PEDRO HENRIQUE DOS SANTOS BASILIO

BENTHIC AND TERRESTRIAL INVERTEBRATES SUPPORT FISH COMMUNITIES OF AMAZONIAN STREAMS

LAVRAS – MG
2025

PEDRO HENRIQUE DOS SANTOS BASILIO

BENTHIC AND TERRESTRIAL INVERTEBRATES SUPPORT FISH COMMUNITIES OF AMAZONIAN STREAMS

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós Graduação em Ecologia Aplicada, área de concentração em Ecologia e conservação de recursos em paisagens fragmentadas e agrossistemas, para a obtenção do título de Mestre.

Prof. Dr. Paulo Santos Pompeu
Orientador

Profa. Dra. Cecília Gontijo Leal
Coorientadora

Profa. Dra. Débora Reis de Carvalho
Coorientadora

LAVRAS – MG
2025

Ficha Catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Basilio, Pedro Henrique dos Santos.
Benthic and terrestrial invertebrates support fish communities of Amazonian streams / Pedro Henrique dos Santos Basilio. - 2025.
46 p.

Orientador: Paulo dos Santos Pompeu
Coorientadora: Cecilia Gontijo Leal
Coorientadora: Débora Reis de Carvalho

Dissertação (Mestrado Acadêmico) - Universidade Federal de Lavras, 2025.
Bibliografia.

1. Isótopos Estáveis. 2. Fluxo de Energia. 3. Estrutura Trófica. 4. Vegetação Ripária. 5. Biomassa. I. Pompeu, Paulo dos Santos. II. Leal, Cecilia Gontijo. III. Carvalho, Débora Reis de. IV. Universidade Federal de Lavras. V. Título.

PEDRO HENRIQUE DOS SANTOS BASILIO

BENTHIC AND TERRESTRIAL INVERTEBRATES SUPPORT FISH COMMUNITIES OF AMAZONIAN STREAMS

INVERTEBRADOS BENTÔNICOS E TERRESTRES SUSTENTAM AS COMUNIDADES DE PEIXES DE RIACHOS AMAZÔNICOS

Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós Graduação em Ecologia Aplicada, área de concentração em Ecologia e conservação de recursos em paisagens fragmentadas e agrossistemas, para a obtenção do título de Mestre.

APROVADO em 14 de fevereiro de 2025
Dr(^{a}) Naraiana Loureiro Benone, UEMG
Dr(^{a}) Alessandra Angélica de Pádua Bueno, UFLA

Prof. Dr. Paulo Santos Pompeu
Orientador

Profa. Dra. Cecília Gontijo Leal
Coorientadora

Dra. Débora Reis de Carvalho
Coorientadora

LAVRAS – MG
2025

À Érica Pellegrini Caramaschi,
Pela amizade, inspiração e dedicação, que foram
fundamentais para minha chegada até aqui.

AGRADECIMENTOS

A Deus, pela oportunidade, saúde e força nos momentos difíceis;

À Universidade Federal de Lavras e ao Programa de Pós-Graduação em Ecologia Aplicada, por proporcionar um ambiente de aprendizado, pesquisa e desenvolvimento que foram essenciais para minha formação pessoal e acadêmica;

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pela bolsa de mestrado concedida, permitindo minha dedicação exclusiva à pesquisa;

À Lancaster University e à *United Kingdom Research and Innovation* (UKRI), pelo financiamento do projeto *Understanding and Conserving Tropical Freshwater Ecosystems*, ao qual sou vinculado;

À Rede Amazônia Sustentável (RAS) e a todos os seus pesquisadores, pelo grande trabalho desenvolvido na região amazônica ao longo desses anos;

Ao meu orientador, Paulo Pompeu, agradeço pela amizade e companheirismo ao longo de todo o mestrado, além de ser um pesquisador e professor ímpar, que se mantém próximo de seus orientados durante todo o percurso e nos dá autonomia para trilharmos nossos primeiros passos como pesquisadores. Sorte a minha ter tido você como guia no mestrado, Pomps! Gratidão pela sua vida!

À Cecília Gontijo Leal, minha coorientadora querida, não tenho palavras para agradecer pelas oportunidades que tive neste projeto incrível, que só é incrível por ser coordenado por você! Obrigado por existir, confiar em mim e no nosso projeto, e por todo apoio ao longo desse percurso. Gratidão pela sua vida também!

Agradeço à minha coorientadora, Débora Reis de Carvalho, pelos momentos incríveis que compartilhamos durante o campo. Nunca vou esquecer nossos dias aplicando protocolos e atolando (hahaha), mesmo irritados com as cabas, carapanãs, cobras e companhia. Ainda assim, sempre dávamos risadas da situação. E o momento de coleta, então? Nem se fala! Era diversão garantida! Nem parecia trabalho, porque o amor e a alegria com que você conduzia a equipe contagiavam a todos. Sou muito grato por tudo o que me ensinou sobre a metodologia de coleta, processamento e análise de dados isotópicos. Sua amizade ao longo desse período foi essencial para que eu chegasse até aqui. Gratidão pela sua vida, "Debra"!

À Dennys Drager e Gabriel Ferraz (Cake), Como diz a atriz Mônica Martelli: "Tem pessoas na vida que a gente esbarra, mas tem pessoas que a gente encontra." E é exatamente isso que vocês significam para mim: um grande encontro. Não consigo nem imaginar toda essa experiência sem vocês ao meu lado. Obrigado pelo suporte emocional nos momentos em que mais precisei e pelo acolhimento desde o dia em que nos permitimos ser amigos. Tenho um orgulho imenso de ser amigo de vocês! Obrigado por tanto carinho, que às vezes nem sei se mereço.

A Renilson Freitas (Graveto), nosso assistente de campo, por toda ajuda e suporte durante as coletas e, também, pelos momentos de descontração, que foram imprescindíveis para o sucesso de nossa equipe.

Agradeço aos demais integrantes que fizeram parte da equipe de campo em Santarém 2023: Gilberto Salvador, Thiago Couto, Everton Silva, Joás Brito, Maria Fernanda, Jady Santos, Guilherme Berger, Marcos Ângelo, Gustavo Ferreira. Aos professores: Rafael P. Leitão, Luciano Montag, José Max, Raphael Ligeiro, Daniel Carvalho e Gabriel Brejão, pela ótima experiência em campo e oportunidade de aprendizado.

Ao Laboratório de Ecologia de Peixes da UFLA, que sorte poder conviver diariamente com tanta gente massa! Obrigado a todos pela maravilhosa recepção e acolhimento quando cheguei, e por todo suporte ao longo desse período. Vocês moram no meu coração.

A Rosalva Sulzbacher, minha grande amiga de mestrado e laboratório, por todos os momentos em que pude dividir com você meus medos e inseguranças em relação à pós-graduação, e por todas as palavras de carinho e conforto.

Aos colegas de mestrado, “Ecolindos”, por terem me proporcionado um mestrado alegre e leve em meio à correria de estar num programa de pós-graduação. Levarei cada lembrança dos momentos felizes que tivemos juntos para sempre. Patrícia Michelle, Ingrid Araújo, Barbara Dutra, Sérgio Pugliano, Gabi Spuri, Yuri Arcova, Barbara Luyse, Amanda Serpa e Mariana Araújo.

A Chivago Inácio, não tenho palavras para explicar o quão importante foi ter você como “família” em Lavras. Você também foi um grande encontro que tive na vida, e espero nunca estar longe de você! Obrigado por tanto amor e carinho, amigo.

À minha família, por sempre me apoiarem, acreditarem em mim. Amo vocês, vó e irmã!

IGARAPÉ

Valdir Santos

Eu tomei banho no igarapé
Já fui picado pela tucandeira
Andei de canoa no rio madeira
Se eu pudesse, eu morava na
Floresta a vida inteira

A vida inteira, a vida inteira
Se eu pudesse, eu morava na
Floresta a vida inteira

Canta bonito o uirapuru
É diferente esse seu cantar
Tanta riqueza tem na região do norte
Eu sinto muita saudade, por isso quero voltar

RESUMO

A rede de riachos amazônicos é impressionante não apenas por sua vastidão, mas também por abrigar uma grande diversidade de espécies de peixes. Apesar da grande diversidade, pouco se sabe sobre aspectos ecológicos fundamentais dos riachos amazônicos, como o fluxo de energia e as fontes de carbono que sustentam sua rica ictiofauna. Neste estudo, usamos isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) para avaliar a estrutura trófica e identificar os principais recursos alimentares que sustentam a ictiofauna de riachos amazônicos. Coletamos assembleias de peixes e uma ampla gama de recursos disponíveis nos riachos, incluindo macrófitas, vegetação ripária, algas filamentosas, matéria orgânica, gramíneas, invertebrados bentônicos e terrestres, perifíton e material em suspensão. Em seguida, estimamos a estrutura trófica das assembléias, incluindo métricas de diversidade, nichos tróficos, posições tróficas, proporções de recursos assimilados e biomassa. A biomassa de peixes foi sustentada principalmente por invertebrados terrestres (31%–55%) e invertebrados bentônicos (22%–40%). Camarões representaram o terceiro recurso mais importante para a biomassa de peixes (9%–22%). Juntos, esses três recursos responderam por 79%–90% da biomassa total de peixes nos riachos estudados. A estrutura trófica e as métricas de diversidade trófica foram semelhantes em todos os pontos amostrais, como evidenciado pela sobreposição substancial nos nichos isotópicos das assembléias de peixes avaliadas. Embora as posições tróficas variassem em certa medida entre os locais, a maioria das espécies apresentaram níveis tróficos intermediários. Nossos resultados destacam a importância tanto dos invertebrados aquáticos quanto dos terrestres no suporte à dinâmica trófica dos peixes em riachos amazônicos, reforçando as interconexões entre os pequenos riachos e as paisagens que os circundam. Também evidenciam o papel desempenhado pelas florestas na manutenção da integridade dos ambientes aquáticos amazônicos, ao fornecerem recursos alimentares que sustentam as assembléias de peixes. Portanto, nosso estudo fornece informações essenciais sobre o fluxo de carbono e a ecologia isotópica em pequenos riachos amazônicos, os quais são vitais para compreender seu funcionamento, orientar esforços de conservação e assegurar o manejo sustentável dos recursos naturais.

Palavras-chave: Isótopos estáveis; Fluxo de energia; Estrutura trófica; vegetação ripária; biomassa.

ABSTRACT

The Amazonian stream network is impressive not only for its vastness but also for harboring a great diversity of fish species. Despite their high diversity, little is known about key ecological aspects of Amazonian streams such as energy flow and the carbon sources that sustain their species-rich fish assemblages. In this study we use carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes to evaluate the trophic structure and to identify the key food resources sustaining the ichthyofauna of Amazonian streams. We collected fish assemblages and a wide range of resources available in streams, including macrophytes, riparian vegetation, filamentous algae, organic matter, grasses, benthic and terrestrial invertebrates, periphyton, and suspended matter. We then estimated the trophic structure of the assemblages, including diversity metrics, trophic niches, positions, assimilated resource proportions, and biomass. Fish biomass was primarily supported by terrestrial invertebrates (31%–55%) and benthic invertebrates (22%–40%). Shrimps represented the third most important resource for fish biomass (9%–22%). Together, these three resources accounted for 79%–90% of the total fish biomass in the studied streams. The trophic structure and trophic diversity metrics were similar across all sampling sites, as evidenced by the substantial overlap in the isotopic niche of the assessed fish assemblages. Although trophic positions varied to some extent among sites, most species fed at intermediate trophic levels. Our results highlight the importance of both aquatic and terrestrial invertebrates in supporting fish trophic dynamics in Amazonian streams, reinforcing the interconnections between small streams and their surrounding landscapes. It also underscores the role played by forests in maintaining the integrity of Amazonian aquatic environments by providing food resources that sustain fish assemblages. Therefore, our study provides essential insights into the carbon flow and isotopic ecology in small Amazonian streams, which are vital for understanding their functioning, guiding conservation efforts, and ensuring the sustainable management of natural resources.

Keywords: Stable isotopes; Energy flow; Trophic structure; Riparian vegetation; Biomass.

