UNIVERSIDADE FEDERAL DE LAVRAS

DÉBORA REIS DE CARVALHO

EFFECTS OF AQUATIC POLLUTION UNDER TROPHIC ASPECTS OF FISH FAUNA: AN APPROACH USING CARBON ($\delta^{13}C$) AND NITROGEN ($\delta^{15}N$) STABLE ISOTOPES

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Tese 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 obtenção do título de Doutor.

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

de Carvalho, Débora Reis.
Effects of aquatic pollution under trophic aspects of fish fauna: An approach using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes / Débora Reis de Carvalho. - 2020.
216 p. : il.

Orientador(a): Paulo dos Santos Pompeu.
Coorientador(a): Alexander Flecker, Jed Sparks.
Tese (doutorado) - Universidade Federal de Lavras, 2020.
Bibliografia.

1. Urban pollution. 2. Food web. 3. Fish communities. I. Pompeu, Paulo dos Santos. II. Flecker, Alexander. III. Sparks, Jed. IV. Título.

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EFEITOS DA POLUIÇÃO AQUÁTICA SOB ASPECTOS TRÓFICOS DA FAUNA DE PEIXES: UMA ABORDAGEM UTILIZANDO OS ISÓTOPOS ESTÁVEIS DE CARBONO ($\delta^{13}C$) E NITROGÊNIO ($\delta^{15}N$)

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DEDICATÓRIA

Aos meus pais, que sempre foram meu alicerce e que nunca mediram esforços para ver uma filha se tornar doutora. Dedico.

AGRADECIMENTOS

Agradeço primeiramente a Deus pela oportunidade de me dedicar inteiramente a algo que me dá tanto prazer e satisfação. Hoje tenho mais do que certeza de que ser bióloga (e ecóloga/peixóloga) é muito mais do que uma profissão. Obrigada pela oportunidade de morar fora e ampliar meus horizontes, aprender outras línguas e conhecer novas culturas. E principalmente, obrigada pelos últimos 4 anos em que pude me dedicar em fazer algo pelo nosso meio ambiente.

Em tempos onde a ciência não tem sido muito valorizada, não poderia deixar de fazer um agradecimento especial as agências de fomento, que permitiram que esse projeto fosse desenvolvido. Agradeço a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) pela concessão da minha bolsa de doutorado (Código de Financiamento 001) e de doutorado sanduíche (88881.190508/2018-01). Agradeço também a AGB Peixe Vivo e ao Comitê da Bacia Hidrográfica do Rio das Velhas, pelo financiamento do presente projeto. Aproveito para agradecer também ao Carlos B. M. Alves, que me convidou para participar do projeto “Biomonitoramento da ictiofauna e Monitoramento ambiental participativo na Bacia do Rio das Velhas” ao qual o meu projeto está vinculado. Obrigada, Cacá, por acreditar no meu trabalho, por liderar todas as amostragens e por ser sempre tão prestativo.

Em especial agradeço ao professor, orientador, amigo, Paulo Pompeu. São 10 anos de orientação e amizade. Nesses 10 anos me inspiro em você, e aprendi o que é ser um bom professor, um mestre de verdade, que ensina com vontade e de maneira inovadora. Nesses 10 anos tenho aprendido muito e pude conhecer uma pessoa alto astral, dedicada, criativa e acessível, que faz todo trabalho ser mais divertido e que torna a convivência do laboratório a melhor possível. Agradeço imensamente por você não ter deixado eu desanimar (logo após uma sofrida defesa de mestrado), hoje trabalho com algo que amo e pretendo continuar trabalhando por muito tempo ainda. Obrigada Paulo, pode ter certeza que levarei seus ensinamentos por toda a minha caminhada.

Agradeço a todos do laboratório de ecologia de peixes (aqueles que já passaram e os que ainda permanecem por lá) pela convivência, pela ajuda e companheirismo. Somos uma verdadeira família! Agradeço a Mirella e a Marina por terem confiado em mim para orientá-las na graduação. Agradeço especialmente as Gatas, Tatau e Aline, que acompanharam de perto todos os dilemas, aflições e conquistas de todo o período do doutorado. Tatau, obrigada por dividir todos os momentos comigo, por ser paciente e sempre uma boa ouvinte, e principalmente por ter trazido a Iarinha ao mundo. A Aline reservo um agradecimento especial, por ter me ajudado em todas as triagens e no processamento das inúmeras amostras do meu projeto. Obrigada também por compartilhar sua vida “sem-limites”, seus dotes futebolísticos e diversos momentos de diversão nesses últimos 4 anos.

Agradeço a todos amigos da Ecologia por todo o apoio e pelos momentos de descontração durante ao doutorado. Agradecimento especial a Nina, Rafa Maciel e Rafa Bastos que sempre estão por perto, mesmo na correria de nossas vidas.

Não poderia deixar de agradecer à Ellen, nossa querida secretária, que não mede esforços para que tudo dê certo na nossa pós-graduação. Obrigada por todo o suporte durantes todos esses anos.

Aos professores, sempre amigos e acessíveis, muito obrigada por passarem tanto conhecimento e por motivarem a mim e aos meus colegas de estudo a sempre dar o nosso melhor! Agradecimento especial aos professores e pesquisadores, Diego Castro, Nelson Curi, Marcelo Passamani, Alessandra Bueno, Eduardo Van Den Berg e Thais Pellegrini, que aceitaram integrar minha banca de defesa.

