the environment, which promotes population stability throughout the year. Instead, the seasonal strategy allows *N. beckfordi* adults to survive during the seasonal periods (increasing reproductive success) and with little food availability (e.g. dry season), also leading to population stability throughout the year. Therein, it seems appropriate to link the larger body sizes in the rainy season to ontogenetic development for seasonal spawning and increases in food availability promoting growth. Furthermore, *N. beckfordi* is a nektonic diurnal surface feeder (BREJÃO et al. 2013) which would benefit from increases in zooplankton abundance.

Polymorphism and plasticity in fish morphological traits is driven in some capacity by habitat (SENAY et al. 2014; SHUAI et al. 2018). Dry season river dynamics are less energetically taxing with regards to swimming than higher flow rainy season regimes. Smaller fins are more common in high flow regimes to reduce hydrodynamic drag (PLAUT, 2000), however the opposite was found in *N. beckfordi.* In the dry season *N. beckfordi* showed a greater variation in morphospace than the rainy season individuals, predominantly along PC1. This dry season related variation may be an evolutionary stable strategy of the species in response to uncertain or limiting resources (PLANK et al. 2016). It may also be considered that the plasticity seen on PC1 could be related to developmental differences. Similar interpretations were made by Bemvenuti and Rodrigues (2002) for the species *Odontesthes bonariensis* (Valenciennes, 1835) and *Odontesthes humensis* de Buen, 1953. Differences in fin ontogeny is seen in the zebrafish *Danio rerio* where the caudal, dorsal and anal fins and pectoral fins develop at different rates which alters muscle complexity development dependent on fish length (DANOS;

LAUDER, 2007). Yearly variation in dry season rainfall can affect fish development as exposure to high flow during development can induce shifts to more streamlined bodies (PERES-NETO; MAGNAN, 2004; DUNN et al. 2020).

Relative difference in trait variation between channel and lagoon populations of Neotropical fish is affected by the distance between sampling sites, i.e. population connectivity (LANGERHANS et al. 2003). Morphotypes of *N. beckfordi* found in the sampled site in dry and rainy seasons should not be considered as distinct but that the species is able to produce phenotypes suited to different hydrological regimes. As PC2 was characterised mainly by body height, with no difference between seasons, this suggests that *N. beckfordi* has no loss in fitness with regards to body height in both dry and rainy season. Flow regimes affect intraspecific phenotypic plasticity in a diversity of species, (for example: *Glossamia aprion* (Richardson 1842) (ABECIA et al. 2018); *Hyphessobrycon ericae* Moreira & Lima 2017 (BARROS et al. 2019), and various cyprinid species (ANDRES et al. 2019)), amongst others. Whereupon the prevailing trend is that slow flows are aligned with deeper body depth as well as deeper caudal peduncles and fins for better manoeuvring capacity and strong flows are aligned with fusiform bodies (VOGEL, 1994; BLAKE, 1983 COLGATE; LYNCH, 2004; LANGERHANS et al. 2003; LANGERHANS, 2008). Despite this, the deeper body and caudal peduncle expectation is not met in lake dwelling rainbow fish (MCGUIGAN et al. 2007). Nor was there a strong association with flow regime and caudal peduncle percent area in *H. ericae* (BARROS et al. 2019). *Nannostomus beckfordi* body height did not vary significantly between seasons as *N. beckfordi* is characterised by its slim shape. Nonetheless, although not included in this research, further influences of abiotic drivers on phenotype should be considered as factors (see review OUFIERO; WHITLOW, 2016).

*Nannostomus beckfordi* showed seasonally induced, and potentially ontogenetically induced, differences in linear morphology. Individuals were longer in the rainy season but had a higher variation in trait values in the dry season. The measurements taken in this study represent ecological relevant traits with regards to locomotion, especially swimming propulsion. We interpret this in relation to seasonal flow regimes and indeed, some differences were expected, and some expected differences were not detected in our sample. Information on the ecology of *N. beckfordi* is relatively depauperate in the literature. This is an issue with regards to conservation of small sized fishes but also in that *N. beckfordi* is an emerging invasive species (MAGALHÃES; JACOBI, 2008; MAGALHÃES et al. 2019). Plasticity in morphology is driven by a cohort of interacting

effects and should be considered further, and in tandem with other species in the community assemblage in order to understand how drivers interact (OUFIERO; WHITLOW, 2016).

## Conclusions

*Nannostomus beckfordi* individuals are longer in the rainy season compared to the dry season. Dry season population is likely comprised of fish of different ontogenies due to the larger variation in size ranges and morphology. These strategies favor success of the specie in in terms of ecomorphology related to seasons and flow regime.

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**Table 1.** Meansure list of *Nannostomus beckfordi.*

| | |
|---|---|
| Standard Length (SL) | From the anterior end of the muzzle to the base of the caudal fin. |
| Body height (BH) | From the origin of the base of the dorsal-fin to the base of the pelvic-fin. |
| Caudal peduncle height (CPH) | Minimum height measured in the caudal peduncle. |
| Caudal-fin length (CFL) | Length between the posterior region of the hypural bones to the tip of the largest ray of the caudal-fin |
| Dorsal-fin length (DFL) | Length from the base of the fin to the largest ray of the dorsal-fin. |
| Anal-fin length (AFL) | Length of the anal-fin from base to end of largest ray. |
| Pelvic-fin length (PELVL) | Length of the pelvic-fin from the base to the end of the largest ray. |
| Pectoral-fin length (PECTL) | Pength of the pectoral-fin from the base to the end of the largest radius. |

**Table 2.** Morphological measurements of *Nannostomus beckfordi* in dry and rainy seasons of the Mata da Itamacaoca. F-values and P-values determined from geometric mean measurements against seasons.  N=866 dry season and N=395 rainy season.

| Variables | Season | Mean ± SD (mm) | F | P |
|-----------|--------|----------------|------|-------|
| SL | Dry | 20.97±3.76 | 114.4 | <0.001 |
|    | Rainy | 24.14±2.45 | | |
| BH | Dry | 4.20±0.96 | 95.39 | <0.001 |
|    | Rainy | 5.04±0.65 | | |
| CPH | Dry | 1.92±0.34 | 19.47 | <0.001 |
|     | Rainy | 2.15±0.27 | | |
| CFL | Dry | 4.42±0.94 | 171.7 | <0.001 |
|     | Rainy | 5.27±0.72 | | |
| DFL | Dry | 4.18±0.98 | 0.441 | >0.506 |
|     | Rainy | 4.97±0.67 | | |
| AFL | Dry | 2.56±0.64 | 3.731 | >0.053 |
|     | Rainy | 3.06±0.65 | | |
| PELVL | Dry | 3.07±0.71 | 0.03 | >0.856 |
|       | Rainy | 3.66±0.48 | | |
| PECTL | Dry | 2.72±0.75 | 4.981 | <0.025 |
|       | Rainy | 3.41±0.59 | | |

**Table 3.** Principle component scores of the eight morphological measurements explained by the first two axes of the PCA.

| Variables | (PC1) | (PC2) |
|-----------|-------|-------|
| BH | 0.24 | 0.55 |
| SL | -0.84 | 0.15 |
| GM | 0.89 | -0.05 |
| CPH | -0.84 | 0.06 |
| CFL | -0.16 | -0.11 |
| DFL | 0.25 | 0.55 |
| AFL | 0.11 | -0.88 |
| PELVL | 0.21 | 0.14 |
| PECTL | 0.67 | 0.02 |
| **%Variation** | 31.7% | 16% |
| **Eigenvalue** | 2.86 | 1.44 |

**Figure 1.** Map of the studied area.
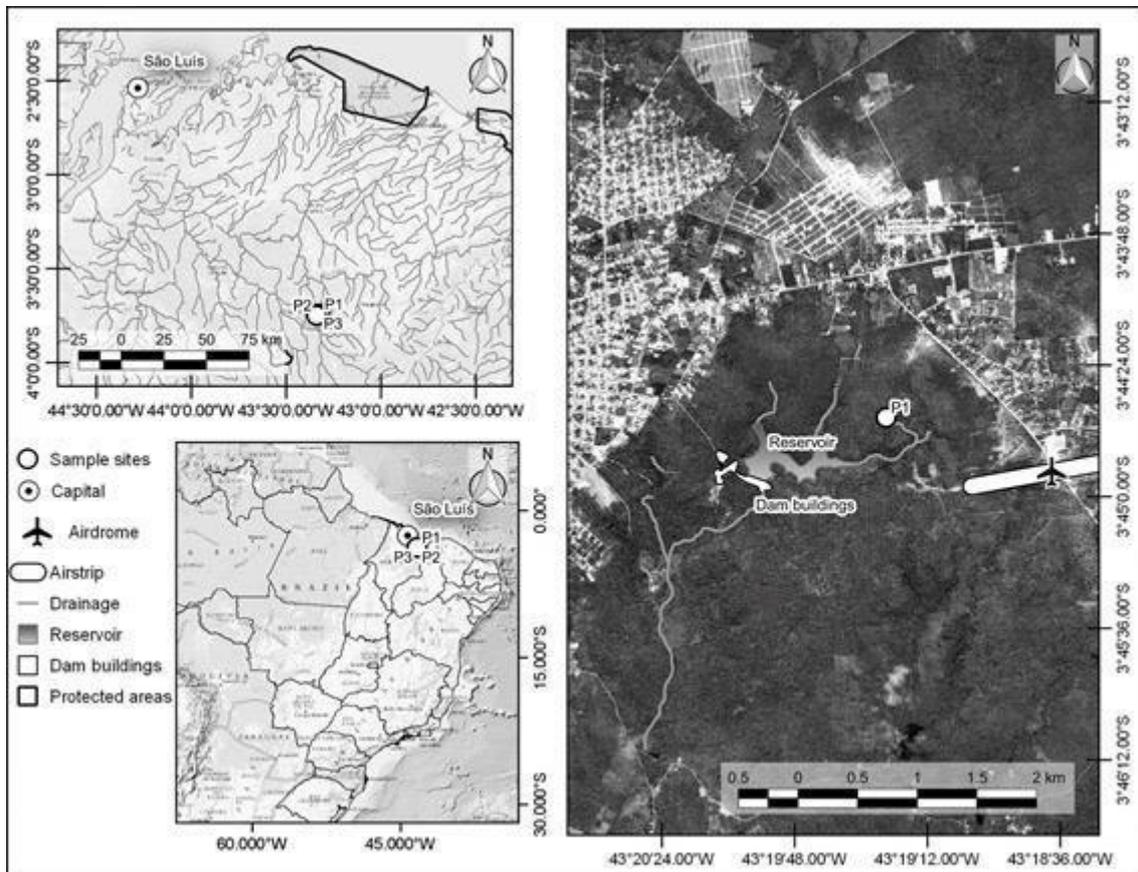
**Figure 2.** Natural log of all morphological measurements taken for *Nannostomus beckfordi* individuals in the rainy and dry seasons. Boxplots indicate median and interquartile ranges, —— indicates mean, and points indicate raw data values.
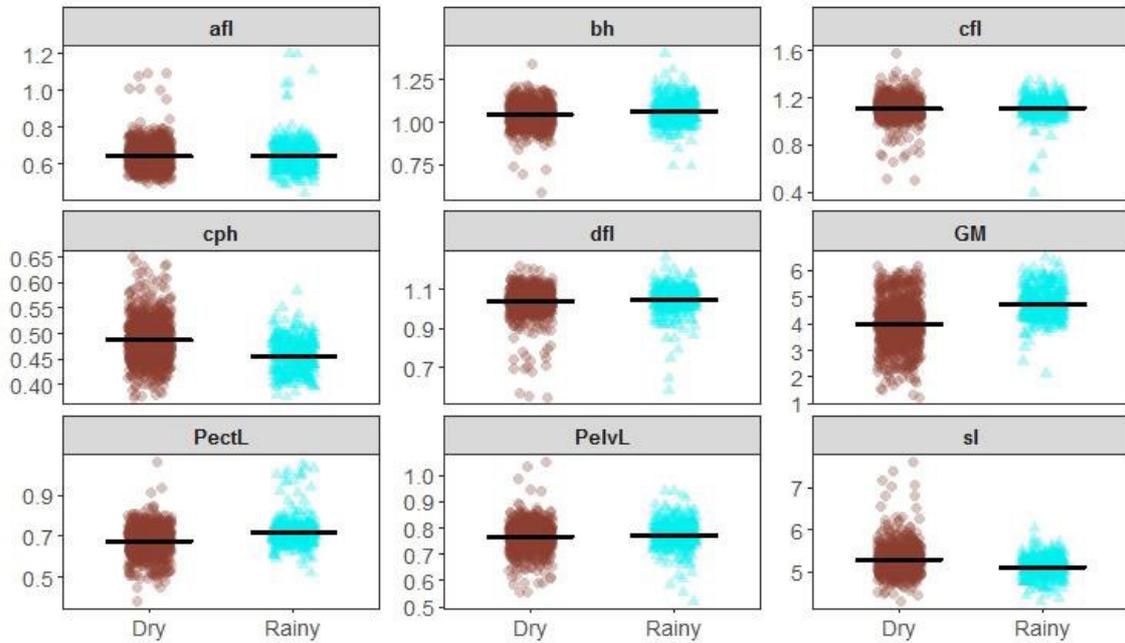


**Figure 3.** a) PCA ordination of *N. beckfordi* individuals in dry (brown) and rainy (blue) season, centroids are indicated by large points. b) Biplot of a principle component analysis (PCA) of morphological characters of *Nannostomus beckfordi*.
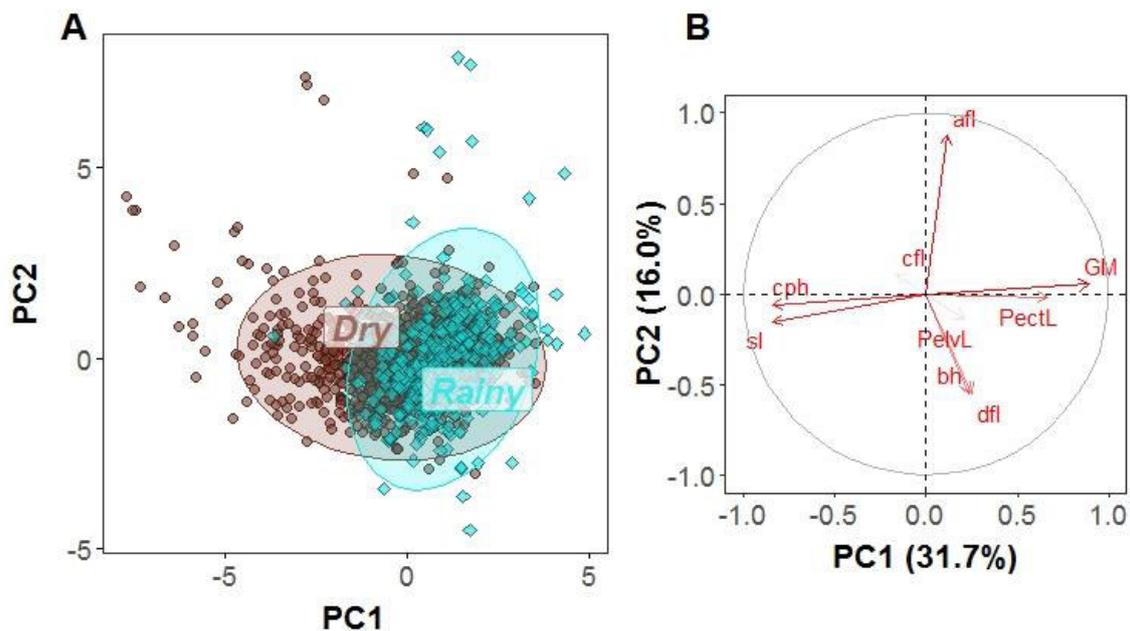
**CAPÍTULO V**

**ECOMORPHOLOGICAL ANALYSIS OF THE FISHES COMMUNITY OF
MATA DE ITAMACAOCA, UPPER MUNIM RIVER BASIN, BRAZIL**

ANÁLISE ECOMORFOLÓGICA DA COMUNIDADE DE PEIXES DE MATA DE
ITAMACAOCA, BACIA DO ALTO RIO MUNIM, BRASIL

## Ecomorphological analysis of the fishes community of Mata de Itamacaoca, upper Munim River Basin, Brazil

**Abstract.** The relationships between morphology, trophic ecology and the use of food resources of ichthyofauna of Mata de Itamacaoca, upper Munim River basin were evaluated. Sampling was performed quarterly of August 2014 to February 2020 in five sampling points of the Mata de Itamacaoca. The stomach contents of 278 individuals belonging to 19 species was analyzed using the volumetric method and a series of diet indices. Fourteen functional feeding traits were measured using electronic calipers and an optical microscope. For the analyses, the community was broken into Family groups to assess phylogenetically similar species niche partitioning, as due to phylogenetic niche conservatism there ought to be a higher degree of overlap, then, the community was broken between the most abundant species of each representative Family to assess niche partitioning amongst species that are less closely related and to get an overview of whole community trophic niche. The food-fish model (FFM) was performed to provide the trophic profile (TP) of each fish species. Generalized Linear Models (GLMs) were used to evaluate changes in die metrics according to the station and location of the dam wall. Non-metric multidimensional scaling (nMDS) were used visualise the differences in gut contents of the fish community and a one-way PERMANOVA was used for whether environmental factors (i.e. season) affected diet. Principal Component Analysis (PCA) was performed on standardized feeding data to compare general trophic morphology, and to predict TPs from morphology. Dietary overlaps between species were high in the dry season and moderate in the rainy season. The fish community consumed terrestrial and aquatic insects in the rainy season, already in the dry season the species of fish also began to feed on of alternative resources, such as plants and seeds. So, the species have changed their diet according to with the station and site of the dam. Morphological variations together with resource availability, determined the dietary differences, as well as the trophic profile between species, which suggests that ecomorphological variations and food availability in the environment are the main mechanisms responsible for trophic overlap and coexistence of fish species of the Mata de Itamacaoca.

**Keywords:** Biodiversity, Diet, Munim River Basin, Morphology.

## INTRODUCTION

The niche partitioning is a mechanism which allows species to coexist in sympatry, and it is evident that phylogenetically related species exhibit niche conservatism (PERES-NETO, 2004; GARCIA et al. 2020). This because, over evolutionary time, these species evolved in the same way, and interspecific competition allowed for displacements of character, facilitating the distribution of resources between species (WIKRAMANAYAKE, 1990). Therefore, these species can coexist and exhibit morphological and ecological segregation. Trophic ecology is considered a strong driver of morphological adaptations in fish, with changes to new behaviors and food strategies, with prey exerting selective pressures on trophic morphology and efficiency in capturing

prey (BENNEMANN; GALVES; CAPRA, 2011; EVANS et al. 2019; GARCIA et al., 2020). These morphological diversifications in fish are useful tool to link the shape of morphological structures with the ecological niche of a species, allowing us to trace the functional role of an organism within a community (EVANS et. al. 2019; BALDASSO et al. 2019). Assessing explicit ecomorphological traits can allow inferences regarding the capacity of a species to exploit a suite of resources (TEIXEIRA; BENNEMANN, 2007; BALDASSO et al. 2019).

Niche differentiation can be characterized by patterns of sizes combined with the different morphological attributes of an individual (SILVA-CAMACHO et al., 2014; NEVES et al., 2015; BALDASSO et al., 2019; GARCIA et al., 2020). In addition, some morphological traits relate to habitat and hydrodynamic aspects, and can be inferred by the relationship between the body shape with the with characteristics of habitat, by the ratio of weight to length, or the combination of morphological characteristics: height and width of the mouth and types of teething with feeding among others (TEIXEIRA; BENNEMANN, 2007; BALDASSO et al., 2019; GARCIA et al., 2020). Therefore, much of the fish that inhabit Neotropical region can alter their diet along of your development biotope changes, such factor propitiates both changes in patterns morphological, as in the function and structure of ichthyophaunistic communities, and believed that in Neotropical streams there is the sharing of resources, such a pattern can facilitates the coexistence of the species in this region (ROSS, 1986; DAUWALTER et al. 2008; AMBROSIO et al. 2008; SILVA-CAMACHO et al. 2014; GARCIA et al. 2020). The coexistence of several species in fish communities of the Neotropical region can be facilitated by morphological divergence which is a mechanism which facilitated niche partitioning of habitat and food resources (SILVA-CAMACHO et al. 2014; GARCIA et al. 2020).

Trophic interactions structure community composition in freshwater environments, however, the strength and direction of these can vary with environmental context (BENNEMANN; GALVES; CAPRA, 2011; SANTOS et al. 2011). Determining consumer-resource interactions and the differences between consumers can help to explain co-existence patterns and trends in biodiversity (SILVA-CAMACHO et al. 2014; GARCIA et al. 2020). For example, diet data helps to identify keystone predators and ontogenetic shifts in resource consumption which is important for conservation (FAYE et al. 2012; SILVA-CAMACHO et al. 2014). Patterns in resource consumption usually reflect temporal patterns of resource abundance and as such gut content data is a snapshot of what a species is eating at that given moment in time (KRIVAN; DIEHL, 2005;

BALDASSO et al. 2019; GARCIA et al. 2020). This spatial and temporal variation in food quality and supply due to the effects of seasonality is a strong driver of selective pressures on species inhabiting limited environments and with little flow especially in streams (NEVES; DELARIVA; WOLFF, 2015). Fish species inhabiting these aquatic systems need to specialize in exploiting a particular resource during a period of seasonal variation (e.g., lepidophagy), or adapt to explore a broad ecological niche (e.g., generalist) to continue to feeding and maintain their positive energy expenditure (EVANS et al. 2019). Food diversification in the environment can promote a series of individual specializations in the use of resources (ARAÚJO et al. 2010; ARAÚJO et al. 2011), and can be exploited at different stages of fish development (ARAÚJO et al. 2010). In this sense, species of fish considered generalist could present specialist individuals at some stage of its development (ARAÚJO et al. 2010), increasing inter-individual variations and expanding the trophic niche of their populations. However, if two generalist species are interacting in a stable environment, they may coexist as a result of niche differentiation, but if a generalist species and a specialist species interact, they end up exploiting the niche specialist, resulting in a decline if the resource isnt abundant enough (ROUGHGARDEN, 1974).

Trophic interactions may be correlated with apparent contributions of allochthonous and autochthonous materials that streams and their surroundings provide to fish communities (FERREIRA et al. 2011). However, anthropogenic actions have strongly impacted aquatic environments around the world (COLLEN et al. 2014), causing water pollution, deforestation of vegetation, and obstruction of water flow amongst other stressors. The sum of all these factors cause both quantity and variety food to decrease (BALDASSO et al. 2019). Therefore, studies that contribute to the knowledge of food ecology of fish present a limitation for streams in pristine areas (CASSATI, 2002). As a consequence, it can be difficult to infer trophic relationships or even morphological reliable in creek ichthyofauna based on the natural availability of food resources, if these predictions are based only on observations of fish fauna in impacted streams (BALDASSO et al. 2019).

The Munim River basin (~16.000 km2) is one of the main hydrological units in the state of Maranhão, Brazil (Hereafter Mrn) (RIBEIRO et al. 2014). Despite its hydrological importance, as well as most of the river channels of the State of Maranhão, are constantly threatened by anthropogenic disturbances. Specifically, deforestation of riparian forests, water pollution and contamination, occurrences of erosion processes

intensified by human activities and the capture and fragmentation of watercourses (SILVA et al. 2008, LIMA et al. 2009; RIBEIRO et al. 2014). These impacts are likely to be significant for regional biodiversity (PELICICE et al. 2017). Although it is now difficult to find pristine streams, Mata de Itamacaoca, upper Munim River basin, Northern Brazilian Cerrado, seems to have conserved its local biodiversity well, especially fish. The Mata de Itamacaoca, is a urban protected area of Mrn, with 23 species native and endemic fish species from the Hydrological Units of Maranhão and Parnaíba (OLIVEIRA et al. 2020). This area within an urban protection area, which is more exposed to human impacts than other protected areas, as a reservoir was constructed help to preserve some of the spring streams that feed into the upper Munim River basin and help supply the city of Chapadinha-MA with drinking water (SILVA et al. 2008; OLIVEIRA et al. 2020). Previous work shows that despite the reservoir there is similar species richness and diversity above and below the dam but there are distinct differences in community composition (OLIVEIRA et al. 2020). The community composition is also influenced by seasonality (OLIVEIRA et al. 2020).

In pristine streams like those in Mata de Itamacaoca it is possible make predictions about the relations between terrestrial arthropods community with the fish community, since ichthyofauna depends on allochthonous resources to continue feeding and keep your energy expenditure positive (CARVALHO et al. 2017). Additionally, environmental heterogeneity and structure of the habitat in pristine streams, together with the input of organic material, are responsible for the great diversification of aquatic invertebrates in them, as well as, the supply of resources, since fish communities depend on allochthonous resources (UIEDA; ALVES; SILVA, 2016; CARVALHO et al. 2017). The availability of food resources can intensify the trophic spectra of the species of fish (MAZZONI et al. 2012), and define the degree of trophic amplitude of the guilds (WOLFF; CARNIATTO; HAHN, 2013). In addition, the variety of food resources in the environment, can promote a range of specializations in fish species for different types of categories or food items, reducing trophic overlap between fish (DIAS et al. 2017).