Impactos sociais, tecnológicos, econômicos e culturais

A Bacia Amazônica é o maior e mais diverso sistema fluvial do mundo e abriga uma rede complexa e extensa de canais fluviais. Além do canal principal do rio Amazonas, o sistema inclui inúmeros pequenos riachos (localmente conhecidos como "igarapés"), que, sozinhos, abrigam aproximadamente 50% da ictiofauna amazônica. Apesar da grande biodiversidade, muitos aspectos ecológicos, como o fluxo de energia e a ecologia trófica dos riachos amazônicos, ainda permanecem pouco explorados. Considerando que a degradação dos habitats naturais é crescente na região, estudos voltados à compreensão do funcionamento desses ecossistemas são essenciais para orientar e fortalecer os esforços de preservação e conservação do bioma. Nosso trabalho teve como objetivo principal a caracterização da dieta das assembleias de peixes em cinco riachos amazônicos com baixos impactos antropogênicos. Tradicionalmente, a dieta de peixes é determinada por meio da análise de conteúdo estomacal, na qual o estômago do peixe é removido e seu conteúdo avaliado por medidas qualitativas e quantitativas. No entanto, esse método revela apenas os itens alimentares consumidos pouco antes da captura, não refletindo com precisão a composição real da dieta desses animais. Neste estudo, adotamos a análise de isótopos estáveis, uma técnica que, por meio de uma análise química do tecido muscular dos peixes, permite inferir os recursos alimentares assimilados ao longo de semanas ou até meses. Para isso, coletamos os peixes e todos os possíveis recursos alimentares disponíveis (vegetação ripária, gramíneas, folhiço no leito do riacho, sedimento, invertebrados aquáticos e terrestres, perifíton, macrófitas etc.), que foram analisados isotopicamente em laboratório. Os resultados revelaram que a biomassa das assembleias de peixes é sustentada principalmente por invertebrados terrestres (31% a 55%), invertebrados aquáticos (22% a 40%) e camarões (9% a 22%). Do ponto de vista tecnológico e científico, a aplicação de isótopos estáveis em estudos ecológicos permite uma compreensão mais robusta da ecologia trófica. Essa abordagem pode incentivar outras pesquisas desenvolvidas no bioma a também adotarem essa técnica, fortalecendo o conhecimento sobre a ecologia trófica dos riachos amazônicos. Além disso, do ponto de vista ambiental, os dados deste trabalho reforçam a importância da proteção da vegetação marginal e do entorno dos riachos, considerando a dependência de recursos alóctones para o sustento das assembleias de peixes. Este estudo e seus resultados estão alinhados ao Objetivo de Desenvolvimento Sustentável 14 — Vida na Água (ODS/ONU)

Social, technological, economic and cultural impacts

The Amazon Basin is the largest and most diverse river system in the world and hosts a complex and extensive network of river channels. Beyond the main stem of the Amazon River, the system includes countless small streams (locally known as *igarapés*), which alone harbour approximately 50% of the Amazonian fish fauna. Despite its remarkable biodiversity, many ecological aspects, such as energy flow and the trophic ecology of Amazonian streams, remain poorly understood. Considering the increasing degradation of natural habitats in the region, studies aimed at understanding the functioning of these ecosystems are essential to guide and strengthen conservation efforts in the biome. Our main objective was to characterise the diet of fish assemblages in five Amazonian streams with low anthropogenic impact. Traditionally, fish diets are assessed through stomach content analysis, in which the fish’s stomach is removed and its contents evaluated using qualitative and quantitative measures. However, this method reveals only the items consumed shortly before capture and does not accurately reflect the long-term composition of the diet. In this study, we adopted stable isotope analysis, a technique that uses chemical analysis of muscle tissue to infer assimilated food resources over weeks or even months. For this, we collected fish and all potential food resources available (riparian vegetation, grasses, leaf litter, streambed sediment, aquatic and terrestrial invertebrates, periphyton, macrophytes, etc.), which were then isotopically analysed in the laboratory. The results revealed that fish biomass is primarily supported by terrestrial invertebrates (31% to 55%), aquatic invertebrates (22% to 40%), and shrimps (9% to 22%). From a technological and scientific perspective, the use of stable isotopes in ecological studies allows for a more robust understanding of trophic dynamics. This approach may encourage other studies in the biome to adopt the technique, strengthening our knowledge of Amazonian stream ecology. Additionally, from an environmental perspective, our findings highlight the importance of protecting riparian vegetation and stream surroundings, given the reliance of fish assemblages on allochthonous resources. This study and its results are aligned with Sustainable Development Goal 14 — Life Below Water (UN/SDG).

SUMÁRIO

PRIMEIRA PARTE 14
1. INTRODUÇÃO GERAL 15
2. CONCLUSÃO 16
REFERÊNCIAS 17
SEGUNDA PARTE – ARTIGO 18
CAPÍTULO 01 – Artigo elaborado de acordo com o periódico *Freshwater biology* 19
ABSTRACT 21
1. Introduction 22
2. Materials and methods 23
2.1 Study area 23
2.2 Fish sampling 25
2.3 Basal resources sampling 25
2.4 Isotopic analyses 26
2.5 Trophic structure and carbon sources 28
2.6 Proportion of food resources assimilated and biomass 28
2.7 Trophic niches and trophic diversity 29
2.8 Trophic position 30
3. Results 30
3.1 Trophic structure and carbon sources 30
3.2 Proportion of resources assimilated and biomass 32
3.3 Trophic niches and trophic diversity 34
3.4 Trophic position 35
4. Discussion 37
Acknowledgements 40
Autor Contribuitions 40
Ethics Statement 40
Conflicts of Interests 40
Data Availability Statement 41
References 41

PRIMEIRA PARTE

1. INTRODUÇÃO GERAL

A floresta desempenha um papel fundamental na manutenção das comunidades aquáticas em riachos amazônicos, uma vez que a vegetação do entorno influencia diretamente os processos ecossistêmicos de fluxo de energia e disponibilidade de nutrientes (Caramaschi et al., 2021; Esteves et al., 2021). Apesar disso, ainda são escassos os estudos que buscam compreender quais são as principais fontes energéticas que sustentam essas comunidades. Compreender as dinâmicas ecológicas de um ecossistema é um dos principais objetivos de ecólogos e pesquisadores, que lançam mão de diversas métricas e ferramentas para investigar interações ecológicas, fluxos de matéria e energia, transferências tróficas e impactos antrópicos (Ballerta et al., 2008).

Entre essas ferramentas, destaca-se a análise de isótopos estáveis, que tem se mostrado essencial para a compreensão do ciclo dos elementos e das relações tróficas em comunidades aquáticas. Essa técnica baseia-se no princípio de que a composição isotópica dos tecidos dos organismos reflete os processos metabólicos que os originaram, funcionando como biomarcadores naturais (Fry, 1999; Fry, 2006; Albrecht et al., 2021). A aplicação de isótopos estáveis em estudos ecológicos tem contribuído significativamente para o avanço do conhecimento sobre processos complexos em diferentes ecossistemas (Pereira & Benedito 2007).

O objetivo geral deste trabalho é caracterizar a ecologia trófica de riachos amazônicos utilizando a técnica de isótopos estáveis. Entre os objetivos específicos, buscamos estabelecer a estrutura trófica, estimar a proporção de recursos assimilados por cada espécie nos riachos estudados, bem como determinar seus nichos e posições tróficas. O capítulo está formatado de acordo com as normas do periódico *Freshwater Biology*, ao qual será submetido.

Os dados analisados nessa dissertação são parte do projeto intitulado, "*Understanding and conserving tropical freshwater ecossystems*" coordenado por pesquisadores da *Lancaster University,* em parceria com a Universidade Federal de Lavras (UFLA). O projeto é financiado pelo *United Kingdon Research and Innovation* (UKRI) e o Programa Ecológico de Longa Duração (PELD) – RAS.

2. CONCLUSÃO

Nossos resultados indicaram que a dieta das assembléias de peixes é sustentada predominantemente por recursos alóctones, especialmente invertebrados aquáticos e terrestres. A estrutura trófica, os nichos e as posições tróficas apresentaram padrões semelhantes entre os igarapés estudados, sugerindo uma possível uniformidade no uso de recursos em ambientes com baixos níveis de impacto antropogênico. Esses achados ressaltam a importância da floresta ripária na manutenção da base alimentar das comunidades aquáticas, destacando seu papel essencial na conservação da integridade ecológica dos riachos amazônicos.

REFERÊNCIAS

BARLETTA, M.; BLABER, S. J. M. Comparison of fish assemblages and guilds in tropical habitats of the Embley (Indo-West Pacific) and Caeté (western Atlantic) estuaries. **Bulletin of Marine Science**, v. 80, p. 647–668, 2008.

FRY, B. et al. Trophic position and individual feeding histories of fish from Lake Okeechobee, Florida. **Canadian Journal of Fisheries and Aquatic Sciences**, v. 56, n. 4, p. 590-600, 1999.

CARAMASCHI, E. P. et al. Ecologia de peixes de riacho. In: CARAMASCHI, E. P.; MAZZONI, R.; LEITÃO, R. P. (org.). Ecologia de Peixes de Riacho. Série **Oecologia Australis**, v. 25, n. 2, 2021.

ESTEVES, K. E.; ARANHA, J. M. R.; ALBRECHT, M. P. Ecologia trófica de peixes de riacho: uma releitura 20 anos depois. **Oecologia Australis**, v. 25, n. 2, p. 266-282, 2021.

FRY, B. Stable Isotope Ecology. New York: **Springer**, 2006. 308 p.

PEREIRA, A. L.; BENEDITO, E. Isótopos estáveis em estudos ecológicos: métodos, aplicações e perspectivas. **Revista Biociências**, v. 13, n. 1, 2007.

SEGUNDA PARTE – ARTIGO

CAPÍTULO 01 – Artigo elaborado de acordo com o periódico *Freshwater biology*

Aquatic and terrestrial invertebrates support fish assemblages of Amazonian streams

Igarapés Amazônicos na região de Santarém, Pará em agosto de 2023

Aquatic and terrestrial invertebrates support fish assemblages of Amazonian streams

Pedro H. S. Basilio[1, 2], Cecília G. Leal[1,3], Débora R. de Carvalho[3], Dennys H. C. Drager[4,5], Gilberto N. Salvador[4,5], Gabriel L. Brejão[6], Rafael P. Leitão[4,5], Paulo S. Pompeu[1,2]

[1] Programa de Pós-Graduação em Ecologia Aplicada, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. hhenriquepedro.2018@gmail.com (corresponding author)

[2] Laboratório de Ecologia de Peixes, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras 37200-900, Minas Gerais, Brazil (PSP)

[3] Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom.

[4] Laboratório de Ecologia de Peixes, Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biólogicas, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, Minas Gerais, Brazil.

[5] Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais, Brazil.

[6] Departamento de Biodiversidade, Universidade Estadual Paulista (UNESP), Instituto de Biociências, Rio Claro. Av. 24 A, 1515, Bela Vista, Rio Claro, São Paulo, Brazil.

Keywords: Stable isotopes, Energy flow, Trophic structure, Riparian vegetation, Biomass

ABSTRACT

1) The Amazonian stream network is impressive not only for its vastness but also for harboring a great diversity of fish species. Despite their high diversity, little is known about key ecological aspects of Amazonian streams such as energy flow and the carbon sources that sustain their species-rich fish assemblages.

2) In this study we use carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes to evaluate the trophic structure and to identify the key food resources sustaining the ichthyofauna of Amazonian streams. We collected fish assemblages and a wide range of resources available in streams, including macrophytes, riparian vegetation, filamentous algae, organic matter, grasses, benthic and terrestrial invertebrates, periphyton, and suspended matter. We then estimated the trophic structure of the assemblages, including diversity metrics, trophic niches, positions, assimilated resource proportions, and biomass.

3) Fish biomass was primarily supported by terrestrial invertebrates (31%–55%) and benthic invertebrates (22%–40%). Shrimps represented the third most important resource for fish biomass (9%–22%). Together, these three resources accounted for 79%–90% of the total fish biomass in the studied streams. The trophic structure and trophic diversity metrics were similar across all sampling sites, as evidenced by the substantial overlap in the isotopic niche of the assessed fish assemblages. Although trophic positions varied to some extent among sites, most species fed at intermediate trophic levels.

4) Our results highlight the importance of both aquatic and terrestrial invertebrates in supporting fish trophic dynamics in Amazonian streams, reinforcing the interconnections between small streams and their surrounding landscapes. It also underscores the role played by forests in maintaining the integrity of Amazonian aquatic environments by providing food resources that sustain fish assemblages.

5) Therefore, our study provides essential insights into the carbon flow and isotopic ecology in small Amazonian streams, which are vital for understanding their functioning, guiding conservation efforts, and ensuring the sustainable management of natural resources.

1. Introduction

Small Amazonian streams run within the forest making the link between aquatic and terrestrial environment and draining impressive volumes of water into larger rivers. These streams and their riparian vegetation are interconnected through the movement of organisms and the flow of organic and inorganic matter (Baxter et al. 2005; Esteves et al. 2021). As a consequence of light shading by the riparian vegetation, upland (*Terra Firme*) Amazonian streams exhibit low primary production and are strongly dependent on the surrounding forest, for instance for carbon resources (Lowe-McConnel, 1999; Casatti et al. 2012; Esteves et al. 2021).