Morphology and diet composition of a species are related, this correlation can be used to determine the trophic niche of the species (OLIVEIRA et al. 2010; NEVES et al. 2015). When these correlations are found, it is assumed that morphologically similar species use similar food categories or items, therefore, should share them to coexist (ROSS, 1986; WOLFF et al. 2013; MAZZONI et al. 2012). In other words, morphology-diet correlations can be interpreted as a result of the numerous selections imposed by the

environment for a species to improve its ability to obtain certain food categories and continue to exist (WAINWRIGHT; REILLY, 1994). To test these expectations, our objectives in this study were to: describe and analyze intra- and inter-specific variations of dietary of fishes in terms of their trophic amplitude and overlap, as well as assessing ecomorphological variations and trophic profiles of fish community of Mata de Itamacaoca, upper Munim River basin, especially regarding the small-sized, rare, and more ecologically demanding species.

## Material and Methods

### Study area

This study was conducted in the Mata de Itamacaoca, an urban protected area of 460 hectares belonging to CAEMA (Companhia de Saneamento Ambiental do Maranhão) and recongnizes as an Area of Relevant Ecological Interest by the Decreto Municipal Nº 05/2018. It is located within the Municipality of Chapadinha, State of Maranhão (24°25'47" S, 58°44'05" W), and is approximately 90 meters above sea level. The predominant biome in the region is the Brazilian Cerrado (Figure 1). This area is formatted for Trees reaching more than 10 meters in height, formeding a mosaic of plant, and are associated with watercourses and streams in this area (SILVA et al. 2008).

### Field sampling

The fish fauna was sampled quarterly from August 2014 to February 2020 at five sample sites (C1-C5) of Mata de Itamacaoca, upper Munim River basin, comprising springs, streams, pools, and a reservoir (Figure 1). All The collection points (C1 to C5) are described in Oliveira et al. 2020 (See, OLIVEIRA et al. 2020, fig. 1). The equipment from consisted of manual trail-net (2 m long × 1.8 m high; mesh size, 2 mm), cast nets (2 m height, mesh size 15 mm), gillnets of various mesh sizes (15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 100 mm), and dip nets (mesh size 5 and 10 mm).The specimens collected for morphological analysis were fixed in formalin and left for 15 days, after which they were preserved in 70% ethanol. The fishes were identified according to Oliveira et al. (2020) for each taxonomic group, and were gutted to remove the gastrointestinal tracts, which were then preserved in 70% ethanol. Voucher specimens were deposited in the Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais of the Universidade Federal do Maranhão (CICCAA).

**Laboratory analysis**

Fourteen functional feeding traits were measured, whenever possible, in twenty indviduals, of the dry season and twenty of the rainy season of each species using electronic calipers and an optical microscope. At the end, 514 individuals of nineteen species were measured (Table 1). All measurements followed Sibbing and Nagelkerke (2001) and Teixeira and Bennemann (2007), except for fishes Cichlidae families that followed Kullander (1986). The metric measurements were dimensioned by dividing them by the standard length (SL) (Tabel 2). Then, twenty individuals of each species (ten of the rainy season; and ten of the dry season) had their stomachs removed with a scalpel for the quantification and qualification from the stomach contents. Food items were identified to the insects order level using an optical microscope along with identification keys for macroinvertebrates (LEHMKUHL, 1976; MUGNAI et al. 2010) and immature insects (Chu, 1949) and their volume were then quantified using the volumetric method (HELLAWEL; ABEL, 1971; HYSLOP1980). Sludge debris and sediment were combined in UI sludge; and fish remains, bones and scales were combined in UI teleost for decrease the number of trophic variety and facilitate statistical analyses. This method expresses the proportion in volume each food item of all food categories found in the samples in millimetre glass plates.

**Data analysis**

Metrics were calculated to determine the Frequency of Occurrence (%FOI), Prey Abundance (%Ni) and Percentage Volume (%V) of each prey category for each fish species (HYSLOP, 1980). A modification of Pinkas' index of relative importance for assessed the importance of prey in fish dietary (PINKAS et al. 1971):

%IRI = (%N + %V) × %F

Shannon-Weiner Index of Diversity (SHANNON, 1948) was calculated for each species gut contents, with relevance to season and dam to assess abiotic drivers of change in dietary composition. This was completed using the raw occurrence of items (NI) in the package 'vegan' version 2.5-5 (OKSANEN, et al. 2019). This index describes the entropy of a given community:

$$(H) = \sum_{i=1}^{s} p_i \ln p_i$$

Where H is the Shannon diversity index, S is the total number of species in the community, Pi is the proportion of S made up of the ith species. GLMs were completed on the H value per species using the same terms as above to assess change in gut content species (item) diversity with regards to season and dam locality. To describe the interactions in H and Sprich between species, season and dam, a network was built only using species present across both seasons and dam locations. The community was broken into Family groups to assess phylogenetically similar species niche partitioning, as due to phylogenetic niche conservatism there ought to be a higher degree of overlap, then, the community was broken between the most abundant species of each representative Family to assess niche partitioning amongst species that are less closely related and to get an overview of whole community trophic niche.

The model Food-Fish (FFM), as described in Nagelkerke (2018), was used to predict what types of aquatic prey can be potentially be exploited by each species of fish. For these predictions, positive and negative values are first assigned (ranging from 2 to -2) for each morphological trait, according to their trophic capacity. These values form the Food Specialist Profiles (FSP). The values FSP were Correlated (Kendall's tau correlation) with the measurements of each fish, obtaining the correlation coefficient which is defined as trophic profile (TP). Before the correlation, the values were standardized (subtracting the average value of each variable and dividing by the standard deviation), resulting in an average value of zero and a standard deviation of one for each variable, thus giving equal weight to all variables (NAGELKERKE et al. 2018). Principal Component Analysis (PCA) was performed on standardized feeding data to compare general trophic morphology, and also in TPs, for the general interpretation of trophic capacities. This allowed for prediction of possible trophic niche and comparison with the actual trophic niche and subsequent overlaps between the species groups. All statistics were performed in an R environment (R CORE TEAM, 2018).

Dietary niche and dietary overlap of the eight most abundant species from each family [(*Anablepsoides vieirai* Nelson, 2016, *Apistogramma piauiensis* Kullander, 1980, *Characidium* sp., *Curimatopsis* aff. *cryptica*, *Hemigrammus* sp.1, *Hoplias malabaricus* (Bloch, 1794), *Megalechis thoracata* (Valenciennes, 1840) and *Nannostomus beckfordi* Günther, 1872)] and familys most abundant (Characiformes and Cichliformes) and

associations regarding season, were visualized using multidimensional non-metricscaling (nMDS) ordination via 'vegan'. A one-way PERMANOVA using Bray-Curtis non-metric similarity and 999 permutations was then used to test for significant effects of environmental factors (species, dam locality, seasons) on fish species gut contents. Community data was square-root transformed and Wisconsin double standardization was applied (vegan::metaMDS). Ordination stress was used to assess whether a two-dimensional ordination biplot was suitable to represent gut contents community data variation. Stress values < 0.15 were considered appropriate (QUINN; KEOUGH, 2002, COUSINS et al. 2017). This allowed visualisation of the trophic niche of each species in each tested group and assessment of whether this was affected by other variables.

**Results**

**Gut content metrics and interactions with abiotic variables**

The fish community of Mata de Itamacaoca have a greater diversification of the dietary in dry season than at the rainy season (Figure 2; Figure 3). Among the items of greatest occurrence in overall diet are aquatic insects, terrestrial insects, plant material and remains of fish in points below of dam (Figure 2). In points above the dam, flying and terrestrial insects were more predominant (Figure 2). Of terrestrial insects, the order Coleoptera showed a higher %IRI value, of flying insects dipterous larva showed a higher %IRI value, at both points (above, below) and seasons (dry, rainy) (Figure 4). There was significant interaction interaction effect in H between species and season (*Anablepsoides vieirai - Apistogramma piauiensis* z= 3.86, p=0.001; *Anablepsoides vieirai - Crenicichla brasiliensis* (Bloch, 1792) z= 2.20, p= 0.02; *Anablepsoides vieirai - Hoplias malabaricus* z= 2.02, p=0.04; *Apistogramma piauiensis - Hemigrammus* sp. 1 z= -3.41, p= 0.001; *Apistogramma piauiensis - Hyphessobrycon piorskii* Guimarães, Brito, Feitosa & Ottoni, 2018 z= -2.20, p= 0.02; *Apistogramma piauiensis - Nannostomus beckfordi* z= -3.11, p= 0.001), dam has no main effect, whereupon, sites below the dam wall had higher H in both dry and rainy seasons compared to above the dam wall (Figure 4). Sprich showed that is no dam effect, but in the gut contentes species richness is significantly higher in the rainy season compared to the dry season but this isn't affected by the dam wall (*Anablepsoides vieirai - Apistogramma piauiensis* z= 3.40, p=0.001; *Anablepsoides vieirai - Crenicichla brasiliensis* z= 3.27, p=0.001; *Anablepsoides vieirai - Hoplias malabaricus* z= 2.36, p=0.01; *Apistogramma piauiensis - Hemigrammus* sp.1 z= -2.93, p= 0.001; *Apistogramma piauiensis - Nannostomus beckfordi* z= -2.19, p=0.02;

*Crenicichla brasiliensis - Hemigrammus* sp.1 z= -2.80, p=0.005; *Crenicichla brasiliensis - Nannostomus beckfordi,* z= -2.07, p= 0.03) (Tabel 3, Figure 5, figure 6).


**Dietary overlap and trophic profiles of representative species**

The NMDS showed that the communities of prey for eight species fish which represent the most abundant species in each family present in the study area have broad dietary overlaps. *Curimatopsis* aff. *cryptica* stands out for being a specialist-consumer of plant matter. *Nannostomus beckfordi* Günther, 1872 appears to be a generalist-consumer as its niche is spread broadly across both dimensions. *Anablepsoides vieirai* Nelson, 2016 overlaps in niche with all other species considered here and appears to be a specialist on Coleoptera. *Hoplias malabaricus* (Bloch, 1794) is a generalist with a broad dietary niche, however, it is able to exploit a more carnivorous diet due to occurrences of tadpoles and juvenile fishes which the others do not (Figure 7). The dietary niche in the dry season is more broad, while that in the rainy season the food niche is moderate (Figure 8).

The PERMANOVA showed that species interacts significantly with season on diet niche, contributing to 39% of the variance explained ($F_{132}$ =1.36, $R^2$=0.39, p<0.05). The seasons (Dry and Rainy) has a significant main effect and was responsible for 1% of the variance ($F_{132}$ = 1.96, $R^2$=0.01, p<0.05). Species has a significant main effect with 26% variance explained ($F_{132}$=6.60, $R^2$=0.26, p<0.001).

The PCA of the morphological variables explained 82.1% of variance along the first and second axes (Figure 9 a, b). There is a morphological separation between the most abundant species, with *Hoplias malabaricus* separating across both PC1 and PC2. Here, oral gape and body area were clearly larger and more dorso-laterally flattened, but there was high morphological variation (Figure 9 a, b). The others specimens (*Anablepsoides vieirai, Apistogramma piauiensis* Kullander, 1980, *Characidium* sp., *Curimatopsis* aff. *cryptica, Hemigrammus* sp1., *Megalechis thoracata* (Valenciennes, 1840), *Nannostomus beckfordi*) generally overlap in morphology as all have small body depth. The PCA of TPs explained 78.4% of total variation explained along the first two axes.

In concordance with the NMDS niche profile, *Hoplias malabaricus* was predicted to be a more specialised predatory in fish pursuit (Figure 9 c, d). The others specimens (*Anablepsoides vieirai, Apistogramma piauiensis, Characidium* sp., *Curimatopsis* aff. *cryptica, Hemigrammus* sp1., *Megalechis thoracata, Nannostomus beckfordi*) exploit a wide variety of smaller-bodied prey types(Figure 9 c, d). TPs were significantly different

between species for all prey types (Kruskall wallis test, P<0.05). In general, the most abundant species have a relatively large capacity to feed on sessile algae and detritus/substratum. *Anablepsoides vieirai* is more suitable for phytoplankton pump and zooplankton townet, but a less suitable for larvae/worms and molluscs. *Characidium* sp. is more suitable for zooplankton townet and less suitable for fish pursuit. *Curimatopsis* aff. *cryptica* is less suitable for fish pursuit, but with a greater capacity to feed of larvae/worms. *Megalechis thoracata* is less suitable for macro-crustaceans, larvae/worms and molluscs, but suitable for phytoplankton pump. *Nannostomus beckfordi* is less suitable for fish pursuit, but showed a greater capacity for feeding of macrophytes, zooplankton townet and larvae/worms (Figure 10).

The NMDS showed a clear separation of niche in Characiformes species (Figure 11). But the vast majority of which are insectivorous, feeding mainly on flying insects/larvae and terrestrial arthropods, as the species *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa & Ottoni, 2018 and *Knodus victoriae* (Steindachner, 1907), with a well-diversified diet but the niche of *Hyphessobrycon piorskii* does not include plants or algal matter (Figure 11). On the other hand, *Hemigrammus* sp.1 is a specialist-consumer, feeding mainly on remains of plants and seeds (Figure 11). The PERMANOVA showed that the species of Characiformes does not interact season ($F_{72}$ = 1.20, $R^2$=0.01, p=0.29) with 1% variance (Figure 12), nor with the dam ($F_{72}$ = 0.55, $R^2$=0.00, p=0.84) with 1% variance. There was a significant main effect of Characiformes species with 19% of variance ($F_{72}$ = 3.99, $R^2$=0.19, p<0.001; (Figure 11).

There is wide overlapping of morphospace between the Characiformes, but *Astyanax* cf. *bimaculatus* and *Moenkhausia oligolepis* (Günther, 1864) are separated from the other species along PC1, showing that they are morphologically larger than the other species of Characiformes (Figure 13 a, b). The PCA of the morphological variables explained 79.5% of variance along the first and second axes. Operculum depth, caudal peduncle depth and gill arch resistance were clearly larger in *Astyanax* cf. *bimaculatus* and *Moenkhausia oligolepis* (Figure 13 a, b). The PCA of TPs explained 76.4% of the total variation explained along the first two axes (Figure 13 c, d), revealed that *M. oligolepis* is predicted to be a more specialised pursuit hunter, while *Knodus victoriae, Hemigrammus* sp1, *Astyanax.* cf. *bimaculatus* and *Hyphessobrycon piorskii*, are best equipped for the exploitation of a wide variety of prey and have broad possible niches, although there is considerable trophic overlap between them (Figure 13 c, d). TPs were significantly different between species for all prey types (Kruskall wallis test, P<0.05).

The five species of Characiformes showed a relatively large capacity to feed on larvae/worms and seeds. But *Astyanax* cf. *bimaculatus* is less suitable for macro-crustaceans, but with a greater capacity to feed of zooplankton pump. *Hemigrammus* sp.1 is less suitable for sessile algae, but with a greater capacity to feed of zooplankton townet. *Hyphessobrycon piorskii* with less capacity for zooplankton pump, but with a greater capacity to feed of macrophytes and zooplankton townet. *Knodus victoriae* is less suitable for fish pursuit and suitable for phytoplankton pump. *Moenkhausia oligolepis* is less suitable for fish pursuit, but with a greater trophic capacity for detritus/substratum. (Figure 14).

The NMDS showed that there is a broader dietary niche in the dry season for Cichliformes and all species overlap in feeding (Figure 15), however, The diet of *Crenicichla brasiliensis* is largely the same in both seasons, *Cichlasoma* cf. *zarskei* changes your diet consume more plant matter, seeds and dipterans in the dry season and but more dipteran larvae in the rainy season. *Crenicichla brasiliensis* (Bloch, 1792) has a smaller niche than the other two, but a generalist specie and *Apistogramma piauiensis* only feeds on hydracarina in the dry season, and in the rainy season is dominated by flying and terrestrial insects (Figure 16). The PERMANOVA showed that the species interacts significantly with season on the diet niche of the Cichlidae, contributing to 7% of the variance explained ($F_{57} = 2.27$, $R^2=0.07$, p<0.001). The seasons (Dry and Rainy) has a significant main effect and was responsible for 3% of the variance ($F_{57} = 2.05$, $R^2=0.03$, p<0.01). Species has a significant main effect with 9% variance explained ($F_{57} = 3.20$, $R^2 =0.09$, p<0.001).

The PCA of the morphological variables explained 79.5% of variance along the first and second axes (Figure 17 a, b). There was morphological separation between the Cichlids, with *Apistogramma piauiensis* separated from the others species along PC1 (Figure 17 a, b) and showed a shorter head and the others species have a longer head and oral gape more width and body more depth, so *Cichlasoma* cf. *zarskei* and *Crenicichla brasiliensis* overlap in terms of gape traits and caudal peduncle but *Cichlasoma* cf. *zarskei* shows a wide variation in morphology and seems ot be driven by *Apistogramma piauiensis* (Figure 17 a, b). The first two axes of the TP PCA explained 85.1% of the total variation (Figure 17 c, d). The TPs show that each cichlid species has a clear predicted trophic niche. *Crenicihla brasiliensis* has a morphology for a specialised predatory diet as it has traits for fish ambush/ pursuit and strong swimming ability. *Ciclasoma* cf. *zarskei* is a generalist and *Apistogramma piauiensis* was predicted to be a bentophage. These

latter two were characterized as having relatively depth caudal peduncle, wider bodies and relatively large head (Figure 17 c, d). TPs were significantly different between species for all prey types (Kruskall wallis test, P\0.05), The three species of Cichlids showed a relatively large capacity to feed on sessile algae and zooplankton townet, but *Apistogramma piauiensis* is less suitable for phytoplankton pump and zooplankton pumps, but with a greater capacity to feed of molluscs and macro-crustaceans. *Cichlasoma* cf. *zarskei* is less suitable for macro-insects and fish ambush, but with a greater capacity to feed of detritus/substratum and zooplankton pump. *Crenicichla brasiliensis* is less suitable for molluscs, but with a greater capacity to feed of zooplankton pump, phytoplankton pump, macro-insects and fish ambush/pursuit. (Figure 18).

**Discussion**

Coexistence in many fish communities is related to morphological (i.e. niche separation) and spatial segregation (i.e. habitat filtering) (SAMPAIO et al. 2013). In aquatic environments with small volume of water, communities are subject to greater abiotic interference and these places tend to generate stochastic communities, this can influence community structure and play a role in species distribution and organization of community (WINEMILLER, 1996; SAMPAIO et al., 2013). Predicting and understanding how functional traits and abundance of resources interact to enable coexistence of multiple similar species in biodiverse environments is a key challenge in conservation. This is especially important to determine prior to anthropogenic disturbances so that community responses can be predicted. In Neotropical systems with an abundance of small and medium-sized fish species, these threat are intensified because their habitats are often of small size and with limited dispersal capacity which makes these environments more sensitive perturbation (ARTHINGTON et al. 2016; CASTRO; POLAZ, 2020). Ecological and biological studies of these small and medium-sized fish species, especially in the Neotropical region, should be considered a study priority before they are extirpated (GELLER et al. 2020). We demonstrate here, that ecomorphological analysis can be used to predict trophic niches and overlaps in a small fish community, but that seasonal and species specific variation in diet occurs probably as a result of changes in resource abundance. All NMDs showed differences in community trophic niche in dry and rainy season, however this not significantly different for the Characiformes group. The dry season caused a broader trophic niche when considering the Cichliformes group and the most abundant representative species group. This is likely a result of decreasing

abundance of resources in the dry season which increases intra and interspecific competition and forces individual niche breadth to widen.

The species diversity (H) increases in the rainy season because of higher diversity and abundance of prey available. In the dry season the niche exploitled (see nMDS) is wider because the prey are likely less abundance in the dry season so that the large niche overlaps between the species are constrained by resouce availability. In the rainy season theres a higher abundance of the prey and more species present to consume which decreases competition. These results suggest that there is an occurrence of food niche partition in the seasons in our study site; therefore, species change their diet according to the season and location of the dam. A wide variety of allochthonous and autochthonous resources were detected in the diet of all ichthyofauna. This is likely strongly associated with the undisturbed condition of the Mata de Itamacaoca. The abundant presence of those resources, such as plants (leaves, seeds) and terrestrial insects (Coleoptera, Hymenoptera), indicates that the Mata de Itamacaoca still has a certain degree of environmental integrity which supports the entire aquatic ecosystem. These patterns are reported in other scientific studies, where unspoiled vegetation has a strong influence on the availability of resources for the ichthyofauna (CASSATI, 2010; LEITE et al. 2015; DALA- CORTE et al. 2016; BALDASSO et al. 2019). This suggests that if the riparian environment is degraded then there will be a loss of resource input which may change the current niche overlaps of the representative fish communities, this is especially concerning for fish which appear to have a small niche now and are specialists.

Ecomorphology has proven to be insightful in predicting of resource use by fishes and which strategies are used as adaptations to environmental conditions (PERES-NETO, 2004). In this study, our results corroborate that different sizes and shapes of the hulls indicate which ones are specialist or generalist. The data from the gut contents broadly matched the predictions from the TPs, this is evidently in *Hoplias malabaricus* that is clearly larger with back laterally flattened is predicted to be a specialised predatory in others fishs diet due to occurrences of tadpoles and juvenile fishes in your feeding. *Anablepsoides vieirai, Apistogramma piauiensis, Characidium* sp., *Curimatopsis* aff. *cryptica, Hemigrammus* sp1*., Megalechis thoracata, Nannostomus beckfordi* were overlap in morphology, as all have small body depth, but with lots of shapes body and exploit a wide variety of prey, as: phytoplankton pump, zooplankton townet, larvae/worms, macrophytes among others. In Characiformes there is a morphological overlapping *Moenkhausia oligolepis* is less suitable for fish pursuit, but with a greater

trophic capacity for detritus/substratum, while *Knodus victoriae, Hemigrammus.* sp1, *Astyanax* cf. *bimaculatus* and *Hyphessobrycon piorskii,* are best equipped for the exploitation of a wide variety of prey. In Ciclídeos There is a morphological separateding *Crenicichla brasiliensis* with specialised predatory diet in fish ambush/ pursuit and with strong swimming ability, *Cichlasoma* cf. *zarskei* generalist and *Apistogramma piauiensis* bentophage.

Our results indicate that medium-sized fish (*H. malabaricus* and *C. brasiliensis*) from the MRN fish community have the capacity to be predatory piscivorous fish. This is due to their ecomorphological characteristics which facilitate active pursuit and a large oral gape. Such characteristics also explain the large distribution of occurrences this species in the studied area, being found above and below the dam as pointed out by Oliveira et al. (2020). Although *C. brasiliensis* was found to be feeding on invertebrates and small fish, their morphology indicates that they are less specialized in piscivory terms than *H .malabaricus*. This implies that *C. brasiliensis* performs better in environments with greater habitat complexity providing different resources as they are more suited for ambush hunting (ALEXANDER et al. 2015; LUGER et al. 2020), in contrast to *H. malabaricus* which has traits for active pursuit which indicates better trophic success in simplified habitats (ALEXANDER et al. 2015; LUGER et al. 2020), when feeding on fish prey (SCHNEIDER et al. 2011; SILVA et al. 2017). Some studies show that *H. malabaricus* can change diet accordingly with the degree of deforestation of enviromental and competition with other predators (POMPEU, 2001; BELIENE; ROCHA; SOUZA, 2014), as well as being an opportunistic species according to the food supply (WINEMILLER, 1989; MACHADO-ALLISON, 1994; BELIENE; ROCHA; SOUZA, 2014). This shows that despite the trait specialisation, the realised niche width of *H. malabaricus* is broad and plastic which facilitates its persistence in the environment when resources fluctuate, as it is able to feed across a range of resources.

The drastic seasonal decline of food may result in individuals increasing their trophic niches in order to reduce inter and intraspecific competition in the face of decreased resources (DAUWALTER et al., 2007). This was observed in the present study, in which trophic overlaps were high in the dry season and overall niches broader in the dry season, although this was not the case for characins which were mainly feeding on zooplankton and organic matter. This food overlap allows the coexistence of different species in aquatic environments, and weakens the competitive pressure (POOL et al. 2017). Invertebrates were food sources for most minor species, however, in the dry season

there is usually a decline in invertebrate abundance (ORTEGA et al. 2015). This is due to seasonal declines in breeding habitat and phenology in insect species, as well as productivity dynamics (SANTANA et al. 2015). Thus, the fish species broaden their niche and consume alternative resources (plants and seeds) which are superabundant and easily accessible in this system (POOL et al. 2017; ORTEGA et al. 2015). As algal and plant matter does not have the same degree of nutritional content the frequency of occurrence of these resources in the diet of the icythyofauna increases. In this situation, some species showed a great degree of overlap, but this is not a reflection of intense competition but rather a series of functionally similar fish, with diet plasticity, which have access to abundant resources at the moment.

The urban protected area of Mrn presents, in its entirety, 23 native fish species endemic to the Hydrological Units of Maranhão an Parnaíba, widely distributed along the river basins of Northeast Brazil and some with known distribution in the Amazon River basin (OLIVEIRA et al. 2020; FRICKE et al. 2021). Although the Mata de Itamacaoca, is within the urban perimeter, this conservation unit appears to have successfully conserved freshwater fish biodiversity. The presence of dam may modify environmental conditions which make the area susceptible to invasive species in the future, however it is currently pristine (BUNN; ARTHINGTON, 2002, DAGA et al. 2020; OLIVEIRA et al. 2020). Small and medium-sized fish species are more abundant, while large fish species are absent from the area, this because only small streams were dammed for the construction of the dam in the area (SILVA et al. 2008; OLIVEIRA et al. 2020). The Mata de Itamacaoca, although it is within the urban area, it presents great potential for conservation due to its importance as a shelter for small and medium-sized fish species. This untouched environment is formed by trees up to 10 metres high and are associated with springs, streams and dam (SILVA et al. 2008). This approach of vegetation to the water bed plays a key role in the structuring of the habitat, providing shelter, food and ensuring water quality (ROLDI et al. 2014). Clearly the integrity of the riparian zone in the Mrn conservation area is supporting rich biodiversity, seen from the persistence of many phylogenetically similar fishes which have vastly overlapping trophic niches. The lack of large predatory fishes is notable as the two medium sized opportunistic predatory fish also feed across trophic levels, therein the lack of co-evolutionary history with predators in this system may leave them susceptible to extirpation if invaded.

Given the multiple anthropogenic pressures, such as deforestation, climate warming, pollution, wild-fires and species invasions, the Mata de Itamacaoca appears to

be ecologically undisturbed. Our results show that currently there are many trophic overlaps in the Characiformes, which suggests that they may reduce interspecific competition within this family, through spatial sorting or habitat filtering as they have phylogenetic niche conservation. On the other hand, the Cichliforme species have clearly defined trophic niches which suggests that they are subject to niche separation as the mechanism for co-existence. When faced with a stressor, generalist species are more likely to persist than specialists as they are able to adjust their trophic and spatial niches, in the future this may contribute to biotic homogenisation of biodiverse neotropical fish communities (HAHN; FUGI, 2007; DAGA et al. 2020). In particular, by characterising the feeding habits of fish species in these systems are useful tools to predict which species may be more at risk than others and allow conservation measures to be put in place to ensure resource abundance and system integrity(HAHN, FUGI, 2007; DAGA et al. 2020). Possible reasons for this differentiation, which ought to be further explored, are the dynamics and trophic plasticity above and below the dam, with specific focus on the temporal changes in resource abundance. This research would promote the detection of possible trophic alterations changes over time and identify whether fish are feeding on whatever is most abundant or if there is substantial prey selection. So far it is possible to state that competition for food, if any, is indirect and minimised by probable habitat filtering and spatial sorting in the characiformes, niche separation and trophic specialisation in the cichliformes. A mixture of habitat filtering and trophic niche separation probably acts on the entire community but the large overlaps in community realised niche means that resource abundance changes could affect the dynamics of the community. This should be monitored in the future to assess how changes in resource dynamics affects the abundance of prey items as well as the ecology of the representative fish species. We would suggest using the characiformes and cichliformes as ecological indicator species in this case as each family shows different trait mechanisms to reduce competition.

**Conclusion**

Finally, we conclude that the varied food composition, the wide range of niche and the high diet overlap in the fish community of the Mata de Itamacaoca, are associated with local availability of food resources in the dry and rainy seasons. We have provided essential baseline information for the entire community trophic ecology. The data from the gut contents broadly matched the predictions from the TPs which shows that

ecomorphological approaches can be a useful tool in the future for assessing niche and diet of Mrn species. This approach, when used in tandem with gut content analysis, can also be used to predict which species are most at risk from environmental change. There was clear difference in seasonal diet composition which should be taken into account with the with the ecomorphological predictions of different species. This is a result of changes in resource abundance, which should also be monitored in the future. We show that different mechanisms act on different fish families, which suggests differential selection pressures on the community which facilitate the coexistence of species. Therefore, our study emphasizes the importance of the integrity of aquatic ecosystems for trophic and ecomorphological structuring of ichthyofauna and provides information that can contribute to comparison with other untouched environments and for the evaluation of disturbed streams.

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**Tabel 1.** Descriptive statistics of the fishes used for ecomorphological analysis.

| Species | Seasons | Number of specimens | Standard length range (mm) | Mean (mm) | SD (mm) |
|---|---|---|---|---|---|
| *Anablepsoides Vieirai* Nelson, 2016 | Dry | 20 | 20.8 - 31.51 | 25.32 | 2.85 |
|  | Rainy | 20 | 22.63 - 32.28 | 26.24 | 2.61 |
| *Apistogramma piauiensis* Kullander, 1980 | Dry | 20 | 24.18 - 33 | 28.86 | 2.34 |
|  | Rainy | 20 | 24.59 - 39.19 | 30.79 | 4.04 |
| *Astyanax* cf. *bimaculatus* | Dry | 20 | 53.75 - 98.81 | 63.70 | 12.86 |
|  | Rainy | 3 | 27.5 - 60.5 | 40.5 | 17.54 |
| *Characidium* sp. | Dry | 20 | 21.95 - 26.84 | 24.52 | 1.61 |
|  | Rainy | - | - | - | - |
| *Cichlasoma* cf. *zarskei* Ottoni, 2011 | Dry | 20 | 43.09 - 90.8 | 71.80 | 12.18 |
|  | Rainy | 20 | 43.95 - 107.69 | 78.98 | 15.87 |
| *Compsura* sp. | Dry | 10 | 21.66 - 25.87 | 22.98 | 1.22 |
|  | Rainy | - | - | - | - |
| *Copella arnoldi* (Regan, 1912) | Dry | 19 | 16.39 - 30.03 | 22.58 | 3.52 |
|  | Rainy | 20 | 21.74 - 31.66 | 27.46 | 3.02 |
| *Crenicichla brasiliensis* (Bloch, 1792) | Dry | 20 | 46.33 - 99.7 | 75.94 | 15.16 |
|  | Rainy | 20 | 59.65 - 141.23 | 95.03 | 21.25 |
| *Curimatopsis* aff. *cryptica* | Dry | 16 | 30.89 - 38.95 | 33.84 | 2.80 |
|  | Rainy | 18 | 31.89 - 43.17 | 36.37 | 3.28 |
| *Hemigrammus* sp.1 | Dry | 20 | 24.72 - 35.5 | 30.04 | 2.77 |
|  | Rainy | 20 | 25.64 - 33.43 | 29.46 | 2.23 |
| *Hoplerythrinus unitaeniatus* (Spix & Agassiz, 1829) | Dry | - | - | - | - |
|  | Rainy | 2 | 130.5 - 133.41 | 131.95 | 2.05 |
| *Hoplias malabaricus* (Bloch, 1794) | Dry | 12 | 66.93 - 164.92 | 98.77 | 27.92 |
|  | Rainy | 11 | 82.77 - 233.13 | 133.92 | 46.16 |
| *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa & Ottoni, 2018 | Dry | 20 | 21.7 - 27.79 | 25.48 | 1.72 |
|  | Rainy | 19 | 16.8 - 31.5 | 24.53 | 4.33 |
| *Knodus victoriae* (Steindachner, 1907) | Dry | 4 | 23.88 - 30.63 | 27.74 | 3.36 |
|  | Rainy | 10 | 24.7 - 38.68 | 32.80 | 4.44 |
| *Megalechis thoracata* (Valenciennes, 1840) | Dry | - | - | - | - |
|  | Rainy | 10 | 17.93 - 28.73 | 23.43 | 3.82 |
| *Moenkhausia oligolepis* (Günther, 1864) | Dry | 11 | 45.86 - 68.44 | 52.65 | 6.02 |
|  | Rainy | - | - | - | - |
| *Nannostomus beckfordi* Günther, 1872 | Dry | 20 | 25.02 - 30.3 | 27.27 | 1.27 |
|  | Rainy | 20 | 25.79 - 31.53 | 27.20 | 1.19 |
| *Pimelodella parnahybae* Fowler, 1941 | Dry | - | - | - | - |
|  | Rainy | 2 | 90.5 - 127.2 | 108.85 | 25.95 |
| *Steindachnerina notonota* (Miranda Ribeiro, 1937) | Dry | 1 | 68.44 | - | - |
|  | Rainy | 5 | 58.5 - 96.1 | 72.23 | 14.63 |

**Tabel 2.** Feeding-associated, morphological traits and their units. Definitions follow Sibbing and Nagelkerke (2001) and Teixeira and Bennemann (2007), Kullander (1986) for Cichlideos.

| Morphological trait | Abreviation/description | Unit |
|---|---|---|
| Oral gape height | OGH | mm |
| Oral gape width | OGW | mm |
| Oral gape diameter | OGD: Average of oral gape height and width (OGH+OGW) / 2 | ration |
| Gill raker length | GiRL | mm |
| Gill arch resistance | GiRL/GiRD: ratio between gill raker length and gill raker distance | ration |
| Eye diameter | ED | mm |
| Body width | BW | mm |
| Body depth | BD | mm |
| Head length | HL | mm |
| Caudal peduncle depth | CPD | mm |
| Post orbital length | POrL | mm |
| Operculum depth | OpD | mm |
| Oral gape body área ration | (OGH∗OGW) / (BD∗BW) | ration |
| Gill raker distance | GiRD | mm |

**Tabel 3.** Summary GLMS used to determine differences per species of fishes in the Mata de Itamacaoca. a) species richness, b) Shannon-Weiner diversity index (H). Significant p-values are highlighted with asterisc.

| Species | a) Sprich | | | | b) H1 | | | |
|---|---|---|---|---|---|---|---|---|
| | Est | SE | t | p | Est | SE | t | p |
| *Apistograma piauiensis* | -0.65 | 0.45 | -1.43 | 0.15 | -0.23 | 0.13 | -1.80 | 0.07 |
| *Astyanax* cf. *bimaculatus* | 1.65 | 0.52 | 3.13 | 0.001* | 0.28 | 0.15 | 1.88 | 0.05 |
| *Characidium* sp. | -0.42 | 0.55 | -0.76 | 0.44 | -0.13 | 0.15 | -0.85 | 0.39 |
| *Cichlasoma* cf. *zarskei* | 0.07 | 0.45 | 0.16 | 0.86 | -0.05 | 0.13 | -0.43 | 0.66 |
| *Compsura* sp. | -1.07 | 0.71 | -1.50 | 0.13 | -0.46 | 0.20 | -2.25 | 0.02* |
| *Copella arnoldi* | -0.36 | 0.45 | -0.80 | 0.42 | -0.12 | 0.13 | -0.95 | 0.34 |
| *Crenicichla brasiliensis* | 0.80 | 0.45 | 1.76 | 0.07 | 0.15 | 0.13 | 1.22 | 0.22 |
| *Curimatopsis* aff. *cryptica* | -0.80 | 0.47 | -1.70 | 0.08 | -0.32 | 0.13 | -2.39 | 0.01* |
| *Hemigrammus* sp.1 | -0.17 | 0.45 | -0.38 | 0.69 | -0.04 | 0.13 | -0.38 | 0.70 |
| *Hoplerythrinus unitaeniatus* | 1.72 | 1.46 | 1.17 | 0.23 | 0.59 | 0.42 | 1.40 | 0.15 |
| *Hoplias malabaricus* | 1.13 | 0.52 | 2.15 | 0.03* | 0.25 | 0.15 | 1.66 | 0.09 |
| *Hyphessobrycon piorskii* | -.067 | 0.45 | -1.47 | 0.14 | -0.25 | 0.13 | -1.91 | 0.05 |
| *Knodus victoriae* | 1.36 | 0.62 | 2.17 | 0.02* | 0.34 | 0.18 | 1.92 | 0.05 |
| *Megalechis thoracata* | -0.17 | 0.71 | -0.24 | 0.80 | -0.15 | 0.20 | -0.73 | 0.46 |
| *Moenkhausia oligolepis* | 0.22 | 0.68 | 0.32 | 0.74 | 0.03 | 0.19 | 0.17 | 0.86 |
| *Nannostomus beckfordi* | -0.07 | 0.45 | -0.16 | 0.86 | -0.02 | 0.13 | -0.16 | 0.86 |
| *Pimelodella parnahybae* | 1.22 | 1.46 | 0.83 | 0.40 | 0.38 | 0.42 | 0.92 | 0.35 |
| *Steindachnerina notonota* | -0.77 | 0.88 | -0.87 | 0.38 | -0.53 | 0.25 | -2.09 | 0.03* |