These ecosystems present a variety of microhabitats (Junk et al. 1983; Montag et al. 2019) that harbour a diverse fish fauna (Vieira et al. 2018; Casatti et al. 2024), which exhibits a wide range of ecological roles (Brejão et al. 2013). In Amazonian streams, fish species exploit a variety of energy sources, including autochthonous food resources and allochthonous inputs supplied by the riparian forest (Cardoso & Couceiro, 2017). Seasonal dynamics also shape trophic interactions, as flood pulses increase the availability of terrestrial subsidies (Junk et al. 1989; Benone et al. 2020; Esteves et al. 2021). Altogether, this ecological diversity reflects a wide range of trophic strategies that sustain complex food webs and energy flows in these systems (Saito et al. 2024).

Stable isotope analysis of Carbon (δ^{13} C) and Nitrogen (δ^{15} N) is a powerful tool for investigating energy flow and trophic ecology in aquatic ecosystems (Pereira & Benedito, 2007; Albrecht et al. 2021). The isotopic composition of fish tissue mirror the isotopic composition of the resources assimilated by them, helping to understand the carbon flow within ecosystems (Fry & Sherr, 1984; Newsome et al. 2007; Albrecht et al. 2021). Carbon isotopes exhibit minimal enrichment along the food web, with an average increase of approximately 0.4‰ ±1.3‰ per trophic level (Post, 2002), enabling the identification of different basal resources that sustain an ecosystem's food web. In contrast, nitrogen isotopes are consistently fractionated along the trophic web, with a typical trophic enrichment of about 3.4‰ ±1‰ per trophic level (Post, 2002). This makes nitrogen isotopes particularly useful for estimating the trophic position of consumers within a food web (DeNiro & Epstein, 1981; Fry et al. 2006; Perkins et al. 2014; Alonso et al. 2020).

In the Amazon basin, one of the first studies using stable isotopes was conducted in the mid-1980s, when Araújo-Lima et al. (1986) analyzed the main carbon sources for detritivorous fish in the Amazon River. They showed that Characiformes species obtain most of their carbon through food chains originating from phytoplankton, while Siluriformes get a significant portion of their energy from other plant sources. Later, other studies applied this tool to investigate fish trophic ecology, but mainly with a focus on species from large rivers (e.g., Forsberg et al. 1993; Leite et al. 2002; Silva et al. 2005; Mortillaro et al. 2015; Zuluaga-Gomez et al. 2016; Seabra et al. 2024). However, the isotopic ecology of Amazonian stream fish remains poorly understood, despite the expected significant role of primary resources in sustaining aquatic communities, particularly allochthonous inputs such as terrestrial invertebrates and leaves (Vannote et al. 1980).

To fill this gap, we conducted the first study using stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ to investigate the trophic structure of Amazonian streams fish assemblages. Specifically, we seek to: a) Determine the main carbon sources sustaining fish assemblages, estimating the proportion of resources assimilated by each fish species and the biomass supported by these resources; b) Evaluate the trophic structure of fish assemblages by estimating trophic niches (isotopic niches), trophic positions, and trophic diversity metrics. We tested the following hypotheses:1) Due to forest shading, allochthonous sources are the primary contributors to fish biomass, and invertivorous species will be the most abundant; and 2) Due to the similarities in environmental conditions across the studied streams, no variation is expected in the trophic positions, trophic diversity, or trophic niches of fish assemblages. Understanding fish diets and nutrient flow is essential for uncovering interactions between fish species and their terrestrial surroundings. These dynamics reflect the complexity of ecological processes that sustain the biodiversity and functionality of streams, highlighting the importance of understanding the interdependencies between terrestrial and aquatic compartments in these tropical ecosystems.

2. Materials and methods

2.1 Study area

The study was conducted in five 150 m stream sites (2nd and 3rd Strahler order) within the Curuá-Una River basin, a tributary of the Amazon River, in the state of Pará, Brazil

(Fig 1). The region is located near the confluence of the Tapajós and Amazon Rivers. The landscape is characterized by a mosaic of land uses, reflecting a history of loss of primary forest. It currently comprises mechanized agriculture, cattle ranching, silviculture, smallholder agricultural settlements, regenerating secondary forests, and undisturbed primary forests mostly located in the Tapajós National Forest protected area (Gardner et al. 2013; Leitão et al. 2018).

The five stream sites are located outside protected areas but within landscapes characterized by low anthropogenic disturbance and similar land-use cover (Table 1). Land-use cover was estimated using MapBiomas (Collection 9, 2024). We calculated the percentage of mature forest (Primary Forest + Degraded Forest + Old Secondary Forest, with Old Secondary Forest defined as secondary forest equal to or older than 10 years) for the catchment upstream of each stream site and within a 100 m buffer (riparian zone of the entire microbasin). Non-forest areas consisted of pasture, wetlands, grassland, soybean and other temporary crops. To characterize the physical habitat of streams we measured depth, width, substrate type and stream shadow, following the field protocol proposed by Kaufmann et al. (1999) and Peck et al. (2006). The samplings were conducted once during the dry season in July and August 2023.

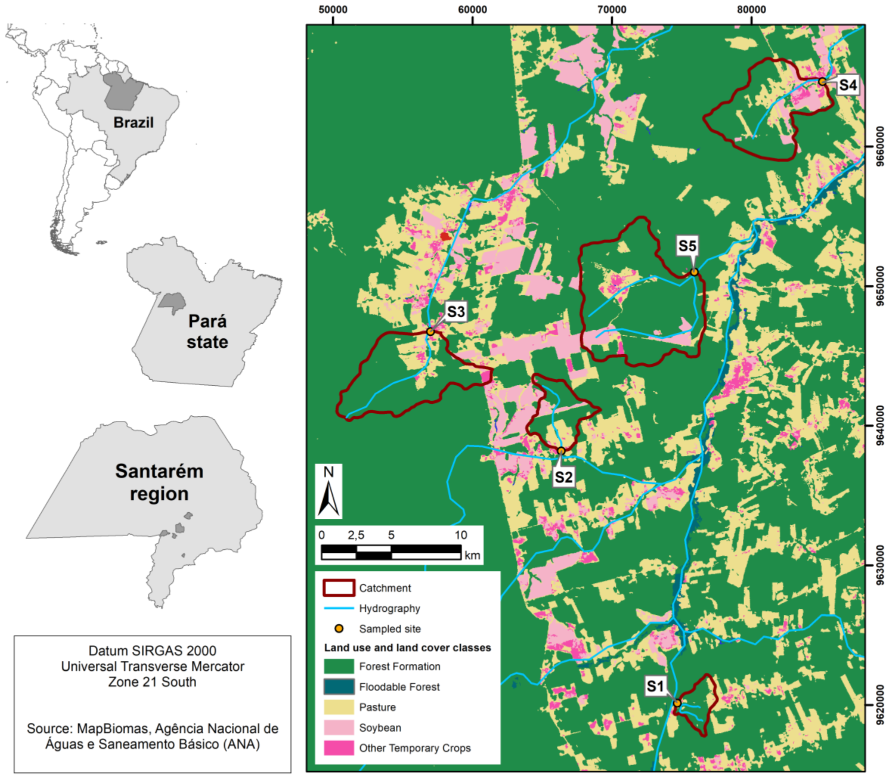

Fig. 1 Stream sites in the Curuá-Una River basin, Pará, Brazil

Table 1 - Land use cover and physical habitat characteristics of the stream sites in the Curuá-Una River basin sampled in July/August 2023.

Environmental variables								
Stream sites	Geographic coordinates	Order (Strahler)	Mean depth (m)	Mean width (m)	Fine subst. (%)	Stream shadow (%)	% Forest	% Others land uses
S1	-54° 49' 34.42" -3° 25' 44.88"	3ª	0.55	2.71	43.80	92.91	82,83	17,17
S2	-54° 54' 0.77" -3° 15' 57.02"	2ª	0.39	2.68	26.31	88.50	62,59	37,41
S3	-54° 59' 2.68" -3° 11' 17.72"	3ª	0.76	12.94	16.19	87.96	78,51	21,49
S4	-54° 43' 52.15" -3° 1' 39.14"	3ª	0.83	3.92	39.04	86.89	56,47	43,53
S5	-54° 48' 51.04" -3° 9' 1.53"	3ª	1.02	5.96	70.47	94.25	80,89	19,11

2.2 Fish sampling

Fish were collected using semicircular sieve (80 cm in diameter, 1-mm mesh) and seine nets (3 m long, 5-mm mesh). Each stream site was sampled by two people for 2 h and the procedures for capture, collection and transportation of biological material were authorized by ICMBio (SISBIO 87389-1) and the Ethics Committee of Federal University of Pará (UFPA) (8293020418).

For stable isotope analysis, we selected up to 5 individuals per species, whenever possible, and stored them immediately on ice for later processing. We weighed, measured and collected muscle samples from each large individual, while the small individuals (< 2 cm) were analyzed whole, after removal of the digestive tract. Voucher specimens were fixed in 10% formalin solution and then transferred to a 70% ethanol solution and deposited in the Fish Ecology Laboratory of Federal University of Minas Gerais (UFMG), where the species identifications were confirmed using identification keys and by consulting specialists. In the laboratory, fish samples were lyophilized for 48 hours and ground into a fine homogeneous powder using a mortar and pestle.

2.3 Basal resources sampling

In each stream site, we collected up to 5 samples of each of the following basal resources, whenever possible: periphyton (PE), filamentous algae (AL), macrophytes (MA), fine particulate organic matter (FPOM), coarse particulate organic matter (CPOM), riparian vegetation (RV), grass (GR), benthic invertebrates (BI), shrimps (SH) and terrestrial invertebrates (TI). Periphyton was sampled by scraping and washing wood or rocks with a small brush and stored in distilled water. FPOM samples were collected by resuspending sediment. Grass, riparian vegetation, coarse particulate organic matter, and macrophytes were manually and randomly collected. Benthic invertebrates were sampled using a D-frame kicking net (30 cm aperture, 500 µm mesh) along the stream site, while terrestrial invertebrates were manually and randomly collected in in the riparian zone. All samples were stored on ice until transferring to freezers.

In the laboratory, periphyton and FPOM samples were filtered through quartz fiber filters (Whatman® QMA quartz filters) using a filtration apparatus connected to a vacuum pump. The samples were then oven-dried at 40 °C for 48 hours and ground into a fine, homogeneous powder using a mortar and pestle. Grass, riparian vegetation, CPOM, filamentous algae, benthic and terrestrial invertebrates were lyophilized for 48 hours and subsequently ground into a fine, homogeneous powder using a ball mill (Retsch MM 400) or a mortar and pestle.

2.4 Isotopic analyses

We analyzed a total of 553 samples for carbon and nitrogen stable isotopes, including 285 individuals from 39 fish species (Table 2), and 268 samples of food resources. We collected 30 samples for each resource type (benthic invertebrates, terrestrial invertebrates, CPOM, FPOM, riparian vegetation, grass, and periphyton), except for macrophytes (20 samples), filamentous algae (14 samples), and shrimps (24 samples). Isotopic analyses were performed at the Stable Isotope Center “Prof. Dr. Carlos Ducatti” at São Paulo State University (Unesp) in Botucatu, Brazil. We used an isotope ratio mass spectrometry system with elemental analyzer EA-IRMS (Flash 2000 - Delta V Advantage, Thermo Scientific, Germany). For each analysis, 3.0-0.5 mg of dry and homogenized samples were weighed in a silver capsule. The system simultaneously determined the isotopic ratios $R({}^{i}E/{}^{j}E)_{sample}$, which were expressed as a relative difference from the isotopic ratio $\delta^{i}E$, in ‰ according to equations (Coplen, 2011):

$$\delta^{i}E_{(‰)} = \frac{R\left({}^{i}E/{}^{j}E\right)_{sample}}{R\left({}^{i}E/{}^{j}E\right)_{standard}} - 1$$

where ${}^{i}E$ is the rare isotope, ${}^{j}E$ is the abundant isotope, and $R({}^{i}E/{}^{j}E)_{standard}$ are the international standard ratios VPDB (PeeDeeBelemnite) for $R({}^{13}C/{}^{12}C)_{VPDB}$ and Air (atmospheric Nitrogen) for $R({}^{15}N/{}^{14}N)_{Air}$. The results were normalized from certified reference standards USGS61, USGS62, USGS63, USGS90 (Paul et al. 2007; Schimmelmann et al. 2016, 2020). The standard uncertainty of the analysis was estimated to be ± 0.10‰ and ± 0.15‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively.

Table 2 - Number of samples collected for stable isotope analysis from fish species at each site. Species were classified into trophic guilds following Leal et al. (2017)

Species	Site 1	Site 2	Site 3	Site 4	Site 5	Total
Algivores / Periphytivores		**2**		**1**	**5**	**8**
Farlowella smithi					5	5
Rhinotocinclus sp. 1				1		1
Rhinotocinclus sp. 2		2				2
Detritivores			**5**	**5**		**10**
Apistogramma taeniata			5	5		10
Invertivores	**56**	**46**	**28**	**36**	**40**	**203**
Anablepsoides cf. *urophthalmus*	4					4
Apteronotus albifrons			4			4
Brachyglanis microphthalmus	1	5			1	7
Brachyhypopomus cf. *brevirostris*			2	1		3
Bryconops caudomaculatus		5	3	4	3	15
Bryconops melanurus			2	1	2	5
Characidium pteroides	5	4	1		2	12
Copella nigrofasciata				5		5
Gymnorhamphichthys rondoni	5	5	1	1	3	15
Gymnotus aff. *coatesi*					1	1
Gymnotus coropinae	3			3	1	7
Helogenes marmoratus	2			2	1	5
Hemigrammus ocellifer		5	2	5	5	17
Hyphessobrycon ericae	5	1	5	5	5	21
Hypopygus lepturus	6				2	8
Ituglanis amazonicus	1					1
Knodus savannensis	5	5			4	17
Melanocharacidium dispilomma		5				5
Moenkhausia comma	3	1		2		6
Moenkhausia conspicua	5	5	5	2	5	22
Nannostomus marginatus	5	5		5	5	20
Synbranchus madeirae	1		2			3
Synbranchus sp. 1	2		1			3
Omnivores	**4**	**6**	**6**	**4**	**5**	**25**
Aequidens epae	2	5			4	11
Aequidens tetramerus	1		5	4	1	11
Rhamdia muelleri	1					1
Rhamdia quelen		1	1			2
Piscivores	**4**		**5**	**1**	**3**	**10**
Hoplias curupira	1		2	1	3	7
Hoplias malabaricus			3			3
Carnivores	**5**	**2**	**10**	**9**	**3**	**29**
Acanthodoras cataphractus			1			1

Bratochoglanis raninus				1		1
Erythrinus erythrinus	3			2	2	7
Hypselecara temporalis			4			4
Lugubria strigata				1		1
Saxatilia brasiliensis	2	2	5	5	1	15
Total	**63**	**56**	**54**	**56**	**56**	**285**

2.5 Trophic structure and carbon sources

Fish were classified into trophic guilds according to Leal et al. (2017), who previously sampled the same streams and evaluated fish diets based on stomach content analysis. To assess the food web structure of the fish assemblages, individuals of all fish species were plotted in a bi-plot space using isotopic values of carbon (x-axis) and nitrogen (y-axis), providing a visual representation of the food web structure at each stream site. Mean $\delta^{13}C$ and $\delta^{15}N$ values were plotted for each fish species, while the food resources were represented by their mean and ± standard deviation values.

2.6 Proportion of food resources assimilated and biomass

To evaluate the trophic partitioning of food resources, we applied a stable isotope mixing model using the SIMMr package in R (Parnell et al. 2013; R Core Team, 2024). A Markov Chain Monte Carlo (MCMC) analysis was conducted with the following parameters: number of chains = 4; number of iterations = 50,000; burn-in = 10,000; thin = 10. For this analysis, we used data on the main food items identified in the stomach content analysis based on Leal et al. (2017) for each guild: Piscivorous/Carnivorous (TI, BI, SH, and fish), Omnivorous (TI, BI, SH, CPOM, and AL), Invertivorous (TI and BI), Periphytivorous (PE, FPOM, and AL), and Detritivorous (CPOM, FPOM, and PE). Given that carbon signature of leaves can vary vertically along tree height (Domingues et al. 2005, Martinelli et al. 2009) and we only sampled leaves at head height, we decided to use CPOM as a proxy for the riparian vegetation. This is because CPOM should reflect the average carbon isotope composition of all the leaves that fall into the stream. The fractionation values (mean ± standard deviation) considered for this analysis were $0.4 \pm 1.3‰$ for $\delta^{13}C$ and $3.4 \pm 0.98‰$ for $\delta^{15}N$ (Post, 2002).

To calculate the local fish biomass supported by food resources in each stream site, we used the percentage contribution of each food resource consumed by each species, applying the following formula:

$$\mathbf{Biomass\ (\%) = (C / S) \times F}$$

Where:

C is the fish assemblage biomass (g),

S is the species biomass (g), and

F is the percentage contribution of each food source to a species diet.

The total percentage contribution of a given food resource to the local assemblage biomass was determined by summing the contribution values of all species that utilized that resource.

2.7 Trophic niches and trophic diversity

We calculated the standard ellipse area (SEA) to quantify the trophic niche (isotopic niche) of each fish assemblage using the SIBER package in R (Jackson et al. 2011). A small sample size correction (referred as SEAc) was applied to SEA to enhance the accuracy of comparisons, facilitating the evaluation of niches across assemblages with varying sample sizes (Jackson et al. 2011; de Carvalho et al. 2024). Bayesian estimates of SEA (referred as SEAb) were computed to assess significant differences in the isotopic niche widths of fish assemblages by comparing their confidence intervals (Jackson et al. 2011).

To compare the trophic structure across stream sites, we used the SIBER package to calculate six metrics originally proposed by Layman et al. (2007) and reformulated within a Bayesian framework by Jackson et al. (2011). The $\delta^{15}N$ range (NR) offers a vertical perspective of the food web structure, reflecting the distance between the most and least enriched $\delta^{15}N$ values across both resources and consumers. A broader $\delta^{15}N$ range typically indicates the presence of multiple trophic levels within a community. The $\delta^{13}C$ range (CR) represents the horizontal variation, indicating the diversity of resources used by the community. In contrast, the $\delta^{13}C$ captures horizontal variation, which highlights the variety of resources used by the community. A wider $\delta^{13}C$ range is generally linked to food webs that utilize a diverse set of resources with distinct $\delta^{13}C$ signatures and/or exhibit greater variability in the $\delta^{13}C$ values of available resources. The mean distance to the centroid (CD) measures the average trophic diversity within the food web, calculated as the mean Euclidean distance of each species from the centroid in a bi-plot. The mean nearest neighbor distance (MNND) reflects the average Euclidean distance to the closest neighbor for each species, providing an estimate of the community's overall trophic density. Smaller MNND suggests a high degree of trophic redundancy, where many species share similar trophic niches. The standard deviation of the nearest neighbor distance (SDNND) gauges the evenness of spatial density and the packing of species within bi-plot space. Unlike MNND, SDNND is less affected by sample size. Lower SDNND values indicate a more uniform distribution of trophic niches across the community (Layman, 2007).

2.8 Trophic position

We estimated the trophic position (TP) of fish species through the Bayesian package tRophicPosition in R (Quezada-Romegialli et al. 2018), using the "two baselines full model”, where a mixing model incorporates $\delta^{13}C$ to differentiate between two distinct nitrogen sources. Autotrophic and particulate resources were used as baselines (baseline 1 = CPOM and baseline 2 = FPOM). For this analysis, we applied trophic discrimination factors of 0.4 ± 1.3‰ for $\delta^{13}C$ and 3.4 ± 0.98‰ for $\delta^{15}N$ (Post, 2002).

3. Results

3.1 Trophic structure and carbon sources

Among the collected fish species, only *Gymnorhamphichthys rondoni, Hyphessobrycon ericae, Moenkhausia conspicua* and *Saxatilia brasiliensis* were sampled in all stream sites (Fig 2). The $\delta^{13}C$ and $\delta^{15}N$ range values for the assemblages were similar across all sites. However, some resources, as macrophytes in S1 and S5, displayed distinct $\delta^{13}C$ values from those of the fish assemblage and other resources at some stream sites. All assemblages were dominated by invertivorous species, followed by carnivorous, omnivorous, and piscivorous species. Periphytivorous species were present at only three stream sites, while detritivorous species were registered in two (Fig. 3).

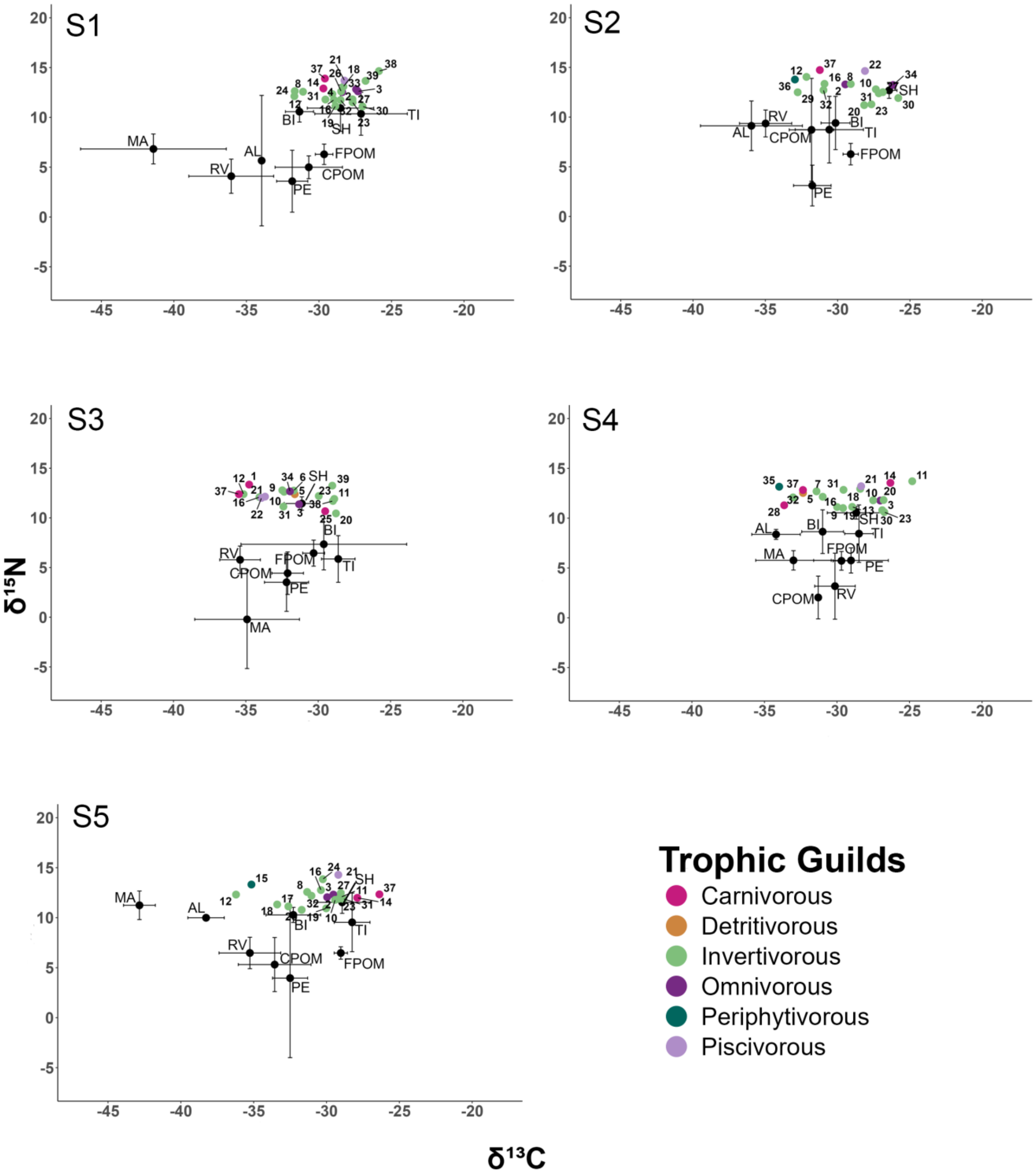

Fig. 2 Trophic structure of the five stream sites sampled in the Curuá-Una river basin considering the isotopic composition of fish (mean) and food resources (mean and standard deviation). Fish species: **1** (*Acanthodoras cataphractus*), **2** (*Aequidens epae*), **3** (*Aequidens tetramerus*), **4** (*Anablepsoides* cf. *urophthalmus*), **5** (*Apistogramma taeniata*), **6** (*Apteronotus albifrons*), **7** (*Batrochoglanis raninus*), **8** (*Brachyglanis microphthalmus*), **9** (*Brachyhypopomus* cf. *brevirostris*), **10** (*Bryconops caudomaculatus*), **11** (*Bryconops melanurus*), **12** (*Characidium* aff. *pteroides*), **13** (*Copella nigrofasciata*), **14** (*Erythrinus erythrinus*), **15** (*Farlowella smith*), **16** (*Gymnorhamphichthys rondoni*),

17 (*Gymnotus* aff. *coatesi*), **18** (*Gymnotus coropinae*), **19** (*Helogenes marmoratus*), **20** (*Hemigrammus ocellifer*), **21** (*Hoplias curupira*) **22** (*Hoplias malabaricus*), **23** (*Hyphessobrycon ericae*), **24** (*Hypopygus lepturus*), **25** (*Hypselecara temporalis*), **26** (*Ituglanis amazonicus*), **27** (*Knodus savannensis*), **28** (*Lugubria strigata*), **29** (*Melanocharacidium dispilomma*), **30** (*Moenkhausia comma*), **31** (*Moenkhausia conspicua*), **32** (*Nannostomus marginatus*), **33** (Rhamdia muelleri), **34** (Rhamdia quelen), **35** (*Rhinotocinclus* sp.1), **36** (*Rhinotocinclus* sp.2), **37** (*Saxatilia brasiliensis*), **38** (*Synbranchus madeirae*), **39** (*Synbranchus* sp.1).