**Figure 1.** Map of the studied area. Collecting sites (C1-C5). Adapted from Oliveira et al. (2020).
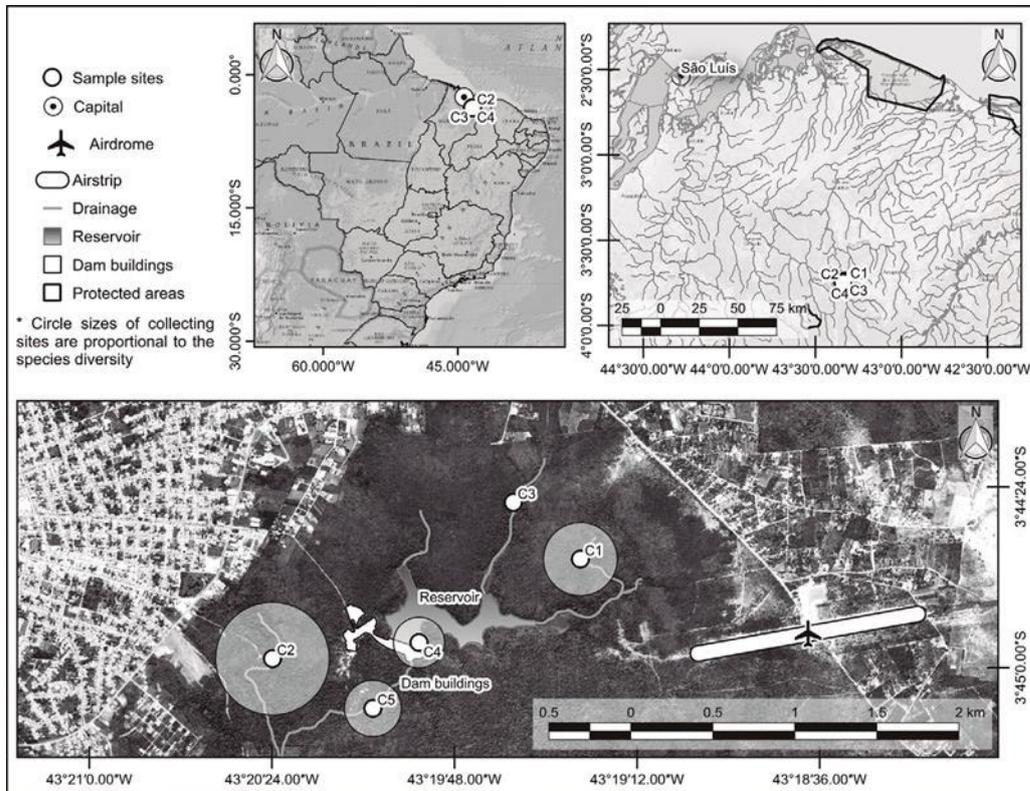
**Figure 2.** Frequency of Occurence of gut content items in the fish species community of with regards to dam wall (Above and Below) and season (Dry and Rainy) in Mata de Itamacaoca.
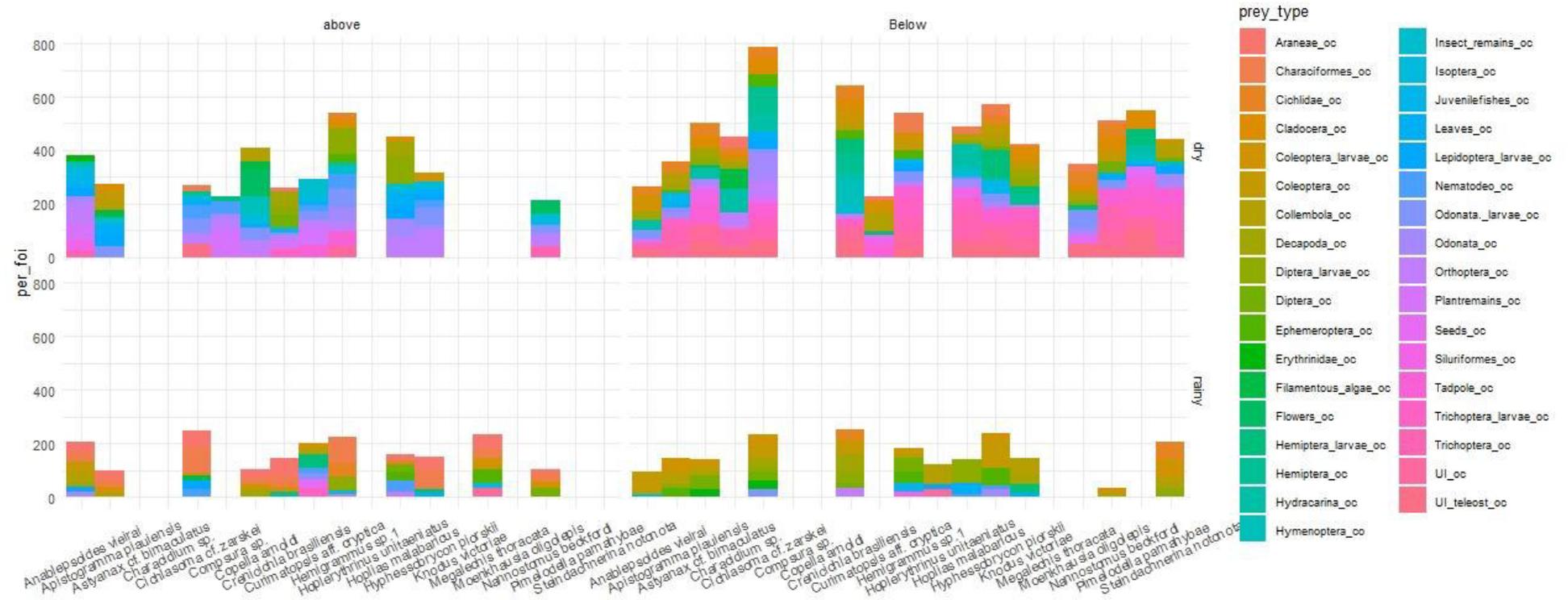
**Figure 3.** Index of Relative Importance (%IRI) for the different species and their prey it by dam (Above and Below) and season (Dry and Rainy) in Mata de Itamacaoca.
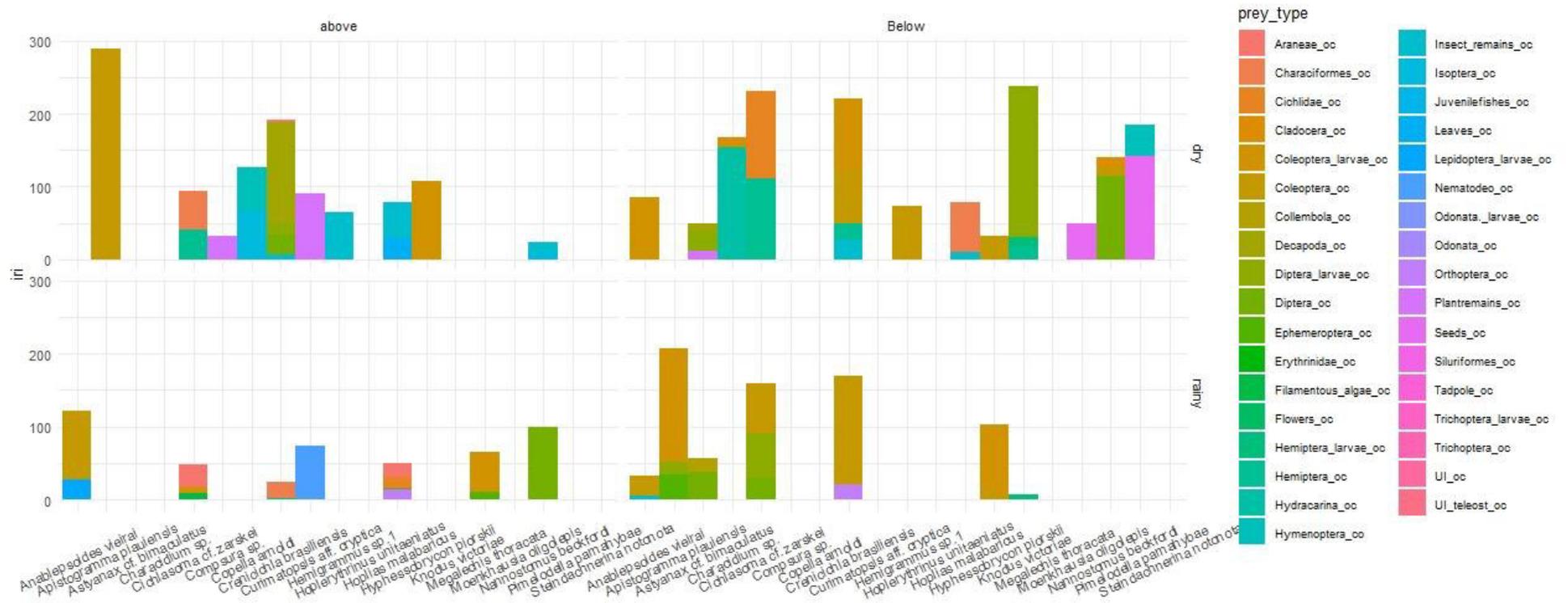
**Figure 4.** Species richness (Sprich), Shannon-Weiner diversity (H) for each species gut contents and season and dam (above and below the dam wall). Boxplot indicates median and inter-quartile ranges while points indicate species richness per site.
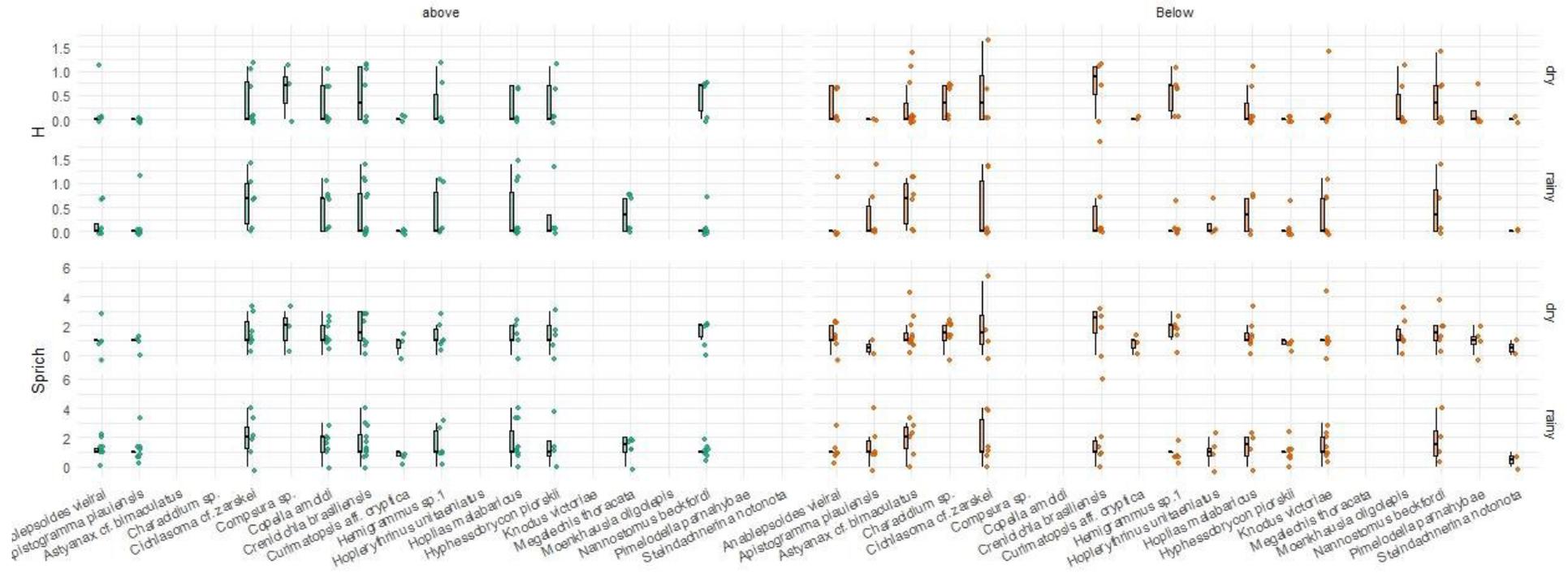
**Figure 5.** Interaction between most abundant species of fishes, Mata de Itamacaoca, during the dry/rainy seasons. Shannon-Weiner diversity (H1. Dotted lines indicate interactions between species.
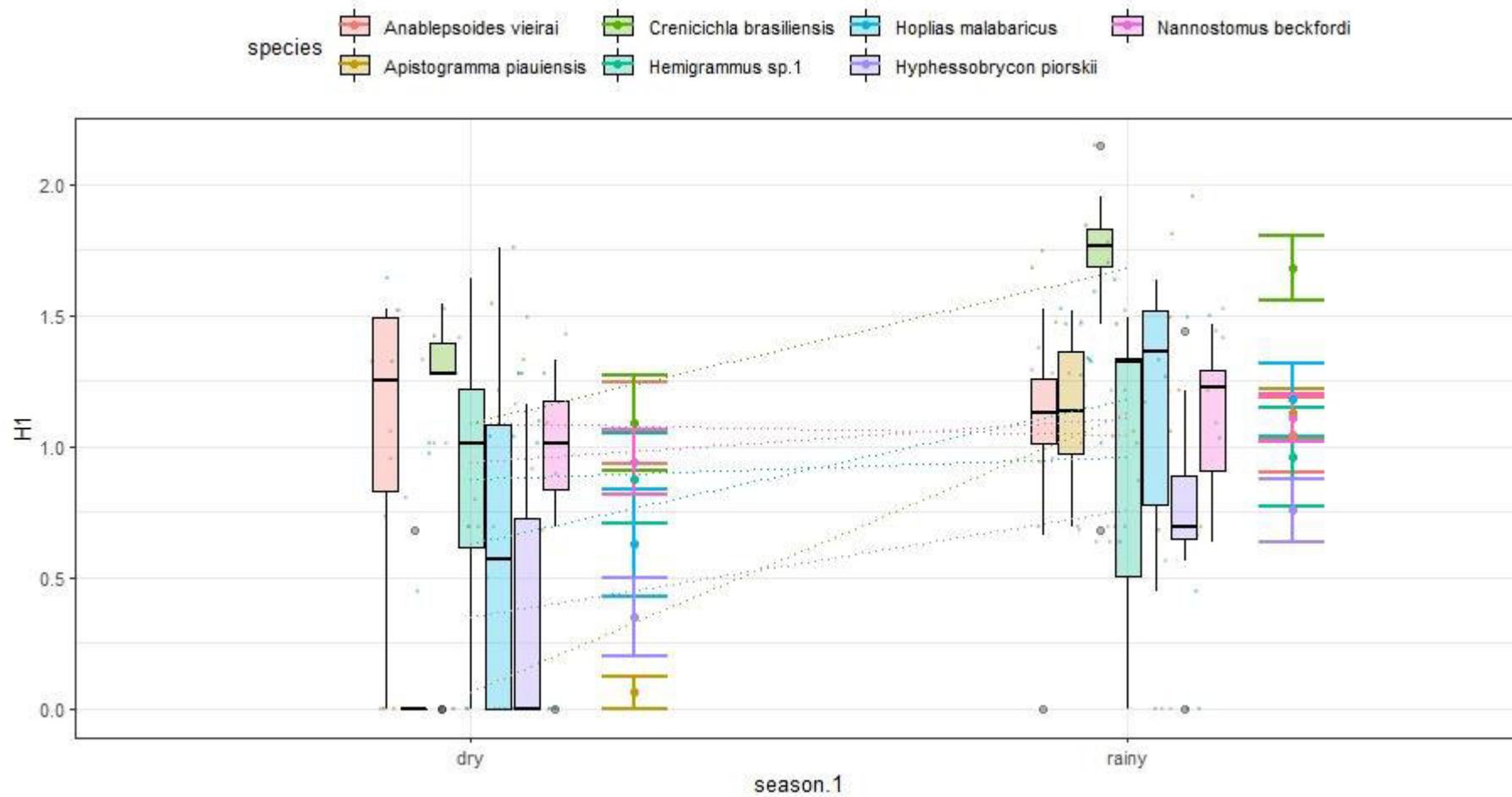
**Figure 6.** GLMs boxplot per species and individual. Species richness (Sprich1), Shannon-Weiner diversity (H1). Boxplot indicates median and inter-quartile.
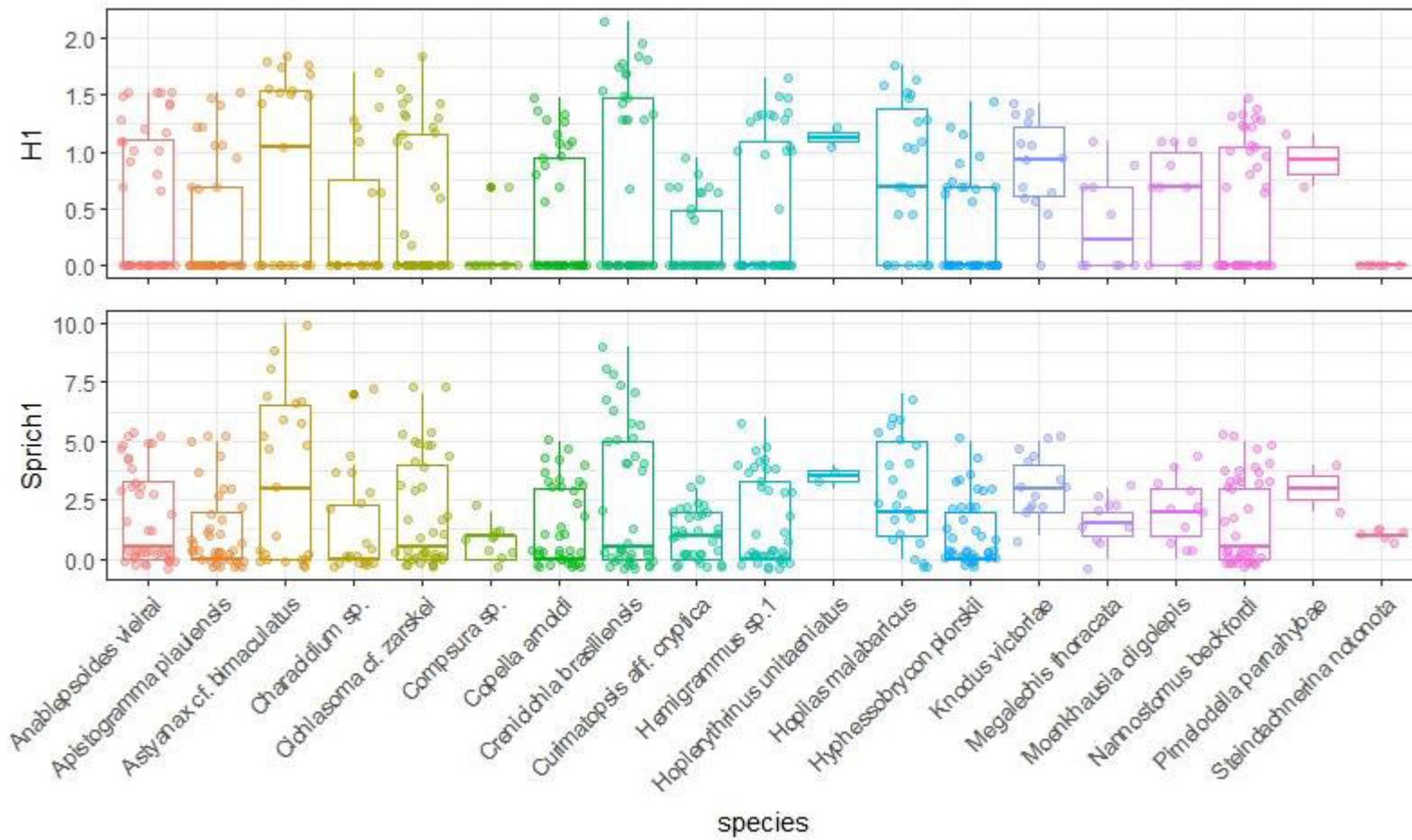
**Figure 7.** Dietary overlap between most abundant species of fishes, Mata de Itamacaoca.
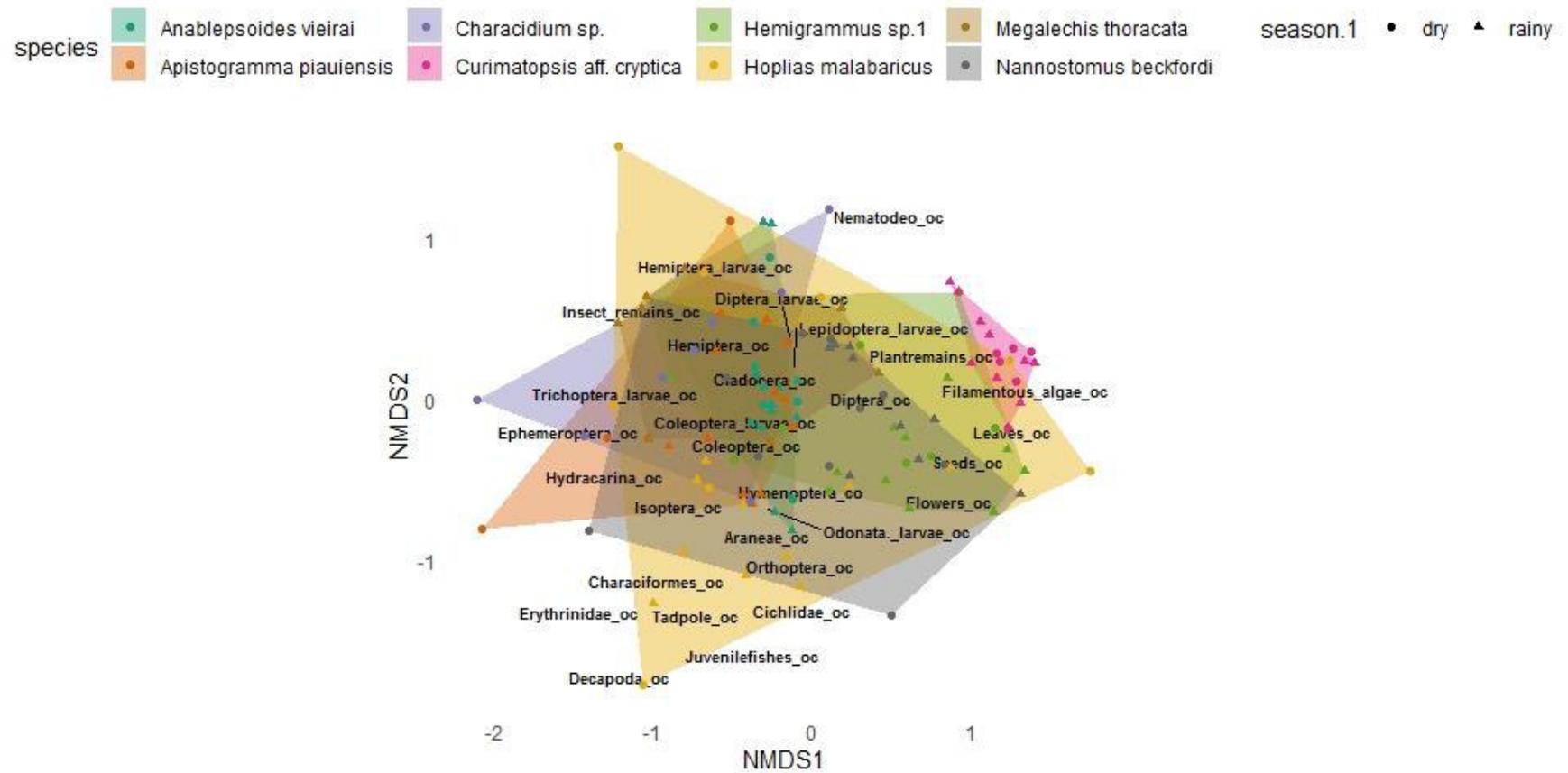
**Figure 8.** Dietary overlap enter dry and rainy seasons between most abundant species of fishes, Mata de Itamacaoca.
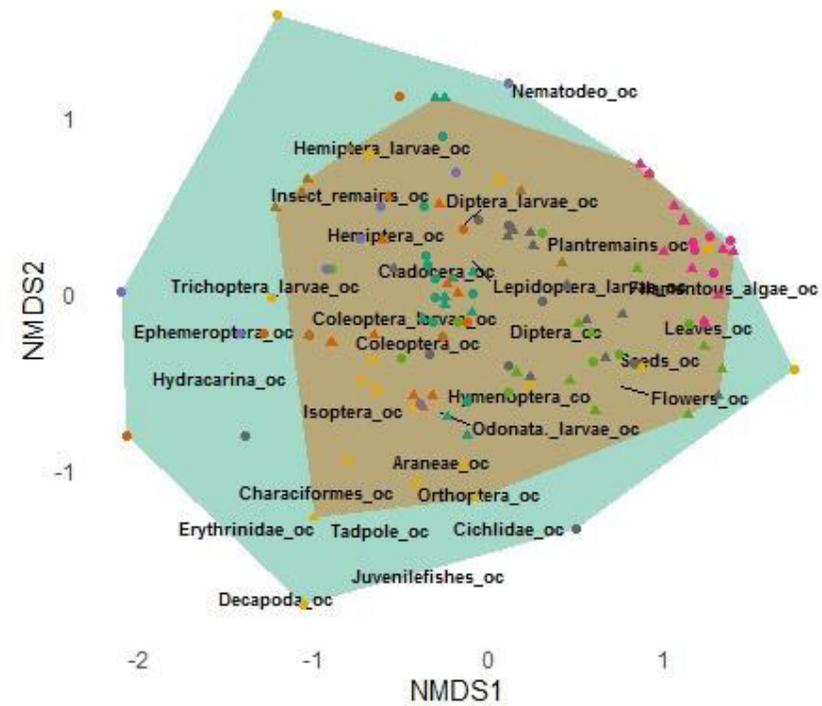
**Figure 9.** Biplot of a principal component analysis (PCA) of trophic morphological traits (a,b) and of derived trophic profiles (TP: c,d). Arrows in the right graphs indicate the loadings of the most influential variables on the PC-axes.
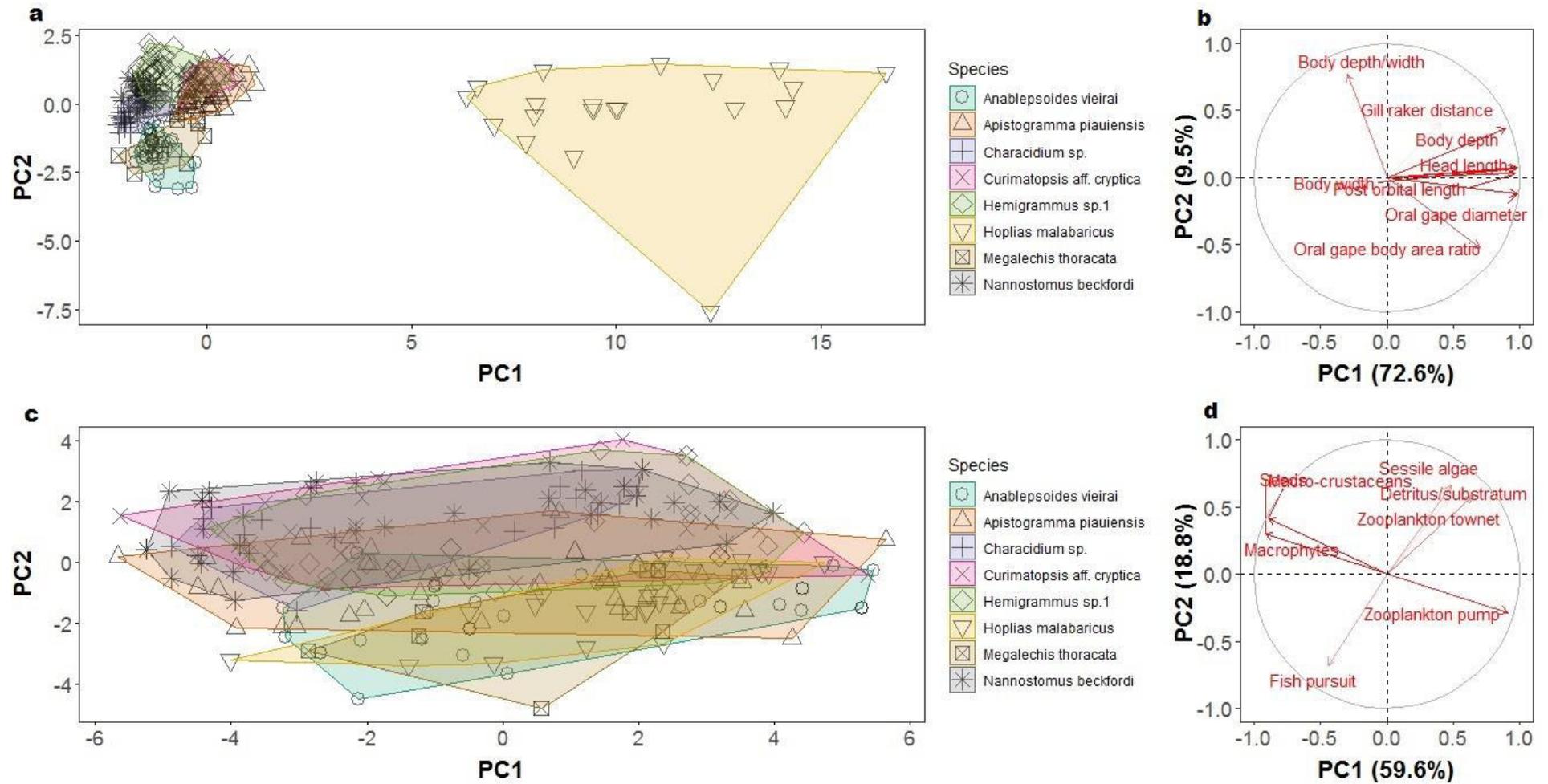
**Figure 10.** Correlations of predicted diet specialisations from the food-fish model (Sibbing and Nagelkerke 2018) between most abundant species of fishes of Mata de Itamacaoca. Differences between species are all statistically significant (P\0.05).
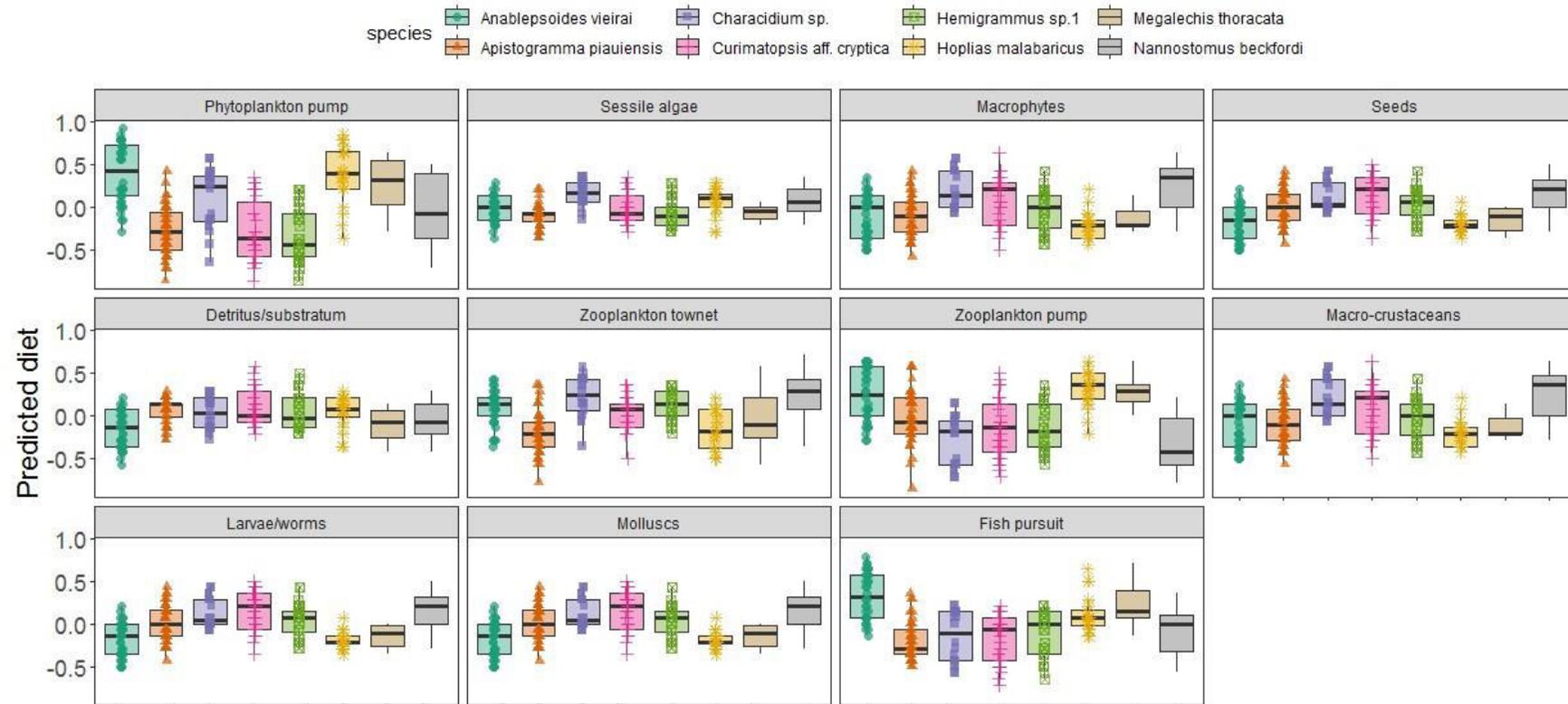
**Figure 11.** Dietary overlap between Characiformes species of fishes, Mata de Itamacaoca.
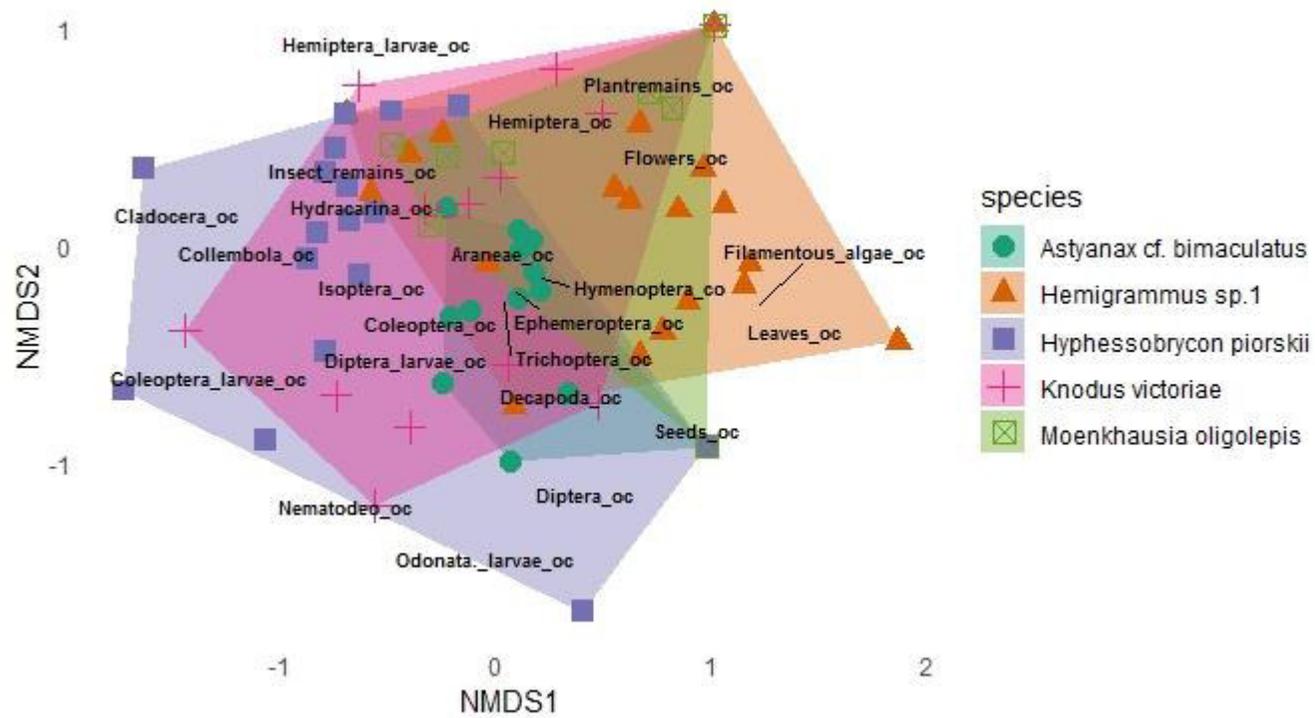
**Figure 12.** Dietary overlap enter dry and rainy seasons between Characiformes species of fishes, Mata de Itamacaoca.
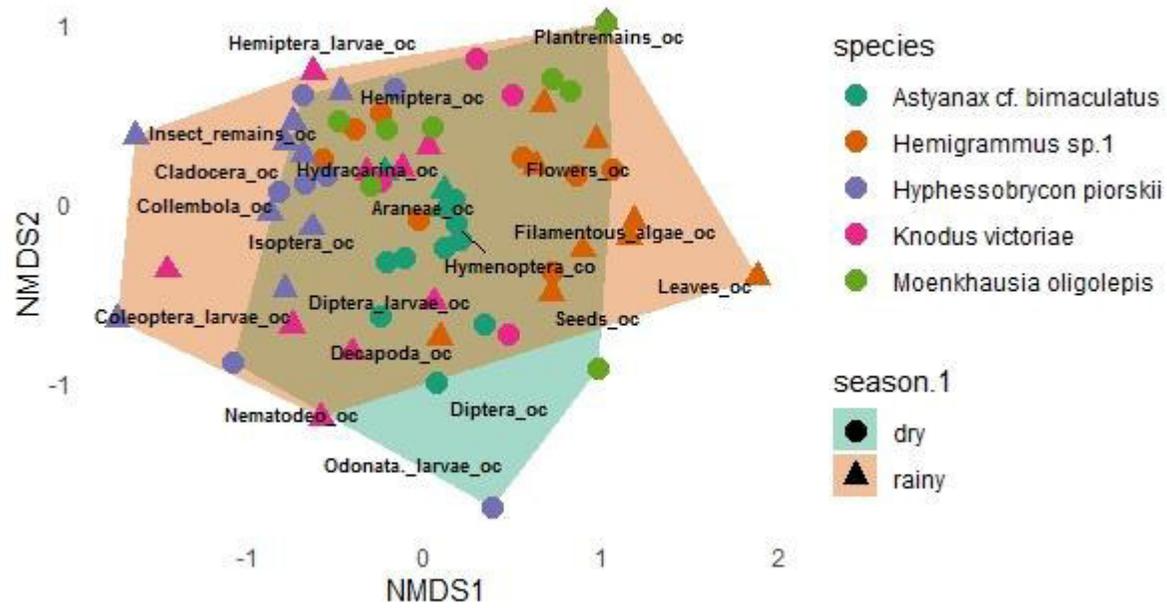
**Figure 13.** Biplot of a principal component analysis (PCA) of trophic morphological traits (a,b) and of derived trophic profiles (TP: c,d) between Characiformes species. Arrows in the right graphs indicate the loadings of the most influential variables on the PC-axes.
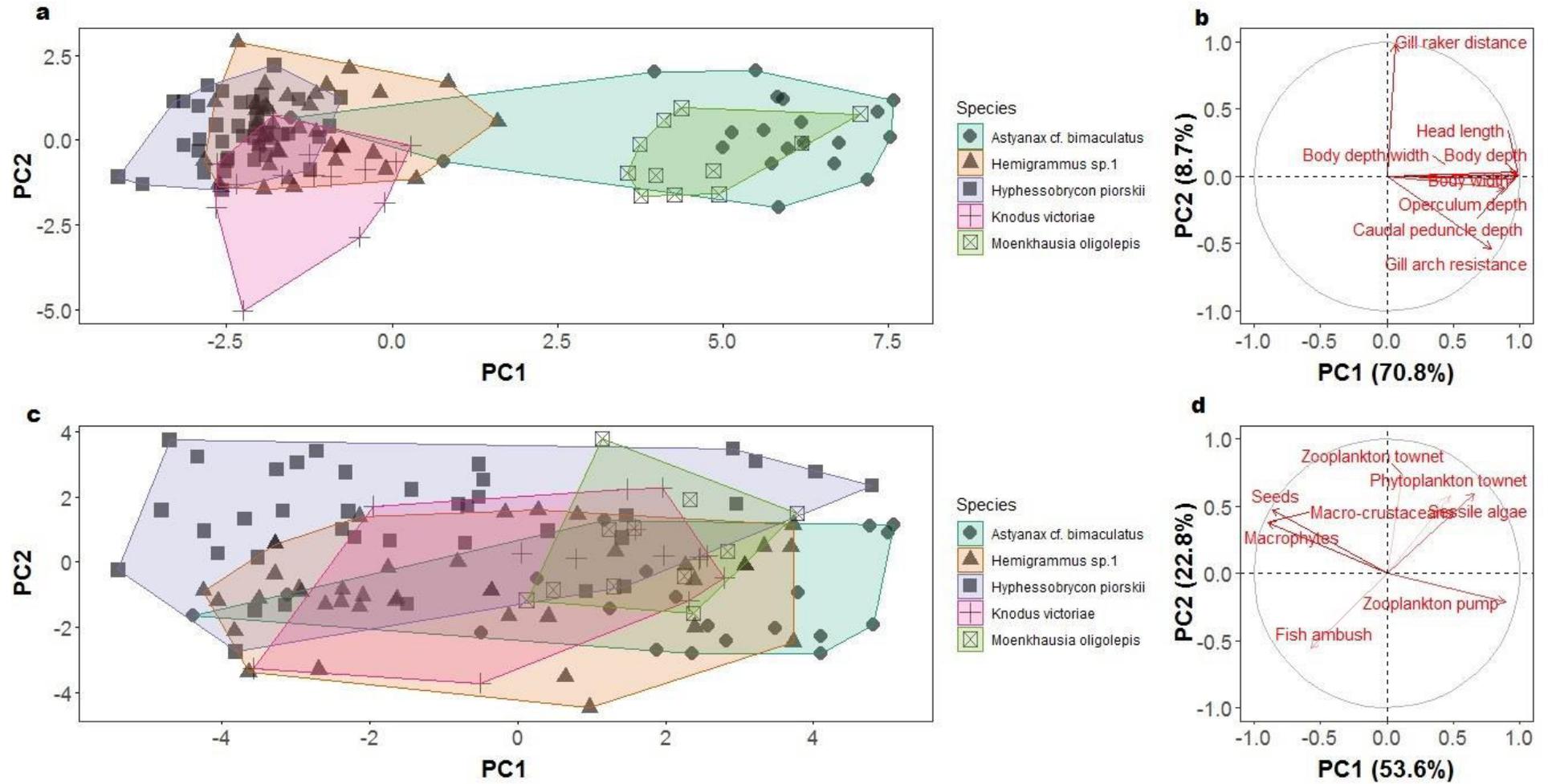
**Figure 14.** Correlations of predicted diet specialisations from the food-fish model (Sibbing and Nagelkerke 2018) between Characiformes species of Mata de Itamacaoca. Differences between species are all statistically significant, except for zooplankton townet (P\0.05).
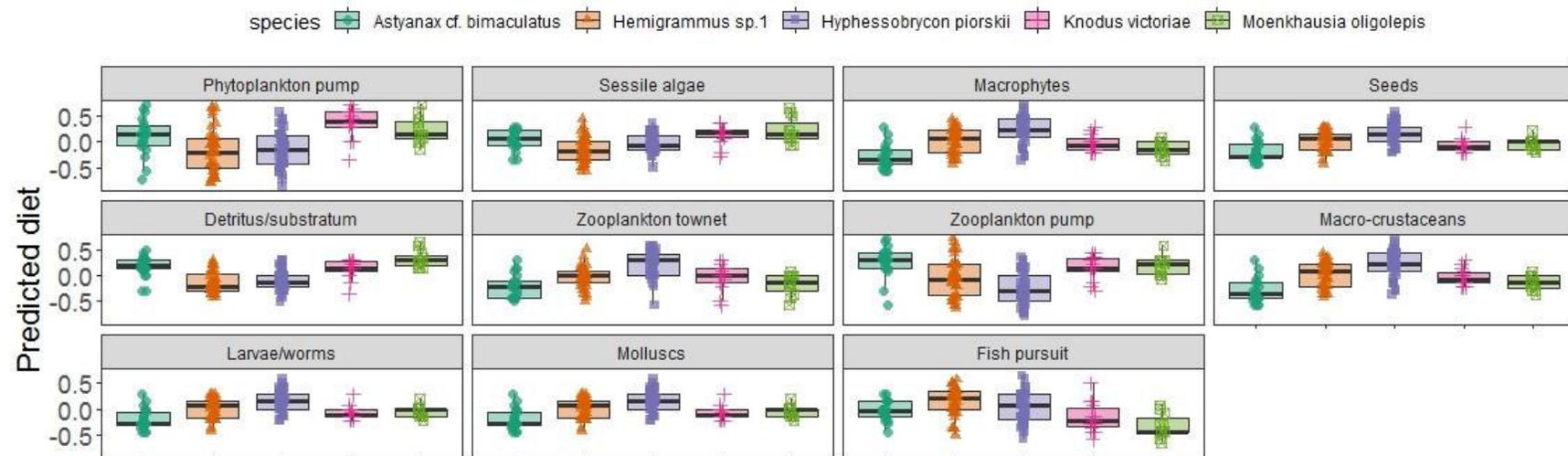
**Figure 15.** Dietary overlap enter dry and rainy seasons between Cichliformes species, Mata de Itamacaoca.
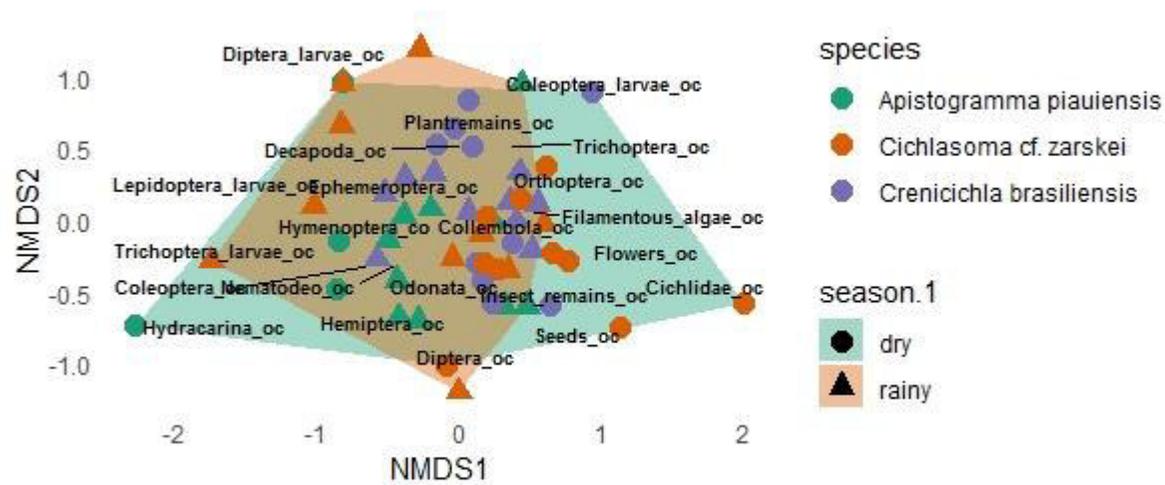
**Figure 16.** Dietary overlap between Cichliformes species, Mata de Itamacaoca.
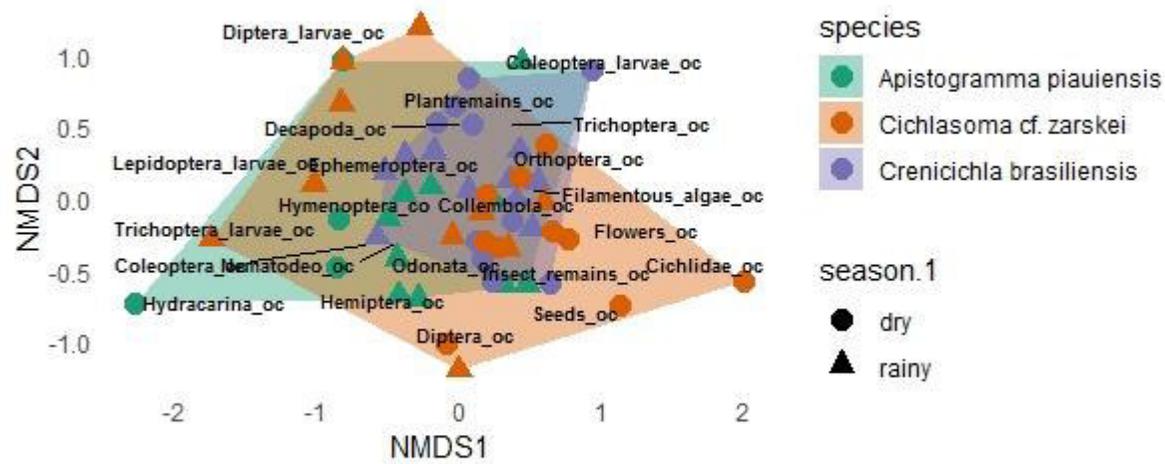
**17.** Biplot of a principal component analysis (PCA) of trophic morphological traits (a,b) and of derived trophic profiles (TP: c,d) between Cichliformes species. Arrows in the right graphs indicate the loadings of the most influential variables on the PC-axes.
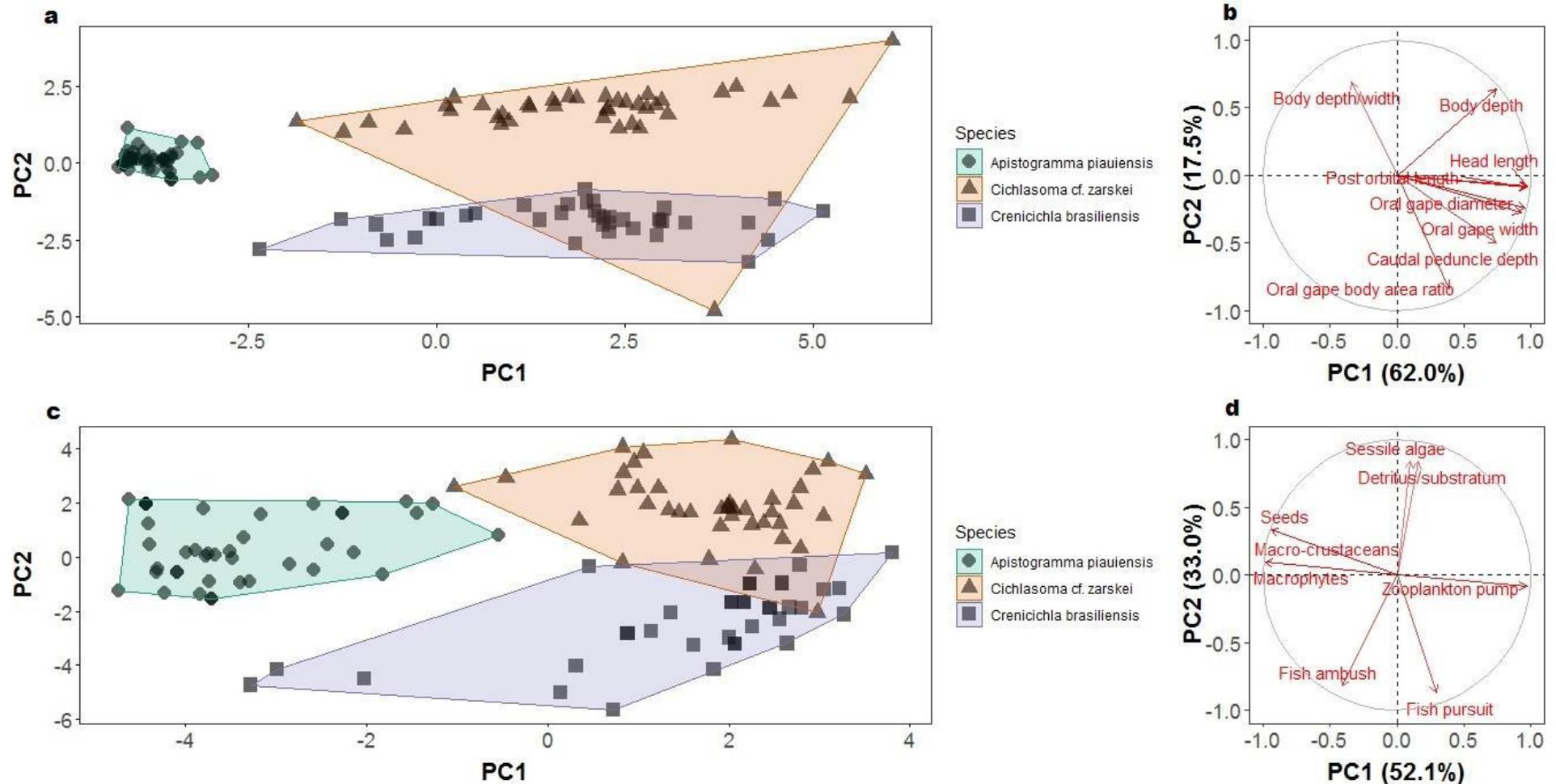
**18.** Correlations of predicted diet specialisations from the food-fish model (Sibbing and Nagelkerke 2018) between Cichliformes species of Mata de Itamacaoca. Differences between species are all statistically significant, except for zooplankton townet (P\0.05).
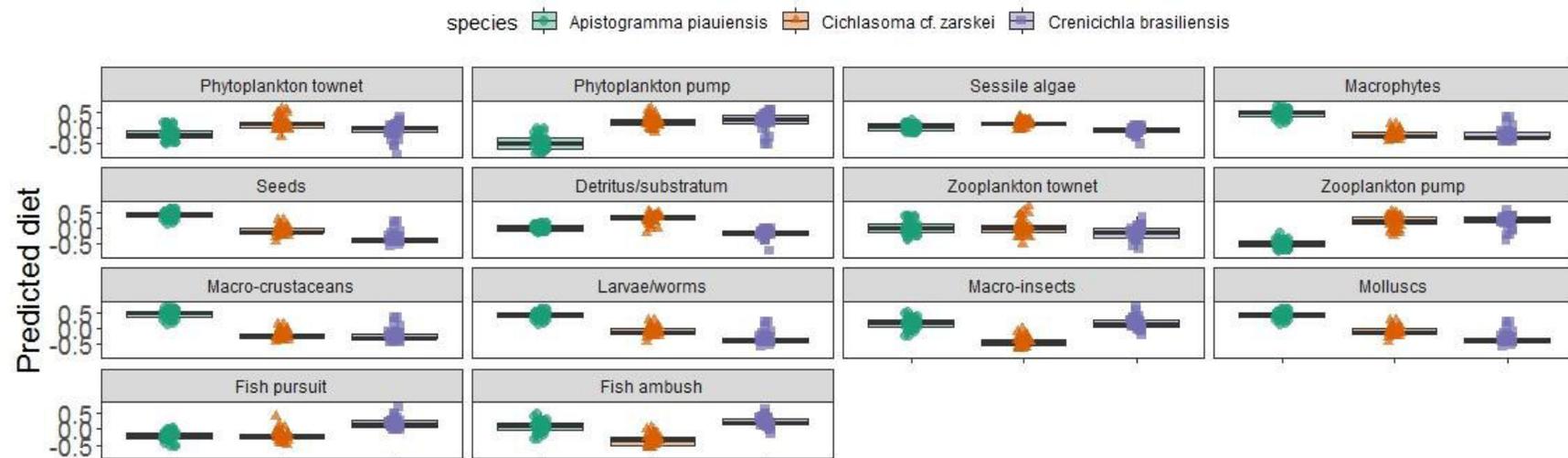
# CAPÍTULO VI

# CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

## Considerações finais e perspectivas futuras

A região Neotropical abriga a maior diversidade de peixes de água doce do mundo, possuindo mais de 6.000 espécies já descritas. Apesar dos estudos da ictiofauna de água doce da região ter avançado em relação à número e qualidade, nas duas últimas décadas, quando comparada com estudos de peixes de água doce de outras regiões do hemisfério sul, ela ainda é pouco conhecida (REIS et al. 2016). A dificuldade de acessibilidade, aliada com a dificuldade de captura e a complexidade morfológica da ictiofauna Neotropical de água doce, dificultam os estudos ictiológicos. Dentro da mega diversidade de peixes de água doce neotropicais, as espécies de pequeno e médio porte apresentam uma abundância e diversidade expressiva nessa região, e podem ser encontrados nos mais variados ecossistemas aquáticos, tais como: rios, riachos, lagoas, piscinas temporárias, planícies alagadas entre outros (CASTRO; POLAZ, 2020). Contudo, a rápida perda e degradação dos ambientes como resultado das ações antrópicas (espécies exóticas introduzidas, mudanças climáticas, assoreamento de rios e riachos, poluição, dentre outros) afetam significantemente a ictiofauna Neotropical, tanto em relação a sua diversidade de espécie, quanto ecológica (AGOSTINHO et al. 2008; CASTRO; POLAZ, 2020).

A Mata de Itamacaoca, localizada na Bacia do Alto Rio Munim, leste do Estado do Maranhão, embora esteja dentro do perímetro urbano do município de Chapadinha, apresenta um grande potencial para a conservação da biodiversidade local, sendo registradas, no presente estudo, 23 espécies de peixes, sendo três ainda não descritas (OLIVEIRA et al. 2020). Apesar da presença da barragem nesta área, criada pela CAEMA (Companhia de Saneamento Básico do Estado do Maranhão) para o abastecimento da cidade de Chapadinha- MA com água potável, nenhuma espécie exótica foi registrada, apontando que a área parece ter conservado a sua biodiversidade local de peixes de água doce (OLIVEIRA et al. 2020), que anteriormente a construção da barragem consistia em uma séria de riachos de pequeno porte. *Nannostomus beckfordi* Günther 1872, uma espécie abundante na área de estudo, exibe variação morfológica em resposta às estações de chuva, usada como adaptação para o regime de vazão. Um outro fator interessante é a presença de dez espécies com crescimento alométrico negativo na área, indicando que o incremento do peso é menor do que em comprimento. De certa forma, a disponibilidade de recursos alimentares no ambiente, explica boa parte dos

padrões de crescimento, uma vez que neste estudo é evidente a partilha de recursos, permitindo que diferentes espécies possam coexistir. Além disso, este estudo demonstrou que a comunidade de peixes da Mata de Itamacaoca podem mudar a sua dieta de acordo com a estação do ano e com local de ocorrência em relação a barragem (acima ou abaixo da barragem).

Finalmente, este estudo apresenta dados relevantes e precisos sobre a ictiofauna da Mata de Itamacaoca. Embora seja difícil encontrar riachos intocados na região, esta área se destaca por ser um ambiente potencialmente conservado, com uma comunidade de peixes amplamente distribuída e endêmica das unidades Hidrológicas do Maranhão e Parnaíba. Porém, a proximidade com a cidade pode modificar as condições ambientais, e a barragem pode facilitar a introdução de espécies exóticas, no futuro, por moradores e pescadores locais. Nesse contexto, a Mata de Itamacaoca, apresenta uma grande importância biológica e ecológica para a ictiofauna local, atuando como verdadeiro centro de refúgio para espécies de peixes de pequeno e médio porte. Os dados da ictiofauna, levantados, neste estudo, podem ser usados como referência e de linha de base para comparação com outras áreas intocadas, ou para a avaliação de riachos perturbados da região.

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**Appendix 1.** List of examined specimens in Chapter II with their collection site (C1-C5 according to the Table 1) and voucher numbers, presented in alphabetical order.

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**C1**

*Anablepsoides vieirai*: CICCAA00007, 18 specimens; CICCAA00008, 46 specimens; CICCAA00014, 5 specimens; CICCAA00017, 4 specimens; CICCAA00026, 2 specimens; CICCAA00073, 5 specimens; CICCAA00075, 7 specimens; CICCAA00080, 1 specimen; CICCAA00083, 2 specimens; CICCAA00127, 2 specimens; CICCAA00182, 11 specimens; CICCAA00205, 11 specimens; CICCAA00230, 22 specimens; CICCAA00563, 15 specimens; CICCAA00651, 4 specimens; CICCAA00661, 6 specimens; CICCAA00788, 26 specimens; CICCAA00884, 27 specimens; CICCAA01443, 18 specimens; CICCAA01556, 9 specimens; CICCAA02011, 131 specimens; CICCAA02805, 12 specimens; CICCAA02826, 56 specimens; CICCAA02964, 11 specimens; CICCAA03103, 5 specimens; CICCAA03449, 12 specimens; CICCAA03729, 11 specimens; CICCAA03876, 32 specimens; CICCAA04700, 25 specimens.

*Apistogramma piauiensis*: CICCAA00009, 8 specimens; CICCAA00010, 7 specimens; CICCAA00011, 1 specimen; CICCAA00013, 4 specimens; CICCAA00027, 6 specimens; CICCAA00078, 1 specimen; CICCAA00122, 1 specimen; CICCAA00176, 5 specimens; CICCAA00206, 6 specimens; CICCAA00229, 70 specimens; CICCAA00564, 36 specimens; CICCAA00640, 12 specimens; CICCAA00645, 4 specimens; CICCAA00656, 14 specimens; CICCAA00744, 3 specimens; CICCAA00789, 18 specimens; CICCAA00880, 13 specimens; CICCAA01554, 49 specimens; CICCAA02016, 82 specimens; CICCAA02801, 14 specimens; CICCAA02830, 32 specimens; CICCAA02963, 11 specimens; CICCAA03101, 3 specimens; CICCAA03730, 1 specimen; CICCAA03825, 1 specimen; CICCAA03871, 1 specimen; CICCAA04585, 32 specimens; CICCAA04699, 6 specimens.

*Cichlasoma* **cf.** *zarskei*: CICCAA00046, 1 specimen; CICCAA00117, 1 specimen; CICCAA00207, 1 specimen; CICCAA00233, 2 specimens; CICCAA00236, 8 specimens; CICCAA00646, 10 specimens; CICCAA00647, 10 specimens; CICCAA00742, 8 specimens; CICCAA00791, 6 specimens; CICCAA00885, 79 specimens; CICCAA01452, 3 specimens; CICCAA01553, 24 specimens; CICCAA02017, 34 specimens; CICCAA02467, 2 specimens; CICCAA02798, 59 specimens; CICCAA02834, 136 specimens; CICCAA02967, 43 specimens; CICCAA03531, 5 specimens; CICCAA03824, 1 specimen; CICCAA03877, 3 specimens; CICCAA04698, 1 specimen.

*Copella arnoldi*: CICCAA00037, 1 specimen; CICCAA00038, 1 specimen; CICCAA00072, 1 specimen; CICCAA00076, 10 specimens; CICCAA00077, 1 specimen; CICCAA00081, 1 specimen; CICCAA00120, 2 specimens; CICCAA00179, 1 specimen; CICCAA00180, 11 specimens; CICCAA00231, 2 specimens; CICCAA00569, 3 specimens; CICCAA00783, 1 specimen;

CICCAA01559, 6 specimens; CICCAA02825, 5 specimens; CICCAA02960, 3 specimens; CICCAA03823, 1 specimen; CICCAA03862, 6 specimens; CICCAA04702, 2 specimens.

*Crenicichla brasiliensis*: CICCAA00128, 2 specimens; CICCAA00232, 1 specimen; CICCAA00648, 16 specimens; CICCAA00784, 1 specimen; CICCAA00882, 5 specimens; CICCAA01555, 11 specimens; CICCAA02010, 3 specimens; CICCAA02799, 6 specimens; CICCAA02832, 1 specimen; CICCAA02968, 5 specimens; CICCAA03104, 1 specimen; CICCAA03402, 6 specimens; CICCAA03870, 1 specimen; CICCAA04704, 4 specimens.

*Curimatopsis* **aff.** *cryptica*: CICCAA00218, 2 specimens; CICCAA00219, 3 specimens; CICCAA00220, 2 specimens; CICCAA00221, 3 specimens; CICCAA00222, 4 specimens; CICCAA00223, 50 specimens; CICCAA00224, 70 specimens; CICCAA00225, 79 specimens; CICCAA00226, 4 specimens; CICCAA00235, 42 specimens; CICCAA00240, 3 specimens; CICCAA00420, 1 specimen; CICCAA00568, 25 specimens; CICCAA00653, 37 specimens; CICCAA00655, 4 specimens; CICCAA00745, 6 specimens; CICCAA00767, 1 specimen; CICCAA00768, 1 specimen; CICCAA00769, 1 specimen; CICCAA00770, 1 specimen; CICCAA00771, 1 specimen; CICCAA00772, 1 specimen; CICCAA00773, 2 specimens; CICCAA00774, 1 specimen; CICCAA00775, 3 specimens; CICCAA00776, 2 specimens; CICCAA00777, 3 specimens; CICCAA00786, 15 specimens; CICCAA00888, 169 specimens; CICCAA01450, 18 specimens; CICCAA01550, 318 specimens; CICCAA02014, 96 specimens; CICCAA02794, 421 specimens; CICCAA02829, 176 specimens; CICCAA02962, 114 specimens; CICCAA03398, 100 specimens; CICCAA03820, 6 specimens; CICCAA03821, 6 specimens; CICCAA03874, 22 specimens; CICCAA03899, 64 specimens; CICCAA04696, 51 specimens.