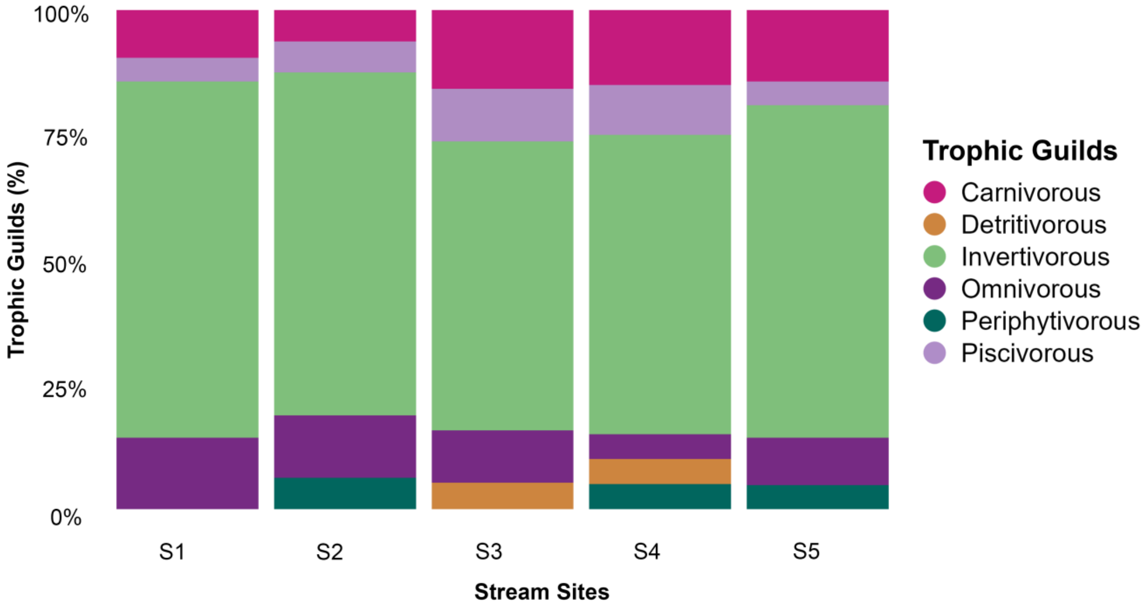

Fig. 3 Relative richness (%) of fish trophic guilds at each stream site in the Curuá-Una River basin. Trophic guilds follow classification by Leal et al. (2017) based on stomach content analysis.

3.2 Proportion of resources assimilated and biomass

Carbon sources supporting fish assemblage at each stream site were similar, with terrestrial invertebrates (TI) and benthic invertebrates (BI) being the most assimilated food resources (Fig. 4). Terrestrial invertebrates were the main food source for *Synbranchus madeirae* (67%, Site 1), *Hyphessobrycon ericae*, and *Helogenes marmoratus* (55% and 58%, respectively, at Site 5). Benthic invertebrates accounted for more than 70% of the assimilation by *Acanthodoras cataphractus* and *Characidium* aff. *pteroides* (Site 3). Filamentous algae were the main food source for *Rhinotocinclus* sp.1 (80%, Site 4) and *Farlowella smithi* (65%, Site 5). Shrimps represented 45% of the assimilated resources by *Rhamdia quelen* (Site 2), while fish accounted for 52% of the assimilated resources for *Lugubria strigata*.

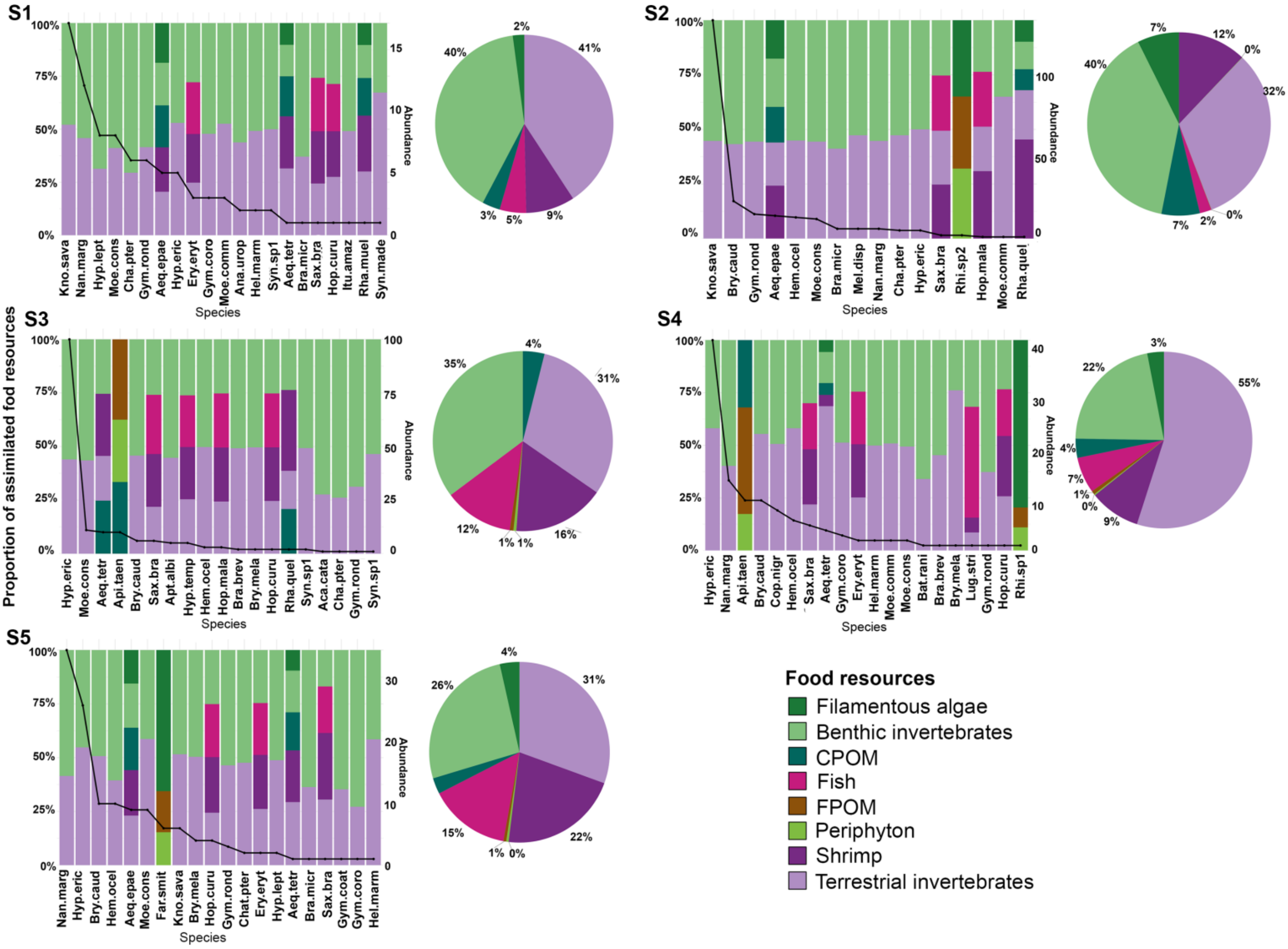

Fig. 4 Proportion of food resources assimilated (bars) and abundance (black line) by fish species, and contribution of food sources to the local assemblage biomass (pie) in five stream sites in Curuá-Una River basin. Fish species: **Aca.cata** (*Acanthodoras cataphractus*), **Aeq.epae** (*Aequidens epae*), **Aeq.tetr** (*Aequidens tetramerus*), **Ana.cf.urop** (*Anablepsoides cf. urophthalmus*), **Api.taen** (*Apistogramma taeniata*), **Apt.albi** (*Apteronotus albifrons*), **Bat.rani** (*Batrochoglanis raninus*), **Bra.micr** (*Brachyglanis microphthalmus*), **Bra.brev** (*Brachyhypopomus cf. brevirostris*), **Bry.caud** (*Bryconops caudomaculatus*), **Bry.mela** (*Bryconops melanurus*), **Cha.pter** (*Characidium aff. pteroides*), **Cop.nigr** (*Copella nigrofasciata*), **Sax.bra** (*Saxatilia brasiliensis*), **Lug.stri** (*Lugubria strigata)* **Ery.eryt** (*Erythrinus erythrinus*), **Far.smit** (*Farlowella smithi*), **Gym.rond** (*Gymnorhamphichthys rondoni*), **Gym.coat** (*Gymnotus aff. coatesi*), **Gym.coro** (*Gymnotus coropinae*), **Hel.marm** (*Helogenes marmoratus*), **Hem.ocel** (*Hemigrammus ocellifer*), **Hop.curu** (*Hoplias curupira*), **Hop.mala** (*Hoplias malabaricus*), **Hyp.eric** (*Hyphessobrycon ericae*), **Hyp.lept** (*Hypopygus lepturus*), **Hyp.temp** (*Hypselecara temporalis*), **Itu.amaz** (*Ituglanis amazonicus*), **Kno.sava** (*Knodus savannensis*), **Mel.disp** (*Melanocharacidium dispilomma*), **Moe.comm** (*Moenkhausia comma*), **Moe.cons** (*Moenkhausia conspicua*), **Nan.marg** (*Nannostomus marginatus*), **Rhi.sp.1** (*Rhinotocinclus sp. 1*), **Par.sp2** (*Rhinotocinclus sp. 2*), **Rha.muel** (*Rhamdia muelleri*), **Rha.quel** (*Rhamdia quelen*), **Syn.made** (*Synbranchus madeirae*), **Syn.sp.1** (*Synbranchus sp. 1*).

Most of the fish biomass was supported by terrestrial invertebrates (TI; 31%–55%) and benthic invertebrates (BI; 22%–40%). Shrimps were the third most important food source, contributing 9%–22% to the biomass. Together, these sources accounted for 79%–90% of the total biomass across all sites, highlighting their crucial role in supporting fish assemblages. In turn, FPOM and periphyton were the least important sources for biomass (Fig. 4)

3.3 Trophic niches and trophic diversity

The trophic niche, or isotopic niche, represented by the standard ellipse area (SEA) showed significant niche overlap among the sites. The highest SEA_b was observed at Site 4, which also exhibited the greatest overlap with other sites (See supplementary materials). Conversely, the lowest SEA_b was observed at Site 1, corresponding to the smallest isotopic niche (Fig. 5).

The trophic diversity metrics for fish were similar across all sampling sites (Table 3). However, Site 1 displayed the lowest Mean Nearest Neighbor Distance (MNND), Standard Deviation of Nearest Neighbor Distance (SDNND), and Centroid Distance (CD), suggesting lower trophic diversity and higher trophic redundancy at this site compared to the others.

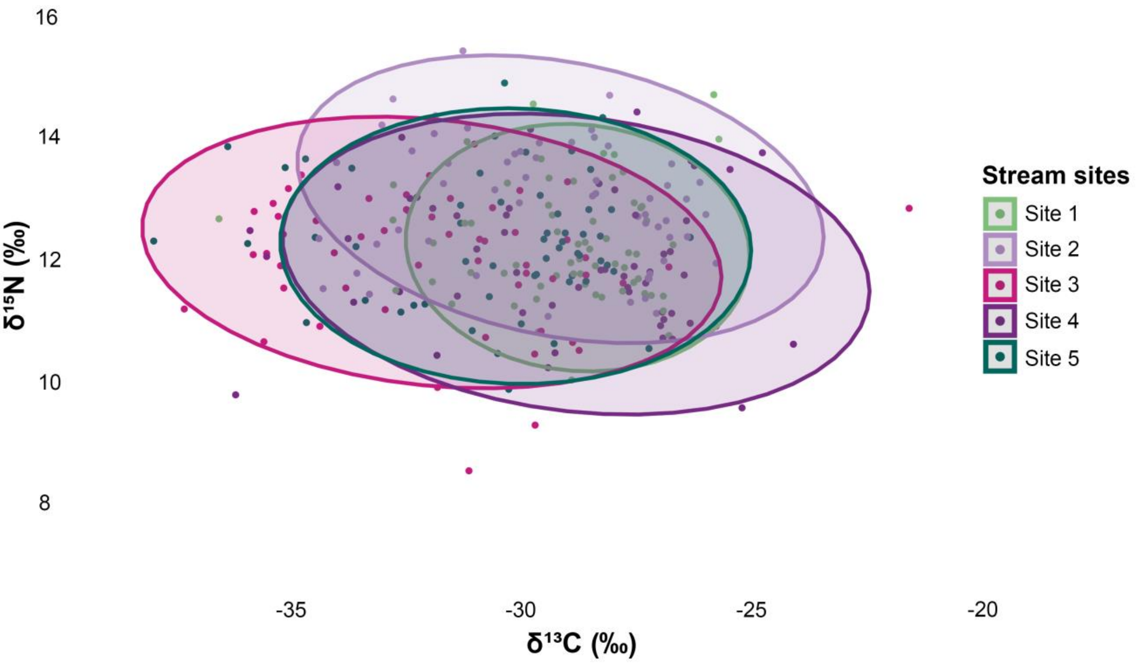

Fig. 5 Isotopic niche (Standard Ellipse Area, SEA, calculated using a 95% confidence interval) of the five stream sites in the Curuá-Una River basin.

Table 3 - Layman metrics of the five stream sites in the Curuá-Una basin. TA (total area), CD (centroid distance), MNND (mean nearest neighbor distance), and SNND (standard deviation of nearest neighbor distance).

	S1	**S2**	**S3**	**S4**	**S5**
N range	3.55	3.53	2.90	3.10	3.50
C range	5.94	7.14	6.78	9.17	9.84
TA	13.43	17.83	13.16	20.03	20.66
CD	1.52	2.30	2.02	2.51	2.04
MNND	0.51	0.75	0.53	0.64	0.77
SDNND	0.23	0.40	0.40	0.45	0.43

3.4 Trophic position

The mean trophic position ranged from 2.55 (*Hemigrammus ocellifer*) to 3.73 (*Parotocinclus* sp.1). The four species common to all stream sites exhibited small variations in trophic positions: *Saxatilia brasiliensis* (2.90–3.65), *Gymnorhamphichthys rondoni* (2.77–3.56), *Hyphessobrycon ericae* (2.31–2.74), and *Moenkhausia conspicua* (2.63–3.59) (Table 4). Among the species common to all stream sites, only *H. ericae* did not show a higher TP in S4. Site 4 displayed the highest trophic positions and the broadest trophic range (2.66–3.92), followed by Site 1 (2.63–3.60), Site 5 (2.41–3.36), Site 3 (2.39–3.32), and Site 2 (2.31–3.21). Overall, the distribution of trophic positions within each stream site was similar, with most species feeding at intermediate trophic levels (Fig. 6).

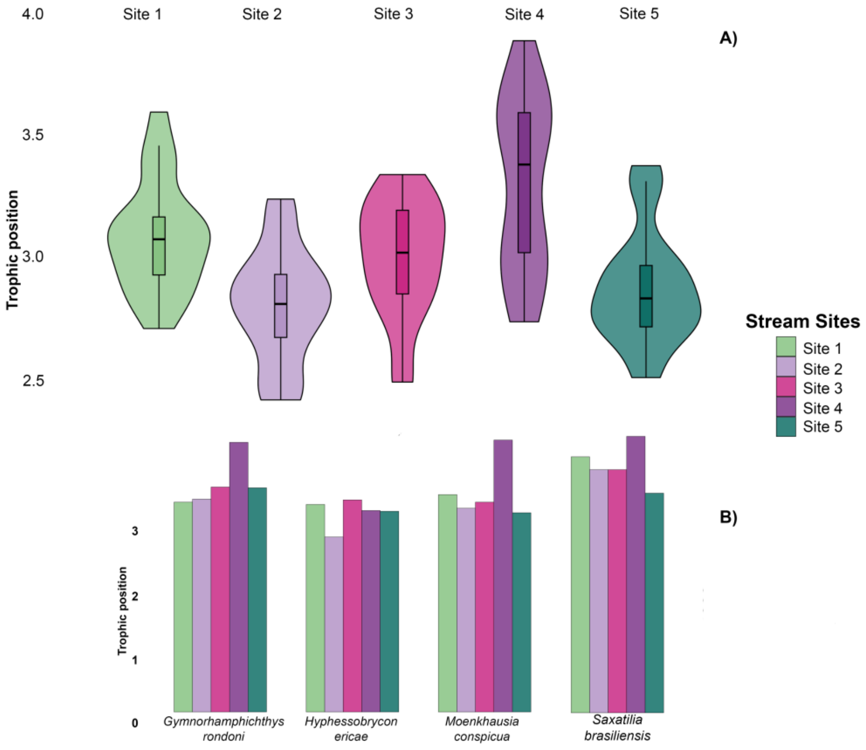

Fig. 6 Food chain length (violin chart) represented by the mean species trophic positions in each site **(A)** and trophic position estimates of fish species common to all stream sites **(B).** FPOM and CPOM were used as baselines.

Table 4 - Trophic position occupied by each fish species in the five stream sites of the Curuá-Una River basin

Species	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Mean**
Acanthodoras cataphractus			3.32			3.32
Aequidens epae	2.97	2.88			2.72	2.86
Aequidens tetramerus	3.03		2.7	3.02	2.86	2.90
Anablepsoides cf. *urophthalmus*	2.93					2.93
Apistogramma taeniata			3.08	3.53		3.31
Apteronotus albifrons			3.1			3.1
Batrochoglanis raninus				3.62		3.62
Brachyglanis microphthalmus	3.06	2.97			2.95	2.99
Brachyhypopomus cf. *brevirostris*			3.21	2.97		3.09
Bryconops caudomaculatus		2.84	3.19	2.97	2.64	2.91
Bryconops melanurus			2.83	3.92	2.69	3.15
Characidium aff. *pteroides*	3	2.76	2.98		2.86	2.9
Copella nigrofasciata				2.8		2.8
Erythrinus erythrinus	3.11			2.67	2.73	2.84
Farlowella smithi					3.25	3.25
Gymnorhamphichthys rondoni	2.77	2.81	2.97	3.56	2.96	3.01
Gymnotus aff. *coatesi*					2.63	2.63
Gymnotus coropinae	3.12			3.44	2.63	3.06
Helogenes marmoratus	2.74			2.99	2.46	2.73
Hemigrammus ocellifer		2.36	2.41	3.02	2.41	2.55
Hoplias curupira	3.45		2.96	3.7	3.36	3.37
Hoplias malabaricus		3.13	2.93			3.03
Hyphessobrycon ericae	2.74	2.31	2.8	2.66	2.65	2.63
Hypopygus lepturus	3.06				3.29	3.18
Hypselecara temporalis			2.39			2.39
Ituglanis amazonicus	3.13					3.13
Knodus savannensis	2.86	2.71			2.8	2.79
Lugubria brasiliensis				3.29		3.29
Melanocharacidium dispilomma		2.38				2.38
Moenkhausia comma	2.63	2.56		2.85		2.68
Moenkhausia conspicua	2.87	2.69	2.77	3.59	2.63	2.91
Nannostomus marginatus	2.88	2.6		3.49	2.81	2.95
Rhamdia muelleri	3.16					3.16
Rhamdia quelen		2.72	3.13			2.93
Rhinotocinclus sp. 2		2.87				2.87
Rhintocinclus sp. 1				3.73		3.73
Saxatilia brasiliensis	3.38	3.21	3.21	3.65	2.9	3.27
Synbranchus madeirae	3.6		2.75			3.18
Synbranchus sp. 1	3.33		3.21			3.27

4. Discussion

Our results show that both aquatic and terrestrial invertebrates play a major role in supporting fish assemblages in Amazonian streams. Allochthonous items are critical to the trophic dynamics of these streams, on average, terrestrial invertebrates and CPOM (coarse particulate organic matter) together accounted for 42% of the local biomass. Their contribution could be even greater since a significant part of the contribution from benthos (33%) is also expected to be primarily derived from allochthonous inputs, as they feed largely on CPOM (Castro et al. 2016). Allochthonous resources played a significant role in the trophic dynamics of Amazonian streams, supporting our first hypothesis. This is because allochthonous inputs serve as both food sources and shelter, not only for fish but also for aquatic invertebrates that support fish biomass (Sabino & Zuanon, 1998; Carvalho et al. 2013). Conversely, macrophytes and riparian vegetation did not appear to be directly assimilated by fish at some sites. Nevertheless, this does not rule out the possibility that these resources are indirectly assimilated, for instance, via invertebrates. The trophic structure, trophic positions and trophic diversity were similar across all stream sites, which were reflected in a great overlap in the trophic niche of fish assemblages of all sites. This confirms our second hypothesis that no variation is expected in the trophic structure of fish assemblages from streams with similar environmental conditions.

Invertivorous species dominated all the stream sites, similar to previous findings that identified invertebrates as primary resources or important components in the diet of Amazonian stream fishes (e.g., Lowe-McConnell, 1999; Ibañez et al. 2007; Barros et al. 2016; Fernandes et al. 2018; Soares et al. 2023). In a study conducted in the Curuá-Una River basin, aquatic insects were found in 100% of the analyzed fish stomachs, while terrestrial insects were present in at least 70% of them (Cardozo & Couceiro, 2017). However, this study analyzed the diet based on stomach content analysis. Through the application of stable isotopes in our study, which reveal the assimilated sources over longer timescales, ranging from weeks to months (Nielsen et al. 2018), we confirmed the assimilation of these invertebrates. We also highlighted the importance of certain often-overlooked items, such as shrimps, while they are usually grouped with benthic invertebrates in isotopic analyses, the high abundance of shrimps during our fieldwork and their distinct $\delta^{13}C$ values compared to benthic invertebrates led us to analyze them as a separate food resource. Shrimps occupied a position in the biplot similar to that of fish, suggesting they might feed on similar resources as invertivorous fish. Additionally, they accounted for 9% to 22% of the local fish biomass, representing the third most important resource contributing to fish biomass. According to Kensley & Walker (1982), Amazonian stream shrimps predominantly prey on arthropods, particularly aquatic insect larvae. Additionally, they represent a significant food resource for carnivorous, piscivorous, and omnivorous species and should be considered in future trophic studies.

The high consumption of invertebrates represents a more nutritious diet for fish and has been reported for assemblages in other pristine Tropical freshwater environments (Correa & Winemiller, 2017; de Carvalho et al. 2019; Paiva et al. 2024). Moreover, the abundance of invertivorous fish may be indicative of good stream integrity (de Carvalho et al. 2017). Invertivore and piscivore species with high swimming abilities have been associated with streams with greater environmental heterogeneity and higher catchment forest cover (Cantanhêde & Montag, 2024). In fact, the links between forest cover and freshwater integrity are well known for Amazonian streams (Leal et al. 2016; Castello & Macedo, 2016; Leal et al. 2018; Brito et al. 2023; Maués-Silva et al. 2023). Thus, disturbances affecting the terrestrial-aquatic interface, such as deforestation, have the potential to alter the availability of resources and nutrients essential for maintaining ecosystem functioning (Machado-Silva et al. 2022) and supporting aquatic assemblages (Zeni & Casatti, 2014). Consequently, those changes on carbon flow may impact aquatic invertebrates and fish assemblages (Lorion et al. 2008; Ceneviva-Bastos et al. 2017; Brejão et al. 2018).

Overall, streams sites exhibited similar trophic niches diversity, density and evenness. The small variations in those metrics suggest that streams with low anthropogenic disturbances display similar trophic structures. However, some variations in resource use are expected due to other factors, as observed in S1, which displayed narrow niches, lower trophic diversity and higher trophic redundancy. The fish assemblage in that stream site probably relies on similar food resources, due to the high richness of invertivorous species (15 out of 21 collected). In contrast, the widest trophic niche was observed at the stream site with the highest recorded number of trophic guilds (S4). This stream site had also the lowest riparian cover, which potentially enhanced primary production and increased the availability of food resources (de Carvalho et al. 2015; de Carvalho et al. 2017; Machado-Silva et al. 2022). This stream site also showed a broader $\delta^{13}C$ range, suggesting the fish assemblage exploits a wider variety of food resources.

The mean trophic positions (TPs) were consistent across all sites, with species feeding at intermediate trophic levels, a pattern already observed in other well-preserved Neotropical streams (Carvalho et al. 2023; Fráguas et al. 2025). However, despite similar overall δ^{15} N ranges, TPs were higher at S4 compared to the others. Among the species present in all sites, three displayed higher TPs at S4, even though their diets showed no significant variation between sites. S4 also exhibited the highest percentage of agricultural land use and CPOM with depleted δ^{15}N values compared to the other sites. Inorganic fertilizers are known to have depleted δ^{15}N values (Nikolenko et al. 2017), which could be influencing the δ^{15}N values observed in CPOM. As we used CPOM as the baseline for estimating trophic positions and fish have enriched δ^{15}N values, the higher TP at S4 was probably influenced by these depleted δ^{15}N values.