*Gymnotus carapo*: CICCAA00879, 1 specimen; CICCAA01558, 1 specimen; CICCAA02802, 1 specimen; CICCAA02959, 1 specimen; CICCAA03450, 1 specimen.

*Hemigrammus* **sp.1:** CICCAA00415, 14 specimens; CICCAA00416, 1 specimen; CICCAA00417, 31 specimens; CICCAA00418, 6 specimens; CICCAA00419, 4 specimens; CICCAA00421, 4 specimens; CICCAA00422, 6 specimens; CICCAA00423, 13 specimens; CICCAA00424, 3 specimens; CICCAA00425, 24 specimens; CICCAA00426, 50 specimens; CICCAA00427, 1 specimen; CICCAA00428, 3 specimens; CICCAA00566, 48 specimens; CICCAA00654, 20 specimens; CICCAA00657, 12 specimens; CICCAA00741, 4 specimens; CICCAA00790, 40 specimens; CICCAA00889, 534 specimens; CICCAA01383, 16 specimens; CICCAA01551, 419 specimens; CICCAA01941, 6 specimens; CICCAA02796, 475 specimens; CICCAA02833, 176 specimens; CICCAA02961, 105 specimens; CICCAA03102, 2 specimens; CICCAA03816, 5 specimens; CICCAA03817, 5 specimens; CICCAA03818, 5 specimens; CICCAA03827, 1 specimen; CICCAA03861, 1 specimen; CICCAA03872, 28 specimens; CICCAA03873, 21 specimens;

CICCAA03897, 16 specimens; CICCAA03898, 9 specimens; CICCAA04601, 200 specimens; CICCAA04697, 34 specimens; CICCAA04701, 8 specimens.

*Hoplias malabaricus*: CICCAA00030, 1 specimen; CICCAA00129, 2 specimens; CICCAA00175, 1 specimen; CICCAA00234, 1 specimen; CICCAA00567, 5 specimens; CICCAA00650, 2 specimens; CICCAA00660, 1 specimen; CICCAA00743, 3 specimens; CICCAA00787, 3 specimens; CICCAA00887, 3 specimens; CICCAA01552, 2 specimens; CICCAA02019, 3 specimens; CICCAA02803, 11 specimens; CICCAA02835, 6 specimens; CICCAA03421, 5 specimens; CICCAA03731, 1 specimen; CICCAA03822, 2 specimens; CICCAA04703, 3 specimens.

*Hyphessobrycon piorskii*: CICCAA00089, 1 (paratype); CICCAA00881, 1 (paratype); CICCAA01444, 11 specimens; CICCAA02800, 1 specimen; CICCAA02965, 1 specimen.

*Megalechis thoracata*: CICCAA00029, 3 specimens; CICCAA00042, 2 specimens; CICCAA00045, 1 specimen; CICCAA00079, 1 specimen; CICCAA00084, 1 specimen; CICCAA00124, 2 specimens; CICCAA00177, 2 specimens; CICCAA00237, 1 specimen; CICCAA00570, 1 specimen; CICCAA00659, 1 specimen; CICCAA00883, 1 specimen; CICCAA01557, 4 specimens; CICCAA02015, 10 specimens; CICCAA02804, 2 specimens; CICCAA02831, 4 specimens; CICCAA03860, 1 specimen.

*Nannostomus beckfordi*: CICCAA00028, 8 specimens; CICCAA00031, 7 specimens; CICCAA00036, 4 specimens; CICCAA00039, 59 specimens; CICCAA00040, 10 specimens; CICCAA00043, 1 specimen; CICCAA00071, 58 specimens; CICCAA00074, 14 specimens; CICCAA00082, 2 specimens; CICCAA00085, 3 specimens; CICCAA00123, 5 specimens; CICCAA00181, 93 specimens; CICCAA00204, 109 specimens; CICCAA00228, 24 specimens; CICCAA00565, 132 specimens; CICCAA00652, 178 specimens; CICCAA00658, 75 specimens; CICCAA00746, 6 specimens; CICCAA00785, 130 specimens; CICCAA00890, 920 specimens; CICCAA00953, 210 specimens; CICCAA01453; 126 specimens; CICCAA01549, 965 specimens; CICCAA01569, 200 specimens; CICCAA02018, 500 specimens; CICCAA02453, 216 specimens; CICCAA02795, 586 specimens; CICCAA02828, 743 specimens; CICCAA02966, 509 specimens; CICCAA03404, 100 specimens; CICCAA03732, 71 specimens; CICCAA03819, 7 specimens; CICCAA03875, 130 specimens; CICCAA04695, 194 specimens.

*Synbranchus marmoratus*:CICCAA00044, 1 specimen; CICCAA00227, 1 specimen; CICCAA00747, 1 specimen; CICCAA00886, 9 specimens; CICCAA02009, 2 specimens; CICCAA02797, 2 specimens; CICCAA02827, 3 specimens; CICCAA03400, 4 specimens; CICCAA03826, 1 specimen.

**C2**

*Anablepsoides vieirai:* CICCAA00188, 3 specimens; CICCAA02517, 5 specimens; CICCAA03889, 20 specimens; CICCAA04692, 6 specimens; CICCAA04732, 4 specimens.

*Apistogramma piauiensis*: CICCAA00196, 4 specimens; CICCAA02423, 1 specimen; CICCAA02446, 2 specimens; CICCAA03652, 4 specimens; CICCAA03884, 2 specimens; CICCAA04687, 5 specimens.

*Astyanax* **cf.** *bimaculatus*: CICCAA03754, 17 specimens; CICCAA03809, 10 specimens; CICCAA03883, 3 specimens; CICCAA04694, 19 specimens; CICCAA04740, 42 specimens.

*Branchyhypopomus* **sp**.: CICCAA02448, 2 specimens; CICCAA02457, 1 specimen; CICCAA03811, 2 specimens.

 *Characidium* **sp**.: CICCAA03721, 24 specimens; CICCAA03751, 17 specimens; CICCAA03888, 3 specimens; CICCAA04693, 11 specimens; CICCAA04735, 19 specimens.

*Cichlasoma* **cf.** *zarskei*: CICCAA02447, 2 specimens; CICCAA02514, 7 specimens; CICCAA03723, 9 specimens; CICCAA03755, 5 specimens; CICCAA03885, 4 specimens; CICCAA04737, 2 specimens:

*Crenicichla brasiliensis*: CICCAA00198, 2 specimens; CICCAA02420, 4 specimens; CICCAA02445, 2 specimens; CICCAA02464, 6 specimens; CICCAA02515, 5 specimens; CICCAA03810, 1 specimen; CICCAA04736, 1 specimen.

*Curimatopsis* **aff.** *cryptica*: CICCAA02419, 1 specimen; CICCAA03746, 1 specimen; CICCAA04728, 6 specimens.

*Hemigrammus* **sp**.**1**: CICCAA02513, 15 specimens; CICCAA03890, 17 specimens; CICCAA03892, 36 specimens; CICCAA03893, 42 specimens; CICCAA03894, 69 specimens; CICCAA03895, 80 specimens; CICCAA03896, 6 specimens.

*Hoplerythrinus unitaeniatus***:** CICCAA02512, 2 specimens.

*Hoplias malabaricus*: CICCAA02422, 1 specimen; CICCAA02458, 1 specimen; CICCAA03752, 2 specimens; CICCAA04730, 2 specimens.

*Hyphessobrycon piorskii*: CICCAA01446, 17 specimens; CICCAA02421, 3 specimens; CICCAA02465, 15 specimens; CICCAA03749, 33 specimens; CICCAA04691, 8 specimens; CICCAA04738, 28 specimens.

*Knodus victoriae*: CICCAA02432, 2 specimens; CICCAA02466, 4 specimens; CICCAA03891, 5 specimens; CICCAA04690, 5 specimens; CICCAA04733, 4 specimens.

*Megalechis thoracata*: CICCAA02456, 1 specimen; CICCAA03747, 1 specimen.

*Moenkhausia oligolepis*: CICCAA03719, 4 specimens; CICCAA03748, 5 specimens; CICCAA04686, 2 specimens; CICCAA04731, 2 specimens.

*Nannostomus beckfordi*: CICCAA00200, 21 specimens; CICCAA03722, 25 specimens; CICCAA03745, 5 specimens; CICCAA03886, 31 specimens; CICCAA04689, 7 specimens; CICCAA04734, 61 specimens.

*Pimelodella parnahybae*: CICCAA03753, 2 specimens.

*Poecilia sarrafae*: CICCAA00187, 3 specimens; CICCAA00199, 15 specimens; CICCAA02449, 10 specimens; CICCAA02516, 3 specimens; CICCAA03720, 16 specimens; CICCAA03744, 8 specimens; CICCAA03887, 12 specimens; CICCAA04688, 5 specimens; CICCAA04739, 16 specimens.

*Steindachnerina notonota*: CICCAA04729, 1 specimen.

*Synbranchus marmoratus*: CICCAA03750, 1 specimen.


**C3**

*Anablepsoides vieirai***:** CICCAA00197, 6 specimens.

*Nannostomus beckfordi***:** CICCAA00201, 25 specimens.

**C4**

*Apistogramma piauiensis***:** CICCAA01562, 1 specimen; CICCAA03107, 1 specimen; CICCAA03724, 2 specimens; CICCAA04586, 13 specimens.

*Cichlasoma* **cf.** *zarskei***:** CICCAA01567, 68 specimens; CICCAA02450, 6 specimens; CICCAA03530, 18 specimens; CICCAA03725, 16 specimens; CICCAA04564, 30 specimens.

*Crenicichla brasiliensis***:** CICCAA01566, 49 specimens; CICCAA03106, 1 specimen; CICCAA03403, 28 specimens; CICCAA03406, 46 specimens; CICCAA03728, 20 specimens; CICCAA03813, 1 specimen; CICCAA03878, 20 specimens; CICCAA03879, 20 specimens.

*Curimatopsis* **aff.** *cryptica***:** CICCAA01565, 22 specimens; CICCAA03105, 4 specimens; CICCAA03451, 13 specimens; CICCAA03726, 14 specimens.

*Hemigrammus* **sp.1.** CICCAA02452, 3 specimens; CICCAA03108, 2 specimens; CICCAA03814, 4 specimens; CICCAA03815, 2 specimens; CICCAA03881, 516 specimens; CICCAA03900, 500 specimens; CICCAA04546, 2 specimens; CICCAA04581, 18 specimens; CICCAA04593, 100 specimens; CICCAA04597, 500 specimens; CICCAA04600, 100 specimens.

*Hemigrammus* **sp.2.** CICCAA02149, 8 specimens, CICCAA02555, 30 specimens.

*Hoplias malabaricus***:** CICCAA01564, 4 specimens; CICCAA03417, 1 specimen; CICCAA03727, 3 specimens; CICCAA03880, 1 specimen.

*Hyphessobrycon piorskii***:** CICCAA01563, 1 (paratype); CICCAA02147, 1 specimen.

*Nannostomus beckfordi***:** CICCAA01568, 565 specimens; CICCAA02451, 20 specimens; CICCAA02480, 500 specimens; CICCAA03397, 500 specimens; CICCAA03399, 500 specimens; CICCAA03882, 416 specimens.

**C5**

*Anablepsoides vieirai***:** CICCAA03863, 3 specimens; CICCAA03923, 1 specimen.

 *Apistogramma piauiensis***:** CICCAA00639, 19 specimens.

*Astyanax* **cf.** *bimaculatus***:** CICCAA03867, 32 specimens.

*Cichlasoma* **cf.** *zarskei***:** CICCAA02504, 6 specimens; CICCAA03865, 1 specimen.

*Gymnotus carapo***:** CICCAA03868, 2 specimens.

*Hemigrammus* **sp.1.** CICCAA02507, 49 specimens.

*Hoplias malabaricus***:** CICCAA03869, 2 specimens; CICCAA03925, 1 specimen.

*Megalechis thoracata***:** CICCAA03924, 1 specimen.

*Nannostomus beckfordi***:** CICCAA02508, 84 specimens; CICCAA03864, 6 specimens.

*Poecilia sarrafae***:** CICCAA02506, 45 specimens; CICCAA03866, 28 specimens. *Steindachnerina notonota***:** CICCAA02505, 6 specimens.

**Appendix 2.** List of examined specimens in the Chapter III and voucher numbers, presented in alphabetical order.

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*Anablepsoides vieirai:* CICCAA00008, CICCAA 00073, CICCAA00080, CICCAA00083, CICCAA00182, CICCAA00205, CICCAA00563, CICCAA00651; CICCAA00788, CICCAA01443, CICCAA01556, CICCAA02826, CICCAA02964.

*Apistogramma piauiensis:* CICCAA00011, CICCAA00564, CICCAA00640, CICCAA00657, CICCAA00789, CICCAA00880, CICCAA01554, CICCAA02016, CICCAA02423, CICCAA02801, CICCAA02830, CICCAA02963, CICCAA04585, CICCAA04586.

*Astyanax cf. bimaculatus:* CICCAA03754, CICCAA03867, CICCAA03873, CICCAA03883, CICCAA04697, CICCAA04740.

*Curimatopsis* **aff.** *cryptica:* CICCAA00219, CICCAA00223, CICCAA00224, CICCAA00235, CICCAA00568, CICCAA00653, CICCAA00786, CICCAA03451.

*Copella arnoldi:* CICCAA00037, CICCAA00038, CICCAA00072, CICCAA00076, CICCAA00077, CICCAA00081, CICCAA00179, CICCAA00180, CICCAA00231, CICCAA00569, CICCAA00783, CICCAA01559, CICCAA02825, CICCAA02960, CICCAA04702.

*Characidium* **sp.** CICCAA03721, CICCAA03754, CICCAA03888.

*Cichlasoma* **cf.** *zarskei***:** CICCAA00207, CICCAA00233, CICCAA00646, CICCAA00647, CICCAA00791, CICCAA01567, CICCAA02017, CICCAA02834, CICCAA02954, CICCAA03530, CICCAA03531, CICCAA04564.

*Hemigrammus* **sp. 1.** CICCAA004597, CICCAA04600.

*Hoplias malabaricus:* CICCAA00175, CICCAA00567, CICCAA00650, CICCAA00660, CICCAA00787, CICCAA00882, CICCAA01552, CICCAA01564, CICCAA02019, CICCAA02422, CICCAAA02803, CICCAA02835, CICCAA02902, CICCAA03421, CICCAA03727, CICCAA03731, CICCAA03752, CICCAA03869, CICCAA03880, CICCAA04703, CICCAA04730.

*Hyphessobrycon piorskii:* CICCAA01444, CICCAA02421, CICCAA02465, CICCAA02800, CICCAA02965, CICCAA03749, CICCAA04738.

*Megalechis thoracata***:** CICCAA00029, CICCAA00079, CICCAA00084, CICCAA00177, CICCAA00570, CICCAA00659, CICCAA00883, CICCAA01557, CICCAA02015, CICCAA02456, CICCA02831, CICCAA03747.

*Nannostomus beckfordi:* CICCAA03404.

*Poecilia sarrafae:* CICCAA00199, CICCAA03720, CICCAA03744, CICCAA03866, CICCAA03887, CICCAA04739.

*Synbranchus marmoratus***:** CICCAA00886, CICCAA02009, CICCAA02797, CICCAA03400, CICCAA03750.

**Appendix 3.** List of examined specimens in the Chapter IV and voucher numbers.

CICCAA00040, CICCAA00204, CICCAA00228, CICCAA00565, CICCAA00652, CICCAA000953, CICCAA01569, CICCAA00028, CICCAA00071, CICCAA00074, CICCAA00082, CICCAA00181, CICCAA00200, CICCAA00201, CICCAA00658, CICCAA00785.

**Appendix 4.** List of examined specimens in the Chapter VI and voucher numbers, presented in alphabetical order.

*Anablepoides vieirai:* CICCAA02011, CICCAA02826.

*Apistogramma paiuiensis:* CICCAA01554, 02016, 02830, 03724, 04586.

*Astyanax* **cf.** *bimaculatus:* CICCAA03883, 04740.

*Characidium* **sp.:** CICCAA03721

*Cichlasoma* **cf.** *zarskei:* CICCAA00233, CICCAA00646, CICCAA00791, CICCAA02017, CICCAA02798, CICCAA02834, CICCAA02967, CICCAA03530, CICCAA03723, CICCAA04564.

*Compsura* **sp.:** CICCAA02555.

*Copella arnoldi:* CICCAA00072; CICCAA00076; CICCAA00179, CICCAA00180, CICCAA00231, CICCAA00569, CICCAA01559, CICCAA02825, CICCAA04702.

*Crenicichla brasiliensis:* CICCAA02464; CICCAA02799, CICCAA02968, CICCAA03728, CICCAA03879, CICCAA04736.

*Curimatopsis* **aff.** *cryptica:* CICCAA02014, CICCAA03451, CICCAA03726, CICCAA03874.

*Hemigrammus* **sp. 1:** CICCAA02796; CICCAA04593.

*Hoplerythrinus unitaeniatus:* CICCAA02512

*Hoplias malabaricus:* CICCAA00175, CICCAA00787, CICCAA00882, CICCAA01552, CICCAA02019, CICCAA02835, CICCAA03417, CICCAA03421, CICCAA03752, CICCAA03869, CICCAA03925.

*Hyphessobrycon piorskii***:** CICCAA01444, CICCAA02465, CICCAA04738.

*Knodus victoriae:* CICCAA02432, CICCAA02466, CICCAA03891, CICCAA04733.

*Megalechis thoracata***:** CICCAA00029, CICCAA00083, CICCAA00084, CICCAA00659, CICCAA02015, CICCAA02804.

*Moenkhausia oligolepis*: CICCAA03748, CICCAA04731.

Nannostomus beckfordi: CICCAA00181, CICCAA01568, CICCAA02828, CICCAA03399.

*Pimelodella parnahybae*: CICCAA03753.

*Steindachnerina notonota*: CICCAA02505, CICCAA04729.

**Appendix 5.** Correlation plot (Kendalls Tau) of all morphological characters of *Nannostomus beckfordi*. Asterisks indicate significant correlation: * = <0.05, ** = < 0.01, *** < 0.001.

**Anexo I.** Instruções para autores.

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# Ichthyofauna of the Mata de Itamacaoca, an urban protected area from the upper Munim River basin, Northern Brazilian Cerrado

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***Abstract:*** The Munim River basin is one of the main river drainages of the Hydrological unit Maranhão, but there are few published studies which focus on ichthyological surveys and taxonomic work within this basin. The present study aims to provide a fish species inventory of the Mata da Itamacaoca, one of the few urban protected areas from the upper Munim River basin, comparing the ichthyofauna with other lists by conducted at the upper Munim River basin. A total of 42 collection expeditions were conducted, the sampling was conducted at five collecting sites distributed within the boundaries of Mata de Itamacaoca, upper Munim River basin. Diversity indices were calculated and generalised linear models (GLMs) were employed to assess differences in species richness, diversity and evenness depending on season and location in relation to the reservoir dam wall. In order to visualize fish community differences, non-metric multidimensional scaling (nMDS) and a one-way PERMANOVA was used to understand whether factors of site, season and location to the dam wall had an effect on fish community compositions. A total of six orders, 13 families, and 23 fish species were found, and the order with the highest species richness, considering all reaches, was Characiformes followed by Cichliformes. The most abundant species was *Nannostomus beckfordi*, while *Pimelodella parnahybae* and *Hoplerythrinus unitaeniatus* were the rarer species sampled. There were no alien invasive species collected within the study area. Species richness was significantly higher below the dam wall, but there were no other significant differences in diversity indices with regards to season or location. Fish community composition was significantly different above and below the dam wall and was significantly affected by sampling site. Season did not have an effect on fish community. This study corroborates other studies conducted in the Unidade Hidrológica Maranhão sensu Hubbert and Renno (2006), that the ichthyofaunal composition and taxonomy of species within this region face major data deficits, anthropogenic impacts, this study may be a baseline for comparing similar environments throughout the region.
***Keywords:*** Biodiversity, Conservation, fish inventory, Maranhão, Neotropical, Species richness.

## Ictiofauna da Mata de Itamacaoca, uma area de proteção urbana da bacia do alto Rio Munim, Cerrado norte brasileiro

***Resumo:*** A bacia do rio Munim é uma das principais drenagens da unidade Hidrológica do Maranhão, mas existem poucos estudos publicados que enfoquem levantamentos ictiológicos e trabalhos taxonômicos dentro desta bacia. O presente estudo tem como objetivo fornecer um inventário de espécies de peixes da Mata da Itamacaoca, uma das poucas áreas protegidas urbanas da bacia do alto rio Munim, comparando a ictiofauna com outras listas realizadas na bacia do alto rio Munim. Foram realizadas 42 expedições de coleta, a amostragem foi conduzida em cinco locais de coleta distribuídos dentro dos limites da Mata de Itamacaoca, bacia do alto rio Munim. Índices de diversidade foram calculados e modelos lineares generalizados (GLMs) foram empregados para avaliar diferenças na riqueza de espécies, diversidade e equitabilidade dependendo da estação e localização em relação à parede da barragem do reservatório. A fim de visualizar as diferenças da comunidade de peixes, escalonamento multidimensional não métrico (nMDS) e um PERMANOVA unilateral foi usado para entender se os fatores de local, estação e localização da parede da barragem afetavam a composição da comunidade de peixes. Um total de seis ordens, 13 famílias e 23 espécies de peixes foram encontradas, sendo que a ordem com maior riqueza de espécies, considerando todos os trechos, foi Characiformes seguida por Cichliformes. A espécie mais abundante foi *Nannostomus beckfordi*, enquanto *Pimelodella parnahybae* e *Hoplerythrinus unitaeniatus* foram as espécies mais raras amostradas. Não houve espécies exóticas invasoras coletadas na área de estudo. A riqueza de espécies foi significativamente maior abaixo da parede da barragem, mas não houve outras diferenças significativas nos índices de diversidade em relação à estação do ano ou localização. A composição da comunidade de peixes foi significativamente diferente acima e abaixo da parede da barragem, e foi significativamente afetada pelo local de amostragem. A estação do ano não afetou a comunidade de peixes. Este estudo corrobora outros estudos realizados na Unidade Hidrológica Maranhão *sensu* Hubbert and Renno (2006), que a composição ictiofaunística e taxonomia das espécies desta região enfrentam grandes déficits de dados. Impactos antropogênicos, este estudo pode ser uma linha de base para comparar ambientes semelhantes em toda a região.

***Palavras-chave:*** Biodiversidade, Conservação, Inventário de peixes, Neotropical, Riqueza de espécies.

## Introduction

The Neotropical freshwater ichthyofauna is the most species-rich of the world, comprising more than 6,000 described species, with estimates of over 9,000 species (Reis et al. 2016, Birindelli & Sidlauskas 2018, Castro & Polaz 2020). Within this huge species assemblage, most (about 70%) are small-sized fishes, with adults around 15 cm or less standard length (SL), which can inhabit a variety of aquatic environments, such as streams, small and large rivers, lagoons, pools, temporary pools, swamps, amongst others (Reis et al. 2003, Castro & Polaz 2020). Literature concerning the diversity and evolution of the Neotropical ichthyofauna has improved in recent years; however, studies are still few and underestimate their real biodiversity (Buckup et al. 2007, Reis et al. 2016, Birindelli & Sidlauskas 2018, Malabarba & Malabarba 2020). In addition to the lack of taxonomic and ecological knowledge, the rapid loss and degradation of natural environments as a result of anthropogenic drivers (i.e. invasive species, climate change, abstraction, pollution etc) has affected many fish species (Agostinho et al. 2008, Nogueira et al. 2010, Azevedo-Santos et al. 2019, Castro & Polaz 2020). Fish species which are particularly at risk are either charismatic megafauna or small sized fish species, the latter of which unfortunately receive rather less conservation attention (He et al. 2019, Castro & Polaz 2020).

Due to the alarming rate of biodiversity loss, combined with multiple and interacting anthropogenic stressors, freshwater ecosystems are facing a "biodiversity crisis" (Darwall et al. 2018, Harrison et al. 2018). In the last two decades, the rate of species extinctions worldwide has been much higher than natural extinction rates, with the subsequent extinction of thousands of species and loss populations, several of them still unknown to the science.

This is of great concern within freshwater environments as the biotic communities represent around 6% of currently described species and yet are vastly understudied commensurate to the species diversity and ecosystem services that they represent (Dudgeon et al. 2006, Lynch et al. 2020). The mis-match in data availability and research output compared to intrinsic value is notable in Brazilian freshwater systems, whereupon species are threatened before they even are described and their ecology characterised (Wilson 1985, 1992, Brooks et al. 2002, Olson et al. 2002, Singh 2002, Brook et al. 2006, Laurance 2007, Wheeler 2008, Costa et al. 2012, Kalinkat et al. 2017, Azevedo-Santos et al. 2019).

Freshwater environments and the biota within are more vulnerable to global change than marine and terrestrial ecosystems, as such they warrant the need for urgent and special attention regarding diversity estimations and conservation actions (Arthington et al. 2016, Darwall et al. 2018, Harrison et al. 2018, Azevedo-Santos et al. 2019, Castro & Polaz 2020). Unfortunately, designation of protected areas combined with current conservation policies, especially in Brazil, have limited efficacy in protecting freshwater biodiversity (Azevedo-Santos et al. 2019, Castro & Polaz 2020). Due to the continued high rate of habitat destruction, the identification of new species and comprehensive compilations of regional inventories as well as improving the taxonomic resolution of under-studied and taxonomically confusing is a research priority. It is imperative that this occurs before these species and populations are extinct, so that appropriate interventions can be actioned, especially in areas of high risk of anthropogenically driven change (Brook et al. 2006, Laurence 2007, Wheeler 2008, Costa et al. 2012, Pimm et al. 2014, Darwall et al. 2018, Oliveira-Silva et al. 2018, Harrison et al. 2018, Frota et al. 2019).