In our study, the use of δ^{13}C and δ^{15}N stable isotopes proved to be efficient in assessing the trophic structure and resources sustaining of fish assemblages from Amazonian streams. While this method provides a comprehensive understanding of resource assimilation and trophic ecology (Pereira & Benedito, 2007; Albrecht et al. 2021), it also presents certain challenges. For example, mixing models, which estimate the probability distributions of resource contributions, can sometimes lead to misinterpretations (Phillips et al. 2014). To avoid biases and inaccurate probability estimates, we incorporated stomach content data from Leal et al. (2017). This helped guide the selection of resources that appeared to be more assimilated by each guild. In the absence of this information in the literature, we recommend thorough evaluation of the isotopic compositions of resources and consumers, using bi-plot graphs, for example, alongside species life history data, to help identify the most relevant resources to be included in the models.

Despite these challenges, the use of carbon and nitrogen stable isotopes in trophic ecology studies has significantly advanced our understanding of complex processes in aquatic ecosystems (e.g., Zuluaga-Gómez et al. 2016; Ceneviva-Bastos et al. 2017; de Carvalho et al. 2019; Urbano et al. 2024). Our study is the first to assess the trophic ecology of fish assemblages from Amazonian stream through stable isotope analyses. We found that both aquatic and terrestrial invertebrates were the most important food resources for fish. Moreover, our results suggest a potential similarity in the trophic structure of streams with low anthropogenic disturbances. Despite some specific differences, the main findings are consistent, including the predominance of the invertivorous guild, niche width, δ^{13}C and δ^{15}N ranges, and trophic positions. Although our stream sites were relatively well preserved, Santarém region is part of the Amazonian agriculture frontier that is under intensive pressure. Future studies investigating how land use changes impact carbon and nitrogen fluxes in this megadiverse ecosystem are crucial specially giving the accelerating anthropogenic changes in the Amazon rainforest (Lapola et al., 2023)

Acknowledgements

We would like to thank, Renilson Freitas (Graveto), Maria Fernanda, Joás Brito and Guilherme Berger for their assistance during our fieldwork, Ivo Figueiredo for his help with sample transportation, the Large-Scale Biosphere-Atmosphere Program (LBA) for logistical and infrastructure support in the Santarém region, and private landowners for giving access to the stream sites. We are also grateful to Leonardo Maeoka for making the map for Figure 1 and helping with the landscape data. This project was funded by PELD-RAS (N° 441573/2020-7) and the United Kingdom Research and Innovation (UKRI) Future Leaders Fellowships (MR/W011085/1) including individual funding to CGL and DRC. Other individual funding included a scholarship from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) to GNS (APQ 00401-19) and PHSB.

Autor Contribuitions

Conceptualization: PHSB, DRC, CGL, PSP; Method development: PHSB, DRC, CGL, PSP; Research execution: PHSB, DRC, CGL, PSP, DHCD, GNS, GLB, RPL; Data analysis: PHSB, DRC, PSP; Data interpretation: PHSB, DRC, CGL, PSP; Figures and tables: PHSB; Writing: PHSB, DRC, CGL, PSP, DHCD, GNS, GLB, RPL.

Ethics Statement

This study was approved by the Ethics Committee for Animal Use of the Federal University of Pará (UFPA), under protocol no. 8293020418, and authorised by the Chico Mendes Institute for Biodiversity Conservation (ICMBio/SISBIO), permit no. 87389-1.

Conflicts of Interests

The authors declare no conflicts of interest.

Data Availability Statement

The authors declare that the dataset used in this manuscript is not pub-licly available yet, but is available from the corresponding author onreasonable request.

References

Albrecht, M. P., Reis, A. S., Neres-Lima, V., et al. (2021). Isótopos estáveis e outras ferramentas em estudos tróficos de peixes em riachos trópicais. Oecologia Australis, 25(2), 283–300. https://doi.org/10.4257/oeco.2021.2502.01

Alonso, M. B., de Carvalho, D. R., Alves, C. B. M., et al. (2020). Trophic structure of a fish assemblage in a reference condition river located in a polluted watershed. Environmental Biology of Fishes, 103, 1437–1452. https://doi.org/10.1007/s10641-020-01021-3

Barletta, M., Jaureguizar, A. J., Baigun, C., Fontoura, N. F., Agostinho, A. A., Almeida-Val, V. M. F., Val, A. L., Torres, R. A., Jimenes-Segura, L. F., Giarrizzo, T., Fabré, N. N., Batista, V. S., Lasso, C., Taphorn, D. C., Costa, M. F., Chaves, P. T., Vieira, J. P., & Corrêa, M. F. M. (2010). Fish and aquatic habitat conservation in South America: A continental overview with emphasis on neotropical systems. Journal of Fish Biology, 76(9), 2118–2176. https://doi.org/10.1111/j.1095-8649.2010.02582.x

Barros, G. G., Silva Araújo, M., Takeshi Yogui, G., Zuanon, J., & Pereira de Deus, C. (2024). Damming of streams due to the construction of a highway in the Amazon rainforest favors individual trophic specialization in the fish Bryconops giacopinii. Journal of Fish Biology, 105(6), 1755–1768. https://doi.org/10.1111/jfb.15319

Barros, G., Zuanon, J., & Deus, C. (2017). Effects of species co-occurrence on the trophic-niche breadth of characids in Amazon forest streams. Journal of Fish Biology, 90, 326–340. https://doi.org/10.1111/jfb.13030

Baxter, C. V., Fausch, K. D., & Saunders, W. C. (2005). Tangled webs: Reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology, 50, 201–220. https://doi.org/10.1111/j.1365-2427.2004.01383.x

Brejão, G. L., Hoeinghaus, D. J., Pérez-Mayorga, M. A., Ferraz, S. F. B., & Casatti, L. (2018). Threshold responses of Amazonian stream fishes to timing and extent of deforestation. Conservation Biology, 32(4), 860–871. https://doi.org/10.1111/cobi.13083

Brito, J. S., Silva, E. C., Ferreira, V. R. S., et al. (2024). The importance of national parks in maintaining the habitat integrity and diversity of Odonata species in Amazonian streams. Journal of Insect Conservation, 28, 315–330. https://doi.org/10.1007/s10841-024-00354-x

Cantanhêde, L. G., & de Assis Montag, L. F. (2024). Effects of deforestation on environmental heterogeneity and its role in the distribution of fish species and functional groups in Amazonian streams. Hydrobiologia, 851, 327–341. https://doi.org/10.1007/s10750-024-05032-0

Cardoso, A. C., & Couceiro, S. R. M. (2017). Insects in the diet of fish from Amazonian streams, in western Pará, Brazil. Marine and Freshwater Research, 68, 2052–2060. https://doi.org/10.1071/MF17049

Araujo-Lima, C. A. R. M., et al. (1986). Energy sources for detritivorous fishes in the Amazon. Science, 234, 1256–1258. https://doi.org/10.1126/science.234.4779.1256

Carvalho, L. N., Fidelis, L., Arruda, R., Galuch, A., & Zuanon, J. (2013). Second floor, please: The fish fauna of floating litter banks in Amazonian streams and rivers. Neotropical Ichthyology, 11(1), 85–94. https://doi.org/10.1590/S1679-62252013000100009

Araujo-Lima, C. A. R. M., et al. (1986). Energy sources for detritivorous fishes in the Amazon. Science, 234(1256-1258). https://doi.org/10.1126/science.234.4782.1256

Carvalho, L. N., Fidelis, L., Arruda, R., Galuch, A., & Zuanon, J. (2013). Second floor, please: The fish fauna of floating litter banks in Amazonian streams and rivers. Neotropical Ichthyology, 11(1), 85–94. https://doi.org/10.1590/S1679-62252013000100009

Casatti, L., Brejão, G. L., Carvalho, F. R., Costa, V. S. da, Cruz, G. M. da, Dias-Silva, K., Langeani, F., Lucena, M. D. L. de, Oliveira-Junior, J. M. B., Michelan, T. S., Montag, L. F. de A., Oliveira, B. da C., Seabra, L. B., & Juen, L. (2024). Stream fish assemblages in the Eastern Amazon: The role of small tetras (Characidae) in alpha diversity and taxonomic structure. Neotropical Ichthyology, 22(3), e240014. https://doi.org/10.1590/1982-0224-2024-240014

Casatti, L., Teresa, F. B., Gonçalves-Souza, T., Bessa, E., Manzotti, A. R., Gonçalves, C. da S., & Zeni, J. de O. (2012). From forests to cattail: How does the riparian zone influence stream fish? Neotropical Ichthyology, 10(1), 205–214. https://doi.org/10.1590/S1679-62252012000100021

Castello, L., & Macedo, M. N. (2015). Large-scale degradation of Amazonian freshwater ecosystems. Global Change Biology, 22(3), 990–1007. https://doi.org/10.1111/gcb.13025

Castro, D. M. P. de, Carvalho, D. R. de, Pompeu, P. S., Moreira, M. Z., Nardoto, G. B., & Callisto, M. (2016). Land use influences niche size and the assimilation of resources by benthic macroinvertebrates in tropical headwater streams. PLOS ONE, 11(3), e0151260. https://doi.org/10.1371/journal.pone.0151260

Ceneviva-Bastos, M., Montana, C. G., Schalk, C. M., Camargo, P. B., & Casatti, L. (2017). Responses of aquatic food webs to the addition of structural complexity and basal resource diversity in degraded neotropical streams. Austral Ecology. https://doi.org/10.1111/aec.12469

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Communications in Mass Spectrometry, 25(17), 2538–2560. https://doi.org/10.1002/rcm.5129

Correa, S. B., & Winemiller, K. (2018). Terrestrial–aquatic trophic linkages support fish production in a tropical oligotrophic river. Oecologia, 186(4), 1069–1078. https://doi.org/10.1007/s00442-018-4161-0

Dalerum, F., & Angerbjörn, A. (2005). Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia, 144(4), 607–614. https://doi.org/10.1007/s00442-005-0111-x

de Carvalho, D. R., de Castro, D. M. P., Callisto, M., et al. (2019). Stable isotopes and stomach content analyses indicate omnivorous habits and opportunistic feeding behavior of an invasive fish. Aquatic Ecology, 53, 365–381. https://doi.org/10.1007/s10452-019-09856-4

de Carvalho, D. R., de Castro, D. M. P., Callisto, M., Moreira, M. Z., & Pompeu, P. S. (2015). Isotopic variation in five species of stream fishes under the influence of different land uses. Journal of Fish Biology, 87(3), 559-578. https://doi.org/10.1111/jfb.12652

de Carvalho, D. R., Leal, C. G., Junqueira, N. T., Castro, M. A., Fagundes, D. C., Bernardo, C., Hughes, R. M., & Pompeu, P. S. (2017). A fish-based multimetric index for Brazilian savanna streams. Ecological Indicators, 77, 386–396. https://doi.org/10.1016/j.ecolind.2017.02.042

de Carvalho, D. R., de Castro, D. M. P., Callisto, M., et al. (2017). The trophic structure of fish communities from streams in the Brazilian Cerrado under different land uses: An approach using stable isotopes. Hydrobiologia, 795, 199–217. https://doi.org/10.1007/s10750-017-3144-0

de Carvalho, D. R., Ferreira, F. F., Dergam, J. A., et al. (2024). Food web structure of fish communities of Doce River, 5 years after the Fundão dam failure. Environmental Monitoring and Assessment, 196(300). https://doi.org/10.1007/s10661-024-10130-9

de Carvalho, D. R., Alves, C. B. M., Moreira, M. Z., & Pompeu, P. S. (2020). Trophic diversity and carbon sources supporting fish communities along a pollution gradient in a tropical river. Science of the Total Environment, 738, 139878. https://doi.org/10.1016/j.scitotenv.2020.139878

DeNiro, M., & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta, 45, 341–351. https://doi.org/10.1016/0016-7037(81)90108-6

Domingues, T. F., Berry, J. A., Martinelli, L. A., Ometto, J. P. H. B., & Ehleringer, J. R. (2005). Parameterization of canopy structure and leaf-level gas exchange for an Eastern Amazonian tropical rain forest (Tapajós National Forest, Pará, Brazil). Earth Interactions, 9(17), 1–23. https://doi.org/10.1175/EI160.1

Ellwanger, J. H., Kulmann-Leal, B., Kaminski, V. L., et al. (2020). Beyond diversity loss and climate change: Impacts of Amazon deforestation on infectious diseases and public health. Anais Da Academia Brasileira De Ciências, 92(1). https://doi.org/10.1590/0001-3765202020190456

Esteves, K. E., Aranha, J. M. R., & Albrect, M. P. (2021). Ecologia trófica de peixes de riacho: Uma releitura 20 anos depois. Oecologia Australis, 25(2), 266-282. https://doi.org/10.4257/oeco.2021.2502.05

Machado-Silva, F., Neres-Lima, V., Oliveira, A. F., & Moulton, T. P. (2022). Forest cover controls the nitrogen and carbon stable isotopes of rivers. Science of The Total Environment, 817, 153040. https://doi.org/10.1016/j.scitotenv.2022.153040

Fernandes, S., Leitão, R. P., Dary, E. P., Guerreiro, A. I. C., Zuanon, J., & Bührnheim, C. M. (2017). Diet of two syntopic species of Crenuchidae (Ostariophysi: Characiformes) in an Amazonian rocky stream. Biota Neotropica, 17(1), e20160281. https://doi.org/10.1590/1676-0611-BN-2016-0281

Forsberg, B. R., Araujo-Lima, C. A. R. M., Martinelli, L. A., Victoria, R. L., & Bonassi, J. A. (1993). Autotrophic carbon sources for fish of the central Amazon. Ecology, 74(3), 643-652. https://doi.org/10.2307/1939550

Frederico, R. G., Dias, M. S., Jézéquel, C., Tedesco, P. A., Hugueny, B., Zuanon, J., et al. (2021). The representativeness of protected areas for Amazonian fish diversity under climate change. Aquatic Conservation: Marine and Freshwater Ecosystems, 31(5), 1158–1166. https://doi.org/10.1002/aqc.3560

Fry, B., & Sherr, E. B. (1984). δ13C measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in Marine Science, 13–47.

Fry, B. (2006). Stable Isotope Ecology. Springer. https://doi.org/10.1007/978-1-4614-0002-8

Gardner, T. A., Ferreira, J., Barlow, J., Lees, A. C., Parry, L., Vieira, I. C. G., Berenguer, E., Abramovay, R., Aleixo, A., Andretti, C., Aragão, L. E. O. C., Araújo, I., de Ávila, W. S., Bardgett, R. D., Batistella, M., Begotti, R. A., Beldini, T., de Blas, D. E., Braga, R. F., & Braga, D. de L. (2013). A social and ecological assessment of tropical land uses at multiple scales: the Sustainable Amazon Network. Philosophical Transactions of the Royal Society B: Biological Sciences, 368(1619), 20120166. https://doi.org/10.1098/rstb.2012.0166

Bunn, S. E., & Hadwen, W. L. (2005). Food web responses to low-level nutrient and 15N-tracer additions in the littoral zone of an oligotrophic dune lake. Limnology and Oceanography, 4(1), 1–9. https://doi.org/10.4319/lo.2005.50.1.0001

Ibañez, C., Tedesco, P. A., Bigorne, R., Hugueny, B., Pouilly, M., Zepita, C., Zubieta, J., & Oberdorff, T. (2007). Dietary-morphological relationships in fish assemblages of small forested streams in the Bolivian Amazon. Aquatic Living Resources, 20(2), 131–142. https://doi.org/10.1051/alr:2007015

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology, 80(3), 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Jepsen, D. B., & Winemiller, K. O. (2002). Structure of tropical river food webs revealed by stable isotope ratios. Oikos, 96(1), 46–55. https://doi.org/10.1034/j.1600-0706.2002.960105.x

Junk, W. J., Soares, M. G. M., & Bayley, P. B. (2007). Freshwater fishes of the Amazon River basin: Their biodiversity, fisheries, and habitats. Aquatic Ecosystem Health & Management, 10(2), 153–173. https://doi.org/10.1080/14634980701318968

Kensley, B., & Walker, I. (1982). Palaemonid shrimps from the Amazon Basin, Brazil (Crustacea: Decapoda: Natantia). Smithsonian Contributions to Zoology, No. 362. Smithsonian Institution Press. https://doi.org/10.5479/si.00810282.362

Lapola, D. M., Pinho, P., Barlow, J., Aragão, L. E. O. C., Berenguer, E., Carmenta, R., Liddy, H. M., Seixas, H., Silva, C. V. J., Silva-Junior, C. H. L., Alencar, A. A. C., Anderson, L. O., Armenteras, D., Brovkin, V., Calders, K., Chambers, J., Chini, L., Costa, M. H., Faria, B. L., & Fearnside, P. M. (2023). The drivers and impacts of Amazon forest degradation. Science, 379(6630). https://doi.org/10.1126/science.abi9223

Latrubesse, E., Arima, E., Dunne, T., et al. (2017). Damming the rivers of the Amazon basin. Nature, 546, 363–369. https://doi.org/10.1038/nature22333

Layman, C. A., Arrington, D. A., Montaña, C. G., & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology, 88(1), 42–48. https://doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2

Leal, C. G., Barlow, J., Gardner, T. A., et al. (2017). Is environmental legislation conserving tropical stream faunas? A large-scale assessment of local, riparian and catchment-scale influences on Amazonian fish. Journal of Applied Ecology, 55(3), 1312–1326. https://doi.org/10.1111/1365-2664.12856

Leal, C. G., Pompeu, P. S., Gardner, T. A., et al. (2016). Multi-scale assessment of human-induced changes to Amazonian instream habitats. Landscape Ecology, 31, 1725–1745. https://doi.org/10.1007/s10980-016-0373-1

Leitão, R. P., Zuanon, J., Mouillot, D., et al. (2018). Disentangling the pathways of land use impacts on the functional structure of fish assemblages in Amazon streams. Ecography, 41, 219–232. https://doi.org/10.1111/ecog.02721

Leite, R. G., Araujo-Lima, C. A. R. M., Victoria, R. L., & Martinelli, L. A. (2002). Stable isotope analysis of energy sources for larvae of eight fish species from the Amazon floodplain. Ecology of Freshwater Fish, 11(1), 56-63. https://doi.org/10.1034/j.1600-0633.2002.01008.x

Letourneur, Y., Fey, P., Dierking, J., Galzin, R., & Parravicini, V. (2024). Challenging trophic position assessments in complex ecosystems: Calculation method, choice of baseline, trophic enrichment factors, season and feeding guild do matter: A case study from Marquesas Islands coral reefs. Ecology and Evolution, 14, e11620. https://doi.org/10.1002/ece3.11620

Lowe-McConnell, R. H. (1999). Ecological studies in tropical fish communities. Cambridge University Press. https://doi.org/10.1017/CBO9780511752551

Martinelli, L. A., & Camargo, P. B. (2009). Desvendando Questões Ambientais com Isótopos Estáveis. Oficina de Textos.

Maués-Silva, R., Barbosa, M., Martins, G., et al. (2024). Evaluation of the environmental quality monitoring protocol for Amazonian streams: a systematic review. Ambiente & Água - an Interdisciplinary Journal of Applied Science, 19, 1–14. https://doi.org/10.4136/ambi-agua.2835

McClain, M. E., & Elsenbeer, H. (2001). Terrestrial Inputs to Amazon Streams and Internal Biogeochemical Processing. In The Biogeochemistry of the Amazon Basin.

Mortillaro, J.M., Pouilly, M., Wach, M., Freitas, C.E.C., Abril, G., & Meziane, T. (2015). Trophic opportunism of central Amazon floodplain fish. Freshwater Biology, 60, 1659-1670. https://doi.org/10.1111/fwb.12603

Newsome, S. D., Rio, C. M. del, Bearhop, S., & Phillips, D. L. (2007). A niche for isotopic ecology. Frontiers in Ecology and the Environment, 5(8), 429–436. https://doi.org/10.1890/060151

Nikolenko, O., Jurado, A., Borges, A., Knöller, K., & Brouyère, S. (2018). Isotopic composition of nitrogen species in groundwater under agricultural areas: A review. Science of the Total Environment, 621, 1415–1432. https://doi.org/10.1016/j.scitotenv.2017.11.243

Paiva, L.C., de Carvalho, D.R., Ferreira, F.F., et al. (2024). Trophic ecology of a small characid reflects the degradation of a basin after the rupture of an ore tailings dam. Aquatic Ecology. https://doi.org/10.1007/s10452-024-09978-4

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2013). Source partitioning using stable isotopes: Coping with too much variation. PLOS ONE, 8(10), e75760. https://doi.org/10.1371/journal.pone.0075760

Paul, D., Skrzypek, G., & Fórizs, I. (2007). Normalization of measured stable isotopic compositions to isotope reference scales – a review. Rapid Communications in Mass Spectrometry, 21(18), 3006–3014. https://doi.org/10.1002/rcm.3153

Pereira, A. L., & Benedito, E. (2007). Isótopos estáveis em estudos ecológicos: métodos, aplicações e perspectivas. Revista Biociências (Taubaté), 13, 16–27.

Perkins, M. J., McDonald, R. A., van Veen, F. J. F., et al. (2014). Application of Nitrogen and Carbon Stable Isotopes (δ15N and δ13C) to Quantify Food Chain Length and Trophic Structure. PLOS ONE, 9(3), e93281. https://doi.org/10.1371/journal.pone.0093281

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., Semmens, B. X., & Ward, E. J. (2014). Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology, 92(10), 823–835. https://doi.org/10.1139/cjz-2014-0065

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology, 83(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Quezada-Romegialli, C., Jackson, A. L., Hayden, B., Kahilainen, K. K., Lopes, C., & Harrod, C. (2018). tRophicPosition, an r package for the Bayesian estimation of trophic position from consumer stable isotope ratios. Methods in Ecology and Evolution, 9(6), 1592–1599. https://doi.org/10.1111/2041-210X.13033

Sabino, J., & Zuanon, J. (1998). A stream fish assemblage in Central Amazonia: Distribution, activity patterns and feeding behavior. Ichthyological Exploration of Freshwaters, 8(3), 201–210.

Sacramento, P.A., Manetta, G.I., & Benedito, E. (2016). Diet-tissue discrimination factors (Δ13C and Δ15N) and turnover rate in somatic tissues of a neotropical detritivorous fish on C3 and C4 diets. Journal of Fish Biology, 89(1), 213–219. https://doi.org/10.1111/jfb.12963

Santos, G. M., Ferreira, E. J. G. (1999). Peixes da Bacia Amazônica. In R. H. Lowe-McConnell (Ed.), Estudos Ecológicos de Comunidades de Peixes Tropicais (pp. 341–362). Edusp.

Schimmelmann, A., Qi, H., Coplen, T. B., Brand, W. A., Fong, J., Meier-Augenstein, W., Kemp, H. F., Toman, B., Ackermann, A., Assonov, S., Aerts-Bijma, A. T., Brejcha, R., Chikaraishi, Y., Darwish, T., Elsner, M., Gehre, M., Geilmann, H., Gröning, M., Hélie, J.-F., … Werner, R. A. (2016). Organic Reference Materials for Hydrogen, Carbon, and Nitrogen Stable Isotope-Ratio Measurements: Caffeines, n-Alkanes, Fatty Acid Methyl Esters, Glycines, l-Valines, Polyethylenes, and Oils. Analytical Chemistry, 88(8), 4294–4302. https://doi.org/10.1021/acs.analchem.6b01031

Schimmelmann, A., Qi, H., Dunn, P. J. H., Camin, F., Bontempo, L., Potočnik, D., Ogrinc, N., Kelly, S., Carter, J. F., Abrahim, A., Reid, L. T., & Coplen, T. B. (2020). Food Matrix Reference Materials for Hydrogen, Carbon, Nitrogen, Oxygen, and Sulfur Stable Isotope-Ratio Measurements: Collagens, Flours, Honeys, and Vegetable Oils. Journal of Agricultural and Food Chemistry, 68(39), 10852–10864. https://doi.org/10.1021/acs.jafc.0c03048

Seabra, L.B., Huckembeck, S., Freitas, T.M.S., et al. (2024). Variation in basal sources contribution to the diet of a predator fish in an altered flood pulse area in the Amazon. Hydrobiologia. https://doi.org/10.1007/s10750-024-04821-9

Soares, B.E., Benone, N.L., Lobato, C.M.C., et al. (2023). Pasture areas reduce the abundance and trophic niche width, but not the body condition of the Amazonian whale catfish. Aquatic Ecology, 57, 187–198. https://doi.org/10.1007/s10452-023-09976-x

Vieira, T. B., Pavanelli, C. S., Casatti, L., Smith, W. S., Benedito, E., et al. (2018). A multiple hypothesis approach to explain species richness patterns in neotropical stream-dweller fish communities. PLOS ONE, 13(9), e0204114. https://doi.org/10.1371/journal.pone.0204114

Winemiller, K. O., Hoeinghaus, D. J., Pease, A. A., et al. (2011). Stable isotope analysis reveals food web structure and watershed impacts along the fluvial gradient of a Mesoamerican coastal river. River Research and Applications, 27, 791–803. https://doi.org/10.1002/rra.1412

Zuluaga-Gómez, M. A., Fitzgerald, D. B., Giarrizzo, T., et al. (2016). Morphologic and trophic diversity of fish assemblages in rapids of the Xingu River, a major Amazon tributary and region of endemism. Environmental Biology of Fishes, 99, 647–658. https://doi.org/10.1007/s10641-016-0537-4