The Munim River basin (~16.000 km2) is a coastal river basin located at the northeastern portion of the State of Maranhão (Ribeiro et al. 2014). It represents one of the main river drainages of the Hydrological unit Maranhão sensu Hubbert and Renno (2006) (Hereafter Mrn). This eastern portion of the Hydrological unit has conspicuously few published fish surveys and ichthyological taxonomic studies. Ribeiro et al. (2014) published a study on an artisanal fishing technique (known as Moita) conducted at the upper Munim River basin, listing 20 fish species, predominantly middle to large-sized species. Matavelli et al. (2015) conducted an inventory of fishes associated with tadpole community in lentic and lotic environments in northeastern Maranhão (some of the sampled areas belonging to the lower Munim River basin) listing 13 fish species. Guimarães et al. (2018a,b) described two new species of Characidae occurring at the upper Munim River basin; and Nunes et al. (2019) carried out a work on length-weight relationship of 15 species from the upper Munim River basin. This gap in knowledge, specifically relating to fish taxonomy and species assemblages is a shared trait throughout the Munim River Basin as well as other river drainages and basins from Mrn (Piorski, 2010, Guimarães et al. 2018a). Therein there is a massive lack of information related to the taxonomy and systematics of the species and groups, species composition, geographical distribution and biogeography of the ichthyofauna from this region.

The fluvial channels in the State Maranhão are constantly threatened by degradation. This includes: removal of riparian forests; pollution and contamination of rivers; occurrences of erosion processes intensified by human activities; and abstraction and fragmentation of watercourses. The Munim River basin is no exception to this trend (Ribeiro et al. 2006, Silva et al. 2008, Lima et al. 2009), and there is a high likelihood that this will significantly impact the regional biodiversity (Pelice et al. 2017).

Pervasive and damaging stressor effects upon a data deficit system means that subjects such as fish biodiversity and taxonomy within the Mrn should be urgently addressed in order to combat further losses within the region.

The present study aims to provide a fish species inventory of the Mata da Itamacaoca and compare the species listed by our survey with the species listed by the other published works conducted at the upper Munim River basin. The study area is an urban protected area from the upper Munim River basin, Northern Brazilian Cerrado, a biome considered as one of the world biodiversity hotspots according to Myers et al. (2000). This study is especially important, being considered a baseline for the region, because it was conducted within an urban protection area, which is more exposed to human impacts than other protected areas. In addition, due to fact the Mata de Itamacaoca is an urban protected area, we believe that it has an ichthyofauna representation closer to the original of the region than the other degraded areas. This thus allowed for us to estimate the ichthyofauna diversity for the upper Munim River basin, especially regarding the small-sized, rare, and more ecologically demanding species. We also assessed fish diversity with regards to season and position above or below the reservoir in order to assess for possible effects of fragmentation.

## Materials and Methods

This study was conducted in the Mata de Itamacaoca, an urban protected area belonging to CAEMA (Companhia de Saneamento Ambiental do Maranhão). It is located within the Municipality of Chapadinha, State of Maranhão (24°25'47" S, 58°44'05" W), and is approximately 90 meters above sea level. The predominant biome in the region is the Brazilian Cerrado (Figure 1).
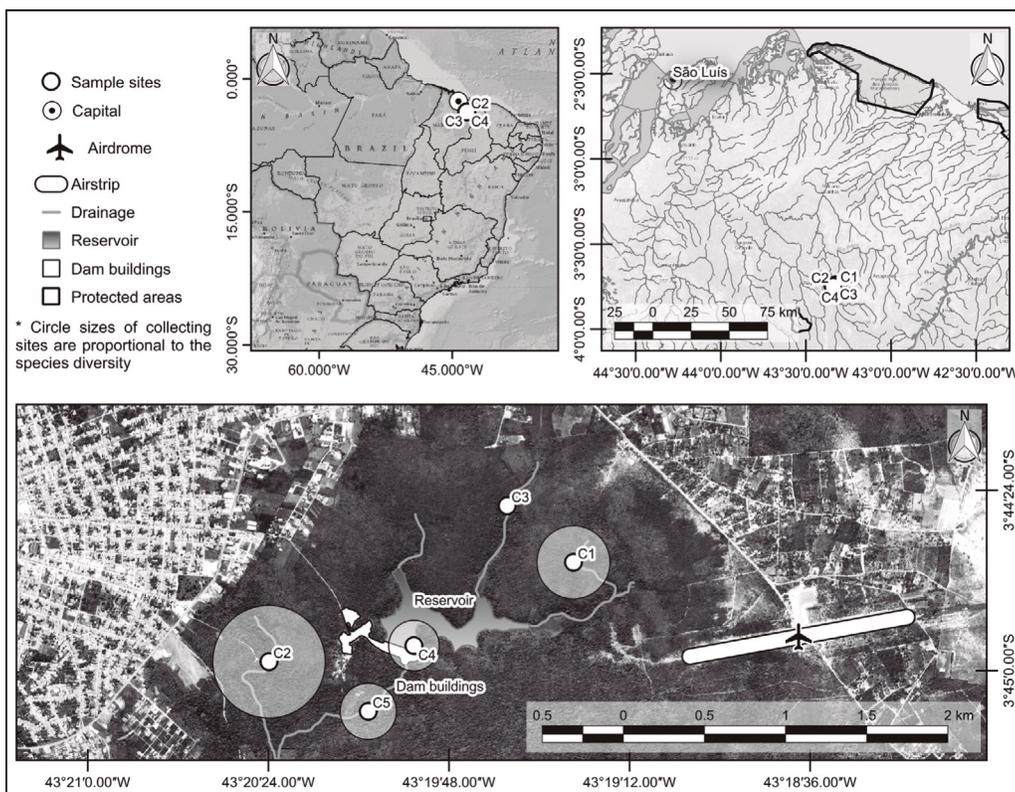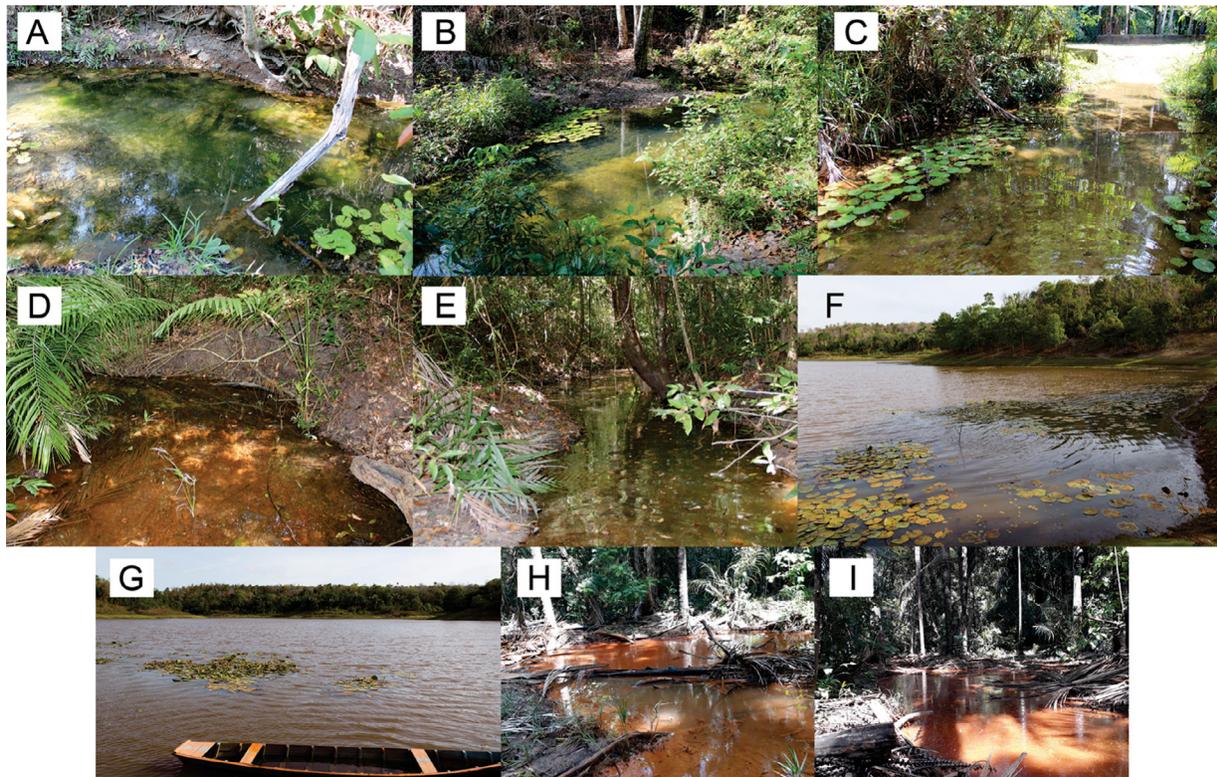


**Figure 1.** Map of the studied area. Collecting sites (C1-C5) listed in Table 1.

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The study area covers about 460 hectares consisting of a mosaic of plant formations including, along with the watercourses, riparian and gallery forests, as well as some stream springs; and formation of closed forest, with trees reaching more than 10 meters in height. The protected area was created to maintain water supply to the city, thus the need to preserve the integrity of vegetation around the springs, water bodies and reservoir (Silva et al. 2008). It is also important to emphasize that this area has been recognized as an Area of Relevant Ecological Interest for the conservation of fauna and flora by the Decreto Municipal Nº 05/2018.

The collection of samples was conducted at five sample sites (C1-C5) distributed within the boundaries of Mata de Itamacaoca, upper Munim River basin, comprising springs, streams, pools, and a reservoir (Table 1, Figures 1 and 2). A total of 42 collection expeditions were conducted. The collections occurred from August 2014 to February 2020, during both dry and wet seasons. All the collection expeditions were conducted during daylight, except for a one off night collection expedition conducted in October 2019 at C4.

**Table 1.** Samples localities at the Mata de Itamacaoca, upper Munim River basin, State of Maranhão, Brazil.

| Collecting site | Coordinates | Altitude (meters) | water temperature (°C) | water ph | Remarks |
|---|---|---|---|---|---|
| C1 | 3°44'45.20"S 43°19'15.10"W | ~80 | ~ 28.1 | ~ 6.2 | Stream near spring, with gallery and riparian forest, at Mata de Itamacaoca, Municipality of Chapadinha, State of Maranhão. |
| | | | | | Obs.: collections on this site were conducted through about 200 meters along the water course. |
| C2 | 3°44'58.24"S 43°20'23.91"W | ~90 | ~26.2 | ~ 6.6 | Stream in the locality Repouso do Guerreiro, at Mata de Itamacaoca, Municipality of Chapadinha, State of Maranhão. |
| C3 | 3°44'27.1"S 43°19'36.4"W | ~80 | ~ 26.8 | ~ 6.4 | Stream near spring, with gallery and riparian forest, at Mata de Itamacaoca, Municipality of Chapadinha, State of Maranhão. |
| C4 | 3°44'55.16"S 43°19'57.10"W | ~80 | ~ 30.7 | ~6.6 | Itamacaoca dam, Municipality of Chapadinha, State of Maranhão. |
| C5 | 3°45'8.20"S 43°20'4.13"W | ~75 | ~28.5 | ~6.6 | Stream, after the dam at Mata de Itamacaoca, Municipality of Chapadinha, State of Maranhão. |
| | | | | | Obs.: collections on this site were conducted along a trail of about 500 along the water course. |



**Figure 2.** Collecting sites (C1-C5) listed in table 1. A and B - C1, C - C2, D and E - C3, F and G - C4, and H and I - C5. Photographed by Felipe Ottoni.

Fishes were collected using manual trail-net (2 m long × 1.8 m high; mesh size, 2 mm), cast nets (2 m height, mesh size 15 mm), gillnets of various mesh sizes (15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 100 mm), and dip nets (mesh size 5 and 10 mm). We tried to collect fishes using traps, such as "fish trap", but we were not successful. The ichthyological material obtained in the samples was euthanized in a buffered solution of ethyl-3-amino-benzoate-methanesulfonate (MS-222) at a concentration of 250 mg/l until completely ceasing opercular movements, according to animal welfare laws and guidelines (Close et al. 1996, 1997, Leary et al. 2013). Specimens selected for morphological analysis were fixed in formalin and left for 15 days, after which they were preserved in 70% ethanol. Molecular data also obtained from specimens, and preserved in absolute ethanol. Sorting and identification of specimens were carried out at the Laboratório de Sistemática e Ecologia de Organismos Aquáticos of the Universidade Federal do Maranhão using specialized bibliography for each taxonomic group and consulting experts. The ichthyological material is deposited in the Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais of the Universidade Federal do Maranhão (CICCAA). A list of all the examined material is presented in Appendix 1. The taxonomic classification, the names of species considered as valid, authors and years of species descriptions, and geographic distribution, were based on the compilations proposed by Fricke et al. (2020a,b), where the authors gather all the most recent classifications for each group of fish.

In order to assess whether this baseline fish community assessment differed between season and location above or below the dam wall, diversity indices were calculated for each discrete sampling events per year. Whereupon, sampling in the dry season above the dam wall was n=27, rainy season above the dam wall n=24, dry season below the dam n=5 and rainy season below the dam wall was n=7.

As the data was unbalanced, a generalised linear model (GLM) was used to determine whether season (dry or rainy) and location with regards to the dam wall (above or below), including an interaction term, affected the species richness (Sprich).

Shannon-Weiner Index of Diversity (Shannon 1948) was calculated for each sampling date at site, season and locality above and below the dam using the package 'vegan' version 2.5-5 (Oksanen et al. 2019). This index describes the entropy of a given community:

$$(H) = \sum_{i=1}^{s} p_i \ln p_i \qquad (1)$$

Where H is the Shannon diversity index, which has no bounded upper value, S is the total number of species in the community, $P_i$ is the proportion of S made up of the $i$th species. Shannon's evenness (eqn 2) was calculated from the results of eqn 1:

$$E_H = H/H_{max} = ln\,S \qquad (2)$$

Where $E_H$ is Shannon's evenness, H is Shannon's diversity index, and $H_{max}$ is the natural log of total species detected in the basin. $E_H$ is bounded between zero and one where one represents complete evenness. In some cases the $E_H$ could not be calculated due to zeros in the dataset. Separate GLMs were completed on the H and $E_H$ values using the same terms as above.

Fish community assemblages and associations regarding season and the dam wall were visualized using non-metric multidimensional scaling (nMDS) ordination via 'vegan' (Okasanen et al. 2019). The community data matrix was compiled using the species counts from the survey and environmental data included was site, season and location to the dam wall, as in the analyses above. A one-way PERMANOVA using Bray-Curtis non-metric similarity and 999 permutations was then used to test for significant effects of environmental factors on fish species abundance. Community data was square-root transformed and Wisconsin double standardization was applied (*vegan::metaMDS*). Ordination stress was used to assess whether a two-dimensional ordination biplot was suitable to represent community data variation. Stress values < 0.15 were considered appropriate (Quinn & Keough 2002, Cousins et al. 2017). All statistical analyses were performed within the R software environment version 4.0.2 (R Core Team 2020).

## Results

We sampled 18,289 specimens representing six orders, 13 families, and 23 fish species (Tables 2 and 3, Figures 3, 4 and 5). The order and family with the highest species richness, considering all reaches, were Characiformes and Characidae, respectively, followed by Cichliformes and Cichlidae, respectively (Figures 4 and 5). *Nannostomus beckfordi* Günther, 1872 was the most abundant species, with about 8,000 specimens sampled. *Pimelodella parnahybae* Fowler, 1941 and *Hoplerythrinus unitaeniatus* (Spix & Agassiz 1829) were the rarest species represented, with only two specimens collected for each species (see appendix 1). We found the highest species richness at sample site (C2), with a total of 20 species, followed by C1 with 13, C5 with 10, C4 with 9, and C3 with only 2 (Table 3). Three putatively undescribed species were collected: *Hemigrammus* sp.1, *Hemigrammus* sp. 2, and *Curimatopsis* aff. *cryptica*. All of the collected species were small/middle-sized native species as there were no exotic nor large-sized species collected in the studied area.

There were three categories of fishs based on their occurence: 1) Only found above the dam wall: *Copella arnoldi* (Regan, 1912), and *Hemigrammus* sp. 2; 2) only found below the dam: *Astyanax* cf. *bimaculatus*, *Brachyhypopomus* sp., *Characidium* sp., *Hoplerythrinus unitaeniatus*, *Knodus victoriae* (Steindachner, 1907), *Moenkhausia oligolepis* (Günther, 1864), *Pimelodella parnahybae*, *Poecilia sarrafae* Bragança & Costa, 2011 and *Steindachnerina notonota* (Miranda Ribeiro, 1937); and 3) Above and below the dam: *Anablepsoides vieirai* Nelson, 2016, *Apistogramma piauiensis* Kullander, 1980, *Cichlasoma* cf. *zarskei*, *Crenicichla brasiliensis* (Bloch, 1792), *Curimatopsis* aff. *cryptica*, *Gymnotus carapo* Linnaeus, 1758, *Hemigrammus* sp. 1, *Hoplias malabaricus* (Bloch, 1794), *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa & Ottoni, 2018, *Megalechis thoracata* (Valenciennes, 1840), *Nannostomus beckfordi* and *Synbranchus marmoratus* Bloch, 1795 (see Table 3).

There was no interaction effect on Sprich by season and location to the dam wall, nor a main effect of season (Table 4, Figure 6). Location to the dam wall did have a significant main effect on Sprich, whereupon sites below the dam wall had higher Sprich than those above (z=1.90, p=0.05; Table 4, Figure 6). There were no significant effects of any factors or interactions on Shannon diversity index (H) or on Shannon evenness ($E_H$) (Table 4, Figure 6).

The nMDs showed obvious distinctions in fish communities above and below the dam wall (Figure 7). The ordination stress was 0.05 and therefore appropriate to display on a two-dimensional scale and and acceptable representation of the community data. The PERMANOVA showed that the sample sites themselves contributed

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**Table 2.** List of species recorded for the Mata de Itamacaoca, upper Munim River basin, State of Maranhão, Brazil. The categories are defined as: native (N), endemic to the Hydrological units Maranhão and Parnaíba *sensu* Hubbert and Renno (2006) (E), widely distributed along Northeastern Brazil river basins (NE), probably undescribed species (U), with known distribution to the Amazon River basin (A), and widely distributed along several river basins of the Neotropical Region, including river basins located south to the Amazon River basin (W). Information obtained from Fricke et al. (2020b).
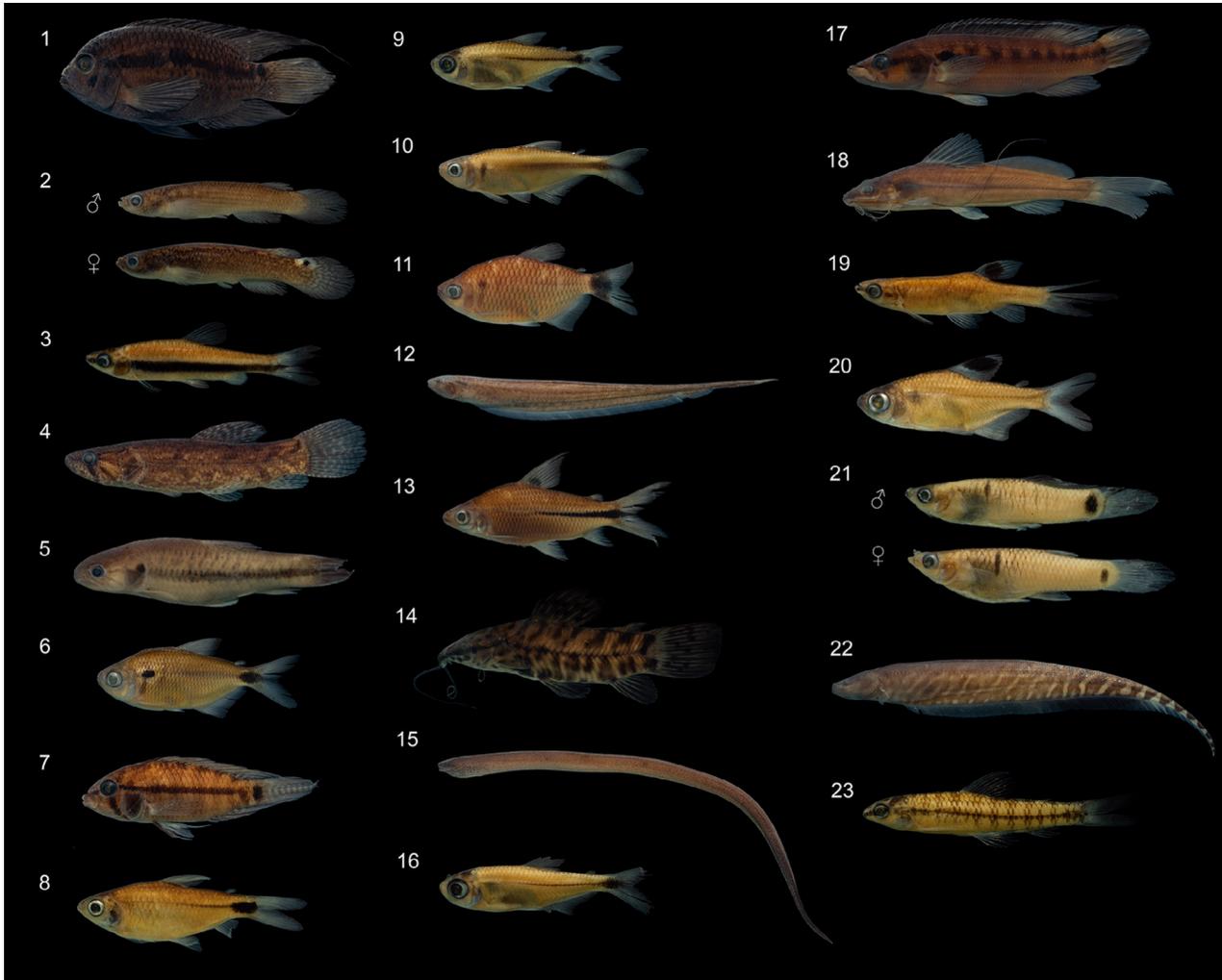
| CLASS/ORDER/FAMILY/GENUS/SPECIES | Category |
|---|:---:|
| **CLASS ACTINOPTERYGII** | |
| **Order Characifomes** | |
| **Family Characidae** | |
| *Astyanax* cf. *bimaculatus* | N |
| *Hemigrammus* sp. 1 | N, U |
| *Hemigrammus* sp. 2 | N, U |
| *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa & Ottoni, 2018 | N, E |
| *Knodus victoriae* (Steindachner, 1907) | N, E |
| *Moenkhausia oligolepis* (Günther, 1864) | N, A |
| **Family Crenuchidae** | |
| *Characidium* sp. | N |
| **Family Curimatidae** | |
| *Curimatopsis* aff. *cryptica* | N, U |
| *Steindachnerina notonota* (Miranda Ribeiro, 1937) | N, NE |
| **Family Erythrinidae** | |
| *Hoplerythrinus unitaeniatus* (Spix & Agassiz, 1829) | N, W |
| *Hoplias malabaricus* (Bloch, 1794) | N, W |
| **Family Lebiasinidae** | |
| *Copella arnoldi* (Regan, 1912) | N, A |
| *Nannostomus beckfordi* Günther, 1872 | N, A |
| **Order Cichliformes** | |
| **Family Cichlidae** | |
| *Apistogramma piauiensis* Kullander, 1980 | N, E |
| *Cichlasoma* cf. *zarskei* Ottoni, 2011 | N |
| *Crenicichla brasiliensis* (Bloch, 1792) | N, NE |
| **Order Cyprinodontiformes** | |
| **Family Poeciliidae** | |
| *Poecilia sarrafae* Bragança & Costa, 2011 | N, E |
| **Family Rivulidae** | |
| *Anablepsoides vieirai* Nelson, 2016 | N, E |
| **Order Gymnotiformes** | |
| **Family Gymnotidae** | |
| *Gymnotus carapo* Linnaeus, 1758 | N, W |
| **Family Hypopomidae** | |
| *Brachyhypopomus* sp. | N |
| **Order Siluriformes** | |
| **Family Callichthyidae** | |
| *Megalechis thoracata* (Valenciennes, 1840) | N, W |
| **Family Heptapteridae** | |
| *Pimelodella parnahybae* Fowler, 1941 | N, E |
| **Order Synbranchiformes** | |
| **Family Synbranchidae** | |
| *Synbranchus marmoratus* Bloch, 1795 | N, W |

**Table 3.** Species collected (X) in each collecting site (C1-C5) according to the Table 1 at Mata de Itamacaoca, upper Munim River basin, State of Maranhão, Brazil.
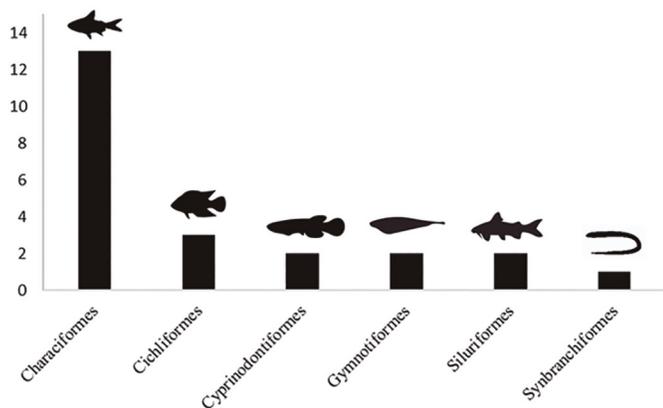
| Species | C1 | C2 | C3 | C4 | C5 |
|---|---|---|---|---|---|
| *Astyanax* cf. *bimaculatus* | | X | | | X |
| *Hemigrammus* sp. 1 | X | X | | X | X |
| *Hemigrammus* sp. 2 | | | | X | |
| *Hyphessobrycon piorskii* | X | X | | X | |
| *Knodus victoriae* | | X | | | |
| *Moenkhausia oligolepis* | | X | | | |
| *Characidium* sp. | | X | | | |
| *Curimatopsis* aff. *cryptica* | X | X | | X | |
| *Steindachnerina notonota* | | X | | | X |
| *Hoplerythrinus unitaeniatus* | | X | | | |
| *Hoplias malabaricus* | X | X | | X | |
| *Copella arnoldi* | X | | | | |
| *Nannostomus beckfordi* | X | X | X | X | X |
| *Apistogramma piauiensis* | X | X | | X | X |
| *Cichlasoma* cf. *zarskei* | X | X | | X | X |
| *Crenicichla brasiliensis* | X | X | | X | |
| *Poecilia sarrafae* | | X | | | X |
| *Anablepsoides vieirai* | X | X | X | | X |
| *Gymnotus carapo* | X | | | | X |
| *Brachyhypopomus* sp. | | X | | | |
| *Megalechis thoracata* | X | X | | | X |
| *Pimelodella parnahybae* | | X | | | |
| *Synbranchus marmoratus* | X | X | | | |
| **Species richness** | **13** | **20** | **2** | **9** | **10** |

**Table 4.** Model terms for all factors and interactions from GLMs used to determine differences in a) species richness, b) Shannon-Weiner diversity index (H) and Shannon's evenness (EH). Significant p-values are highlighted in bold.

| Model term | Est | SE | *t*-value | *p*-value |
|---|---|---|---|---|
| **a) Species richness** | | | | |
| Season | -0.14 | 0.77 | -0.12 | 0.90 |
| Location to dam | 4.71 | 1.95 | 2.41 | **0.02** |
| Season*Location to dam | -4.46 | 2.60 | -1.71 | 0.09 |
| **b) Shannon-Weiner (H)** | | | | |
| Season | -0.00 | 0.28 | -0.01 | 0.99 |
| Location to dam | 0.77 | 0.49 | 1.57 | 0.12 |
| Season*Location to dam | -0.77 | 0.66 | -1.17 | 0.25 |
| **c) Shannon's Evenness (EH)** | | | | |
| Season | -0.01 | 0.01 | -0.92 | 0.36 |
| Location to dam | -0.00 | 0.02 | 0.20 | 0.84 |
| Season*Location to dam | 0.01 | 0.02 | 0.55 | 0.58 |

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**Figure 3.** Fish species collected at Mata de Itamacaoca: 1- *Cichlasoma* cf. *zarskei* (CICCAA 03877, 97.3 mm SL), 2 - *Anablepsoides vieirai* (CICCAA 03729, male 29.9 and female 39.4 mm SL), 3- *Nannostomus beckfordi* (CICCAA 03732, 28.9 mm SL), 4- *Hoplias malabaricus* (CICCAA 03880, 96.2 mm SL), 5- *Hoplerythrinus unitaeniatus* (CICCAA 02512, 116.5 mm SL), 6- *Astyanax* cf. *bimaculatus* (CICCAA 03754, 54.2 mm SL), 7 - *Apistogramma piauiensis* (CICCAA 04585, 39.9 mm SL), 8- *Curimatopsis* aff. *cryptica* (CICCAA 02014, 33.6 mm SL), 9 - *Hemigrammus* sp.1. (CICCAA 04593, 26.0 mm SL), 10 – *Knodus victoriae* (CICCAA 02466, 32.5 mm SL), 11- *Moenkhausia oligolepis* (CICCAA 04731, 53.1 mm SL), 12 - *Brachyhypopomus* sp. (CICCAA 02457, 95.1 mm TL), 13- *Steindachnerina notonota* (CICCAA 04729, 67.15 mm SL), 14 - *Megalechis thoracata* (CICCAA 03447, 47.5 mm SL), 15 - *Synbranchus marmoratus* (CICCAA 03400, 137.8 mm TL), 16 - *Hemigrammus* sp.2 (CICCAA 02555, 22.9 mm SL), 17 - *Crenicichla brasiliensis* (CICCAA 03402, 104.3 mm SL), 18 - *Pimelodella parnahybae* (CICCAA 03753, 60.1 mm SL), 19 – *Copella arnoldi* (CICCAA 00081, 26.2 mm SL), 20 - *Hyphessobrycon piorskii* (CICCAA 02421, 22.7 mm SL), 21 - *Poecilia sarrafae* (CICCAA 02506, male 20.6 and female 24.5 mm SL), 22 - *Gymnotus carapo* (CICCAA 00879, 96.8 mm TL), and 23 - *Characidium* sp. (CICCAA 03751, 26.1 mm SL). Photographed by Felipe Ottoni.



**Figure 4.** Ranking of richness by orders observed in the studied area. The numbers in the left column correspond to the number of species.



**Figure 5.** Ranking of richness by families observed in the studied area. The numbers below the graphic correspond to the number of species. The grey bar evidences the most species-rich family of this study.

Ichthyofauna of the Mata de Itamacaoca
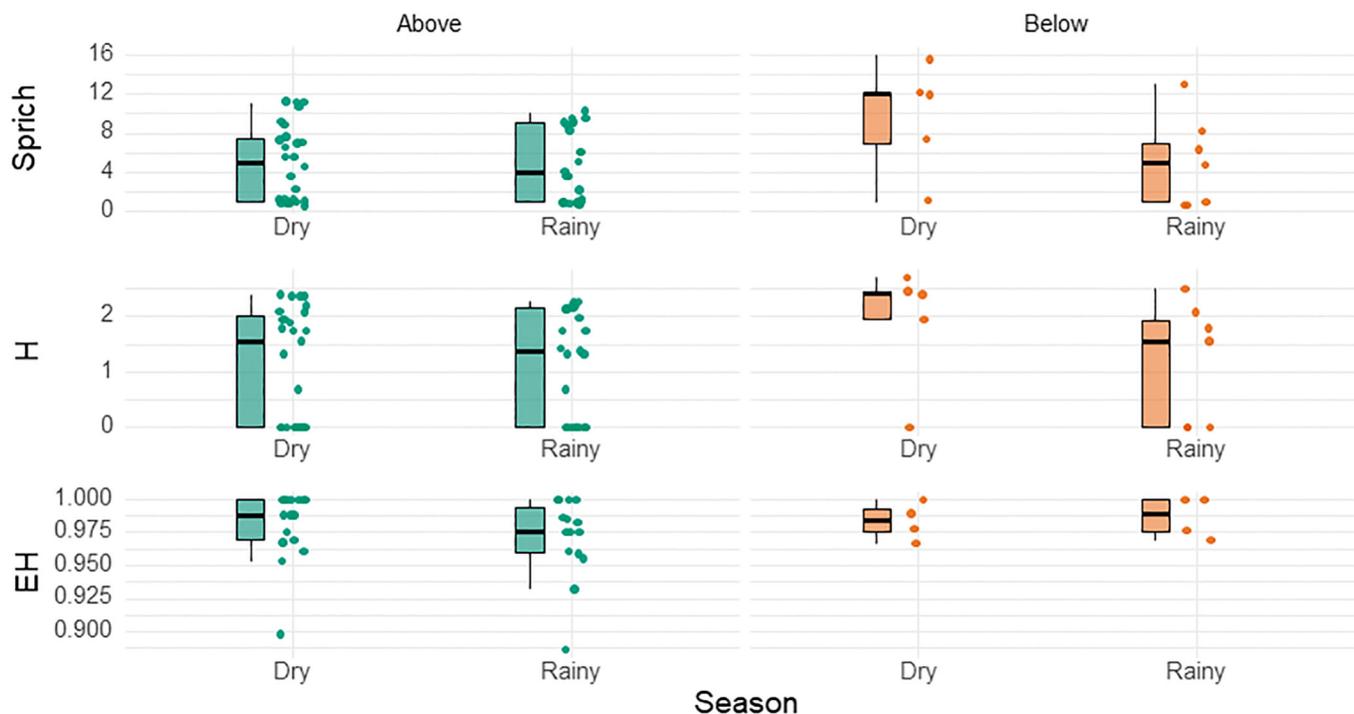


**Figure 6.** Species richness (Sprich), Shannon-Weiner diversity (H), and Shannon's Evenness (EH) of sampling sites in the dry and rainy seasons, above and below the dam wall. Boxplot indicates median and inter-quartile ranges while points indicate species richness per site.
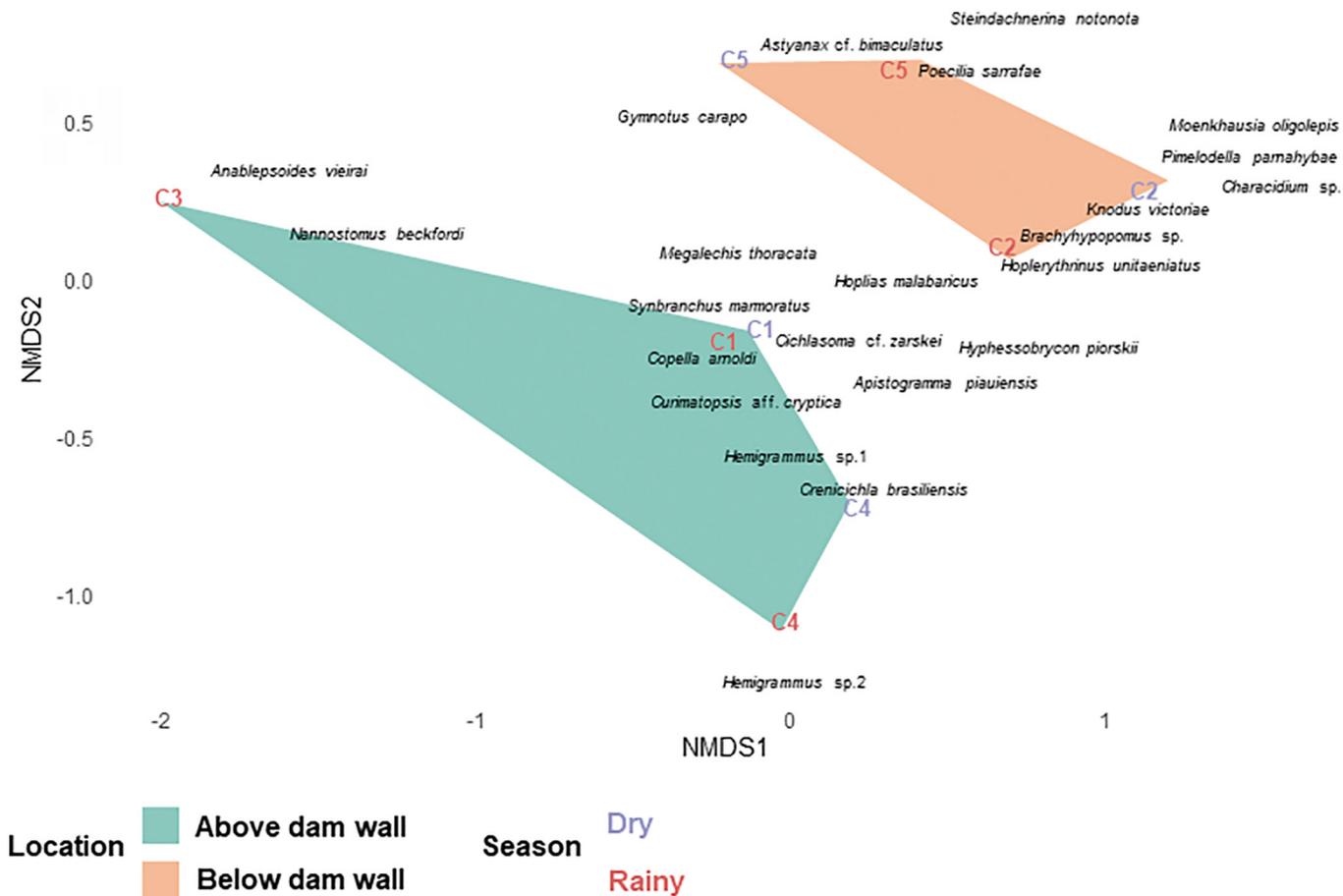


**Figure 7.** Biplot of nMDS ordination of fish communities of the Mrn above and below the dam wall in dry and rainy seasons. Convex hulls denote sites with relation to the dam wall.

to 70% of the variance in fish community ($R^2 = 0.70$, $F_{3,8} = 11.00$, p < 0.001), and position to the dam wall was responsible for 18% of the variance ($R^2 = 0.18$, $F_{3,8} = 8.54$, p < 0.01). Season did not have an effect on fish community ($R^2 = 0.04$, $F_{3,8} = 2.26$, p = 0.13).

## Discussion

The Mrn urban protected area appears to have efficiently conserved freshwater fish biodiversity, as all of the 23 fish species registered to in the study area are native species (there are no introduced species). Another curious fact is the absence of large-sized species in the studied area. This could be explained by the history of the construction of the dam, which was formed by the damming of small streams, not including originally large rivers. In addition to the high sampling effort, information provided by local fishermen confirms the absence of large-sized fish species in the area. Small-sized fish species are the most threatened among the Neotropical freshwater fish fauna (small-sized fish species comprise around 250 species - about 80% - of the total endangered fish species). This size class of fish represents about 70% of fish species richness of the Neotropical Region, however, they are usually overlooked by the general public as well as conservation agencies and organizations in favour of large charismatic species (Kalinkat et al. 2017). Further, threats to small fish species are enhanced due to their habitats being far smaller, often fragmented, linear systems that are usually more vulnerable to human impacts (Arthington et al. 2016, Castro & Polaz 2020). Generating robust baseline data of ecological, biological, geographic distributional and taxonomic aspects of these species ought to be a conservation priority, especially in under-studied Neotropical regions.

The Mata da Itamacaoca is an urban protected area from the upper Munim River basin, Northern Brazilian, located at the Municipality of Chapadinha-MA, in the Northern Brazilian Cerrado. This protected area is efficient in protecting freshwater biodiversity, since it includes not only the main tributaries of the area, but also the reservoir itself aswell as associated springs (Azevedo-Santos et al. 2018). This has protected the system from a series of urban impacts in this protected area, such as pollution and introduced species, which are present in tributaries, rivers and small streams not included in the protected area. The designated area was specifically designated by the CAEMA (Companhia de Saneamento Ambiental do Maranhão) with the aim to supply the city of Chapadinha-MA with water (including potable water) (Silva et al. 2008). The CAEMA built the reservoir to store water for use during the regions severe dry season, thus providing the city with access to water throughout the year. The region where the dam was created was composed of several streams and spring, the main one being the Itamacaoca stream (Silva et al. 2008) and includes streams and fountains in the vicinity to prevent the water pollution of the reservoir. While the study area is currently in pristine condition with regards to non-native alien species, the proximity to the city, Brazilian legislation changes and the nature of the reservoir puts the protected area at risk of invasion in the future (Garcia et al. 2017, Pelicice et al. 2018, Geller et al. 2020). Dams can act as invasion hubs as well as modifying environmental conditions which allow non-native species to proliferate, all of which can contribute towards biotic homogenisation (Bunn & Arthington 2002, Daga et al. 2020), as well as acting as a barrier for natural species, which are restricted to parts above or below the dam. Indeed, the clear different fish community composition above and below

the dam wall indicates that despite the comparable species diversity and richness, there is evidence of alteration in the communities. Future work should identify whether these communities are also separated in terms of functional trait based metrics, as this can provide information on community resilience and biotic resistance (Moyle & Light 1996, Olden et al. 2010, Brito et al. 2020).

None of the species inventoried in the present study have the status of threat of extinction (ICMBIO, 2018). Some species could not be categorized because we were unable to reach species-level identification, or were described after the publication of the red book. However, the species *Hyphessobrycon piorskii* recorded by the present study is probably not threatened with extinction, since it does not have a restricted distribution and also occurs in another federal protected area, the Lençóis Maranhenses National Park (Guimarães et al. 2018b, 2019, Brito et al. 2019, 2020).

The order and family with the highest species richness in the Mata de Itamacaoca, was Characiformes and Characidae, respectively, followed by Cichliformes and Cichlidae, respectively. Characiformes and Characidae are usually the most species-rich groups (Order and Family, respectively) found in any freshwater fish survey of the Neotropical Region. Usually, the second most representative Order is Silurifomes (e.g. Lucinda et al. 2007, Ferreira et al. 2011, Claro-García & Shibatta 2013, Ramos et al. 2014). This trend diverges in the Mata de Itamacaoca, where Cichliformes and Cichlidae (Order and Family, respectively) are the second most diverse groups. This difference found here in the composition pattern of the fish community is probably due to the absence of the Family Loricariidae in the studied area, which is usually one of the most representative freshwater fish families in Neotropical region inventories (e.g. Lucinda et al. 2007, Ferreira et al. 2011, Claro-García & Shibatta 2013, Ramos et al. 2014).

Published studies on fish inventories and taxonomic studies are very scarce in the upper Munim River basin. Recently, two new species were described which occur in the river portion (Guimarães et al. 2018a,b). However, only *Hyphessobrycon piorskii* occurs at Mata the Itamacaoca, including type material (see Appendix 1, Tables 2 and 3, Figure 5, Guimarães et al. 2018b). The dataset from the present study documented more species than previous work by Ribeiro et al. (2014) and Nunes et al. (2019) which list between 15-20 middle to large sized species present in the upper Munim River basin, of which, the majority of the species were not the same. Comparing the present study to Ribeiro et al. (2014) only four species were the same: *Crenicichla brasiliensis* [*Crenicichla menezesi* Ploeg, 1991 in Ribeiro et al. (2014)], *Hoplias malabaricus*, and *Synbranchus marmoratus*. Whereas, compared to Nunes et al. (2019), only three species were the same: *Astyanax* cf. *bimaculatus*, *Crenicichla brasiliensis*, and *Hoplias malabaricus*. This study represents the first time that an ictythological survey conducted at the upper Munim River basin has presented photographs of all of the recorded species and provided specific voucher number for each examined fish lot. By including this type of information it promotes transparency by allowing other researchers to confirm the identifications, as well as facilitating further research by providing a much needed resource for fish identification. Currently, besides the present study, any records of fish inventories for the Mrn, which include illustrations, let alone photographs of the species collected are rare (e.g. Soares, 2013, Matavelli et al. 2015, Piorski et al. 2017).

We found the highest species richness at collecting site (C2), with a total of 20 species, followed by C1 with 13, C5 with 10, C4 with 9, and C3 with only 2 (see Appendix 1). The collecting sites C1, C2, C3 and C5 have the most preserved ecological integrity, while C4 (the reservoir) is the most modified area in relation to the original conditions. The reservoir dam inhibits dispersion of fishes occurring in C2, which is located below the reservoir dam. It is likely that this is driving the greater species richness in C2 compared to C1, C3 and C4. One other site, C5, is also located below the reservoir dam, however, its lower species richness compared to C2 could be explained by two reasons. Firstly, this collecting site was less sampled than C2, and secondly it dries almost completely during the dry season, while C2is permanently inundated. The low number of species in C3 is likely to be due to the sample being collected in one collection expedition due to issues in access to the site.

There were three categories of fishes based on their occurrence: 1) only found above the dam wall, 2) only found below the dam, and 3) above and below the dam. Despite these three distinct categories, it was not possible to observe any ecological pattern that correlates these species with these three distribution patterns. Similarly, despite the different fish community composition above and below the dam wall there is no concrete evidence nor immediate explanation for this. This is compounded by the regional data deficit and lack of historical data prior to the dam construction. Nonetheless, it is important to emphasize that the absence of *Anablepsoides vieirai* and *Copella arnoldi* at locality C4 was already expected, due to the ecological requirements of these species, which do not occur in this type of environment. In addition, the absence of *Pimelodella parnahybae* in the rainy season could be explained because catfishes usually inhabit caves or burrows and are usually associated with the benthos. As in the dry season the water level is lower, it is easier to collect catfishes, since we can access the bottom of the river more easily. Possible reasons for this differentiation, which ought to be further explored, are the effects of habitat filtering, river continuum concept and functional traits of the fish community. This research would further both the conservation of Neotropical freshwaters but also challenge or confirm whether these concepts subscribe to theories that have generally been developed in temperate systems and have data gaps in the tropics (Boulton et al. 2008, Dudgeon 2008, Sternberg & Kennard, 2013).

From the 23 species herein recorded, we were not able to accurately identify seven species to the species level. Three of them correspond to new species (*Curimatopsis* aff. *cryptica*, *Hemigrammus* sp.1, and *Hemigrammus* sp.2), and are in the process of being described. The other four species need a more comprehensive taxonomic investigation, especially comparing with specimens from other populations from other river basins and regions, as already proposed for other fish groups occurring along the coastal river basins of the State of Maranhão by Guimarães et al. (2020). The State of Maranhão (northeastern Brazil), compared to other Brazilian regions, has distinctly few studies related to its freshwater ichthyofauna, especially in taxonomic studies (Piorski, 2010, Guimarães et al. 2018a, 2020). Thus, at the outset it was anticipated that some species would remain taxonomically indeterminate in this study and therefore was

a motivation for carrying out this inventory. Of the other 16 species we were able to identify accurately at the species level, six of them are endemic to the Hydrological units Maranhão and Parnaíba sensu Hubbert and Renno (2006) (hereafter Mrn and Prn, respectively). Two are widely distributed along Northeastern Brazil river basin. Five are widely distributed along several river basins of the Neotropical Region, including river basins located south to the Amazon River basin, and three also have their known distribution to the Amazon River basin. Therefore, we would like to emphasize that a significant number of the species registered here are endemic to the Mrn and Prn; and in addition, the composition of the ichthyofauna in the studied area has little influence from the Amazon basin. Thus, the present study contributes considerable addition to the knowledge around endemic small sized fishes. This information is integral for future planning and conservation endeavours as these species are disproportionately at risk (Arthrington et al. 2016). Further, our data mirrors trends seen in other tropical protected areas, wherein the fish species diversity is as yet undescribed but under high potential risk (Rico-Sánchez et al. 2020).

Considering the small size and similarity oh habitats within the sampled area, the diversity of fish species found in the Mata de Itamacaoca was surprisingly high. Comparing the number of species found by our study (23 species) with other studies that carried out inventories of entire river basins or much larger areas of the region. For example, Barros et al. (2011) (69 species) and Nascimento et al. (2016) (64 species) both inventoried the fish fauna of the entire Itapecuru River basin, a of the significant coastal river basins of the Mrn and Prn. Further, Brito et al. (2019, 2020) inventoried 56 species of freshwater fish from the Parque Nacional dos Lençóis Maranhenses and the adjacent areas. Therein, the Mata de Itamacaoca holds around a third of the number of the species reported in far larger systems, thus demonstrating the importance of the studied area in the protection of the fish fauna inhabiting it.

## Conclusions and Future Perspectives

This study corroborates other studies carried out in the Mrn that the ichthyofauna of this region still has many knowledge gaps, especially concerning the composition and taxonomy of the fish groups occurring in the basins. Especially regarding the diversity of small-sized fishes (Piorskii 2010, Guimarães et al. 2018a). The present study reveals three species not yet described in this study area, and one species recently described (in 2018). This shows the potential of the region in still having freshwater fish species which need a formal description. In addition, the fact that small streams can host a comparatively high species richness combined with the possibility of finding endemic and undescribed fish species emphasises need for these environments to be treated as priority in conservation policies. Further work should focus on the diversity of neglected small sized fish species with particular focus on taxonomy and community ecology in vulnerable Mrn stream environments. All species listed here are small and medium sized and some attention is needed for these species (Castro & Polaz 2020). Problems like the lack of knowledge about them, as well as the reduction of the original riparian vegetation cover lead to changes that affect the existence of these smaller species (Castro & Polaz 2020). An important facet to note is the success in the protected area designation, which is currently free

from harmful non-native species. With this respect, this area appears to be a valuable refuge for small fish species. Moreover, due to the constant anthropogenic change, this study may be a baseline for similar environments in the region as it presents a native fish assemblage unaffected by biotic drivers yet exposed to other abiotic drivers.

## Supplementary Material

The following online material is available for this article:
Appendix 1 - Examined material

## Acknowledgements

## Author Contributions

Elioenai da Silva Oliveira: Specimen Collection, expedition organization, data analysis, statistical analysis, manuscript writing.

Erick Cristofore Guimarães: Specimen Collection, expedition organization, data analysis, specimens identification, manuscript writing.

Pâmella Silva de Brito: Specimen Collection, expedition organization, data analysis, specimens identification, manuscript writing.

Lucas de Oliveira Vieira: Specimen Collection, expedition organization, data analysis, specimens identification.

Rafael Ferreira de Oliveira: Specimen Collection, expedition organization, data analysis, specimens identification.

Diego Sousa Campos: Specimen Collection, expedition organization, data analysis, specimens identification, map preparation.

Axel Makay Katz: Specimens identification, data analysis, image preparation, manuscript writing.

Josie South: Data analysis, Statistical analysis, manuscript writing, language revision.

Jorge Luiz Silva Nunes: Specimen Collection, expedition organization, data analysis, manuscript writing.

Felipe Polivanov Ottoni: Specimen Collection, expedition organization, data analysis, specimens identification, manuscript writing, project financing.

## Conflicts of Interest

The authors declare that they have no conflict of interest.

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