Agradeço aos professores, Alex Flecker e Jed Sparks, pela co-orientação durante o doutorado sanduíche na Cornell University e por terem me acolhido e recebido tão bem em seus laboratórios. A ajuda de vocês foi essencial para que este trabalho fosse desenvolvido. Agradeço também aos amigos Americanos, em especial a Michelle Wong e a Amelia Weiss, por toda amizade, carinho e suporte desde o primeiro momento que cheguei nos Estados Unidos. Agradeço as minhas vizinhas do Highland 316, Priscila e Diéssy, e as minhas amigas de sneebs no Corson Hall, em especial a Nina, a Manu, e a Jéssica, por todos momentos vividos durante esse período morando em Ithaca.

Agradeço as minhas amigas de infância, que apesar de não entenderem muito o que a amiga bióloga faz (e muitas vezes ficarem horrorizadas como os lugares em que eu me meto nos meus campos), sempre estiveram ali me incentivando e motivando. Agradeço a minha amiga e afilhada Andressa, por toda a amizade e parceria que seu intensificou ainda mais nos últimos anos.

Agradecimento especial aos meus pais, Benito e Norma, e minhas irmãs, Mariana e Jacqueline, por estarem sempre ao meu lado me incentivando e aguentando os estresses e dilemas da vida de um pós-graduando. Aos meus sobrinhos, por trazerem tanto amor e alegria para nossa família. Obrigada por serem meu alicerce e por não medirem esforços para que tudo desse certo. Amo vocês imensamente! Aos meus avós, que já não se encontram aqui conosco, obrigada por torcerem por mim aí de cima.

Agradeço ao Douglas, por todo amor e companheirismo durante os últimos 3 anos de doutorado. Inclusive, sou muito grata pelo doutorado ter favorecido o nosso encontro. Agradeço também a todos de Santa Catarina que estiveram presentes em minha vida nos últimos anos, em especial ao Robson por sempre ter me acolhido e recebido tão bem em SC.

Por fim, agradeço à Universidade Federal de Lavras (minha casa nos últimos 13 anos) e ao Programa de Ecologia de Aplicada, pela oportunidade, investimento e suporte durante todo o período em que fui aluna dessa instituição.

RESUMO

A transferência da capital de Minas Gerais para Belo Horizonte em 1898, teve como consequência a expansão de um polo de urbanização e industrialização nas margens do Rio das Velhas, o maior afluente em extensão da Bacia do São Francisco. Com isso, a região que antes era conhecida por sua abundância de peixes, nos dias atuais se encontra em adiantado processo de degradação. O principal objetivo deste estudo foi utilizar os isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) para avaliar se as diversas atividades antrópicas presentes na bacia do Rio das Velhas (em especial a poluição urbana) afetam a estrutura trófica das comunidades de peixes. Além disso, objetivou-se também avaliar se a composição isotópica dos peixes e de outros organismos aquáticos são bons indicadores de presença e intensidade de poluição aquática. Nos quatro capítulos que constituem esse estudo, foram avaliados os impactos da poluição desde nível específico até à nível de comunidade. No primeiro capítulo, observou-se que a poluição afeta o comportamento alimentar de uma espécie exótica (*Poecilia reticulata*) e de uma espécie endêmica (*Phalloceros uai*) de forma similar, entretanto a espécie exótica se alimenta diretamente do esgoto (além de ser encontrada em grande abundância em locais poluídos), o que pode favorecer o estabelecimento dessa espécie em locais muito poluídos. No segundo capítulo, observou-se que a assinatura de nitrogênio ($\delta^{15}N$) do perifíton e dos cascudos (consumidores de perifíton) varia temporalmente e espacialmente em toda bacia em resposta a variação nas concentrações de nitrato e amônia. E, portanto, ambos podem ser utilizados como bioindicadores de poluição. Entretanto, o perifíton é mais sensível a mudanças nas concentrações de nitrato e amônia entre estações do que os peixes. No terceiro capítulo, observou-se que a poluição promove alterações nos nichos tróficos das comunidades de peixes de diferentes pontos da bacia. Em pontos poluídos, observou-se uma grande variação no eixo do $\delta^{15}N$, o que provavelmente ocorre devido a elevada concentração de nitrogênio (especialmente nitrato) proveniente de atividades antrópicas. Nestes pontos, também houve um maior enriquecimento de $\delta^{15}N$ nos peixes detritívoros, o que não é esperado para peixes que se alimentam na base da teia trófica. No quarto e último capítulo, observou-se que os recursos autotróficos (algas, macrófitas e perifíton) são as principais fontes de carbono para os peixes do Rio das Velhas. Entretanto, nos pontos imediatamente após a Região Metropolitana de Belo Horizonte, o esgoto é uma fonte alimentar importante para peixes de todas as guildas alimentares. Métricas indicadoras de diversidade trófica também foram afetadas pela poluição. Apesar de haver uma redução no número de espécies nos locais mais poluídos, houve um aumento na diversidade trófica, e uma menor redundância nos itens consumidos pelos peixes. Concluímos que os isótopos estáveis constituem uma importante ferramenta na avaliação dos efeitos da poluição sobre as comunidades aquáticas. Além disso, os peixes, que alteram seu comportamento trófico em resposta a poluição, se mostraram bons indicadores de qualidade da água, podendo ser utilizados em programas de monitoramento ambiental não só do Rio das Velhas, mas em outras bacias hidrográficas.

Palavras – chave: Teia trófica. Bioindicadores. Espécies exóticas. Esgoto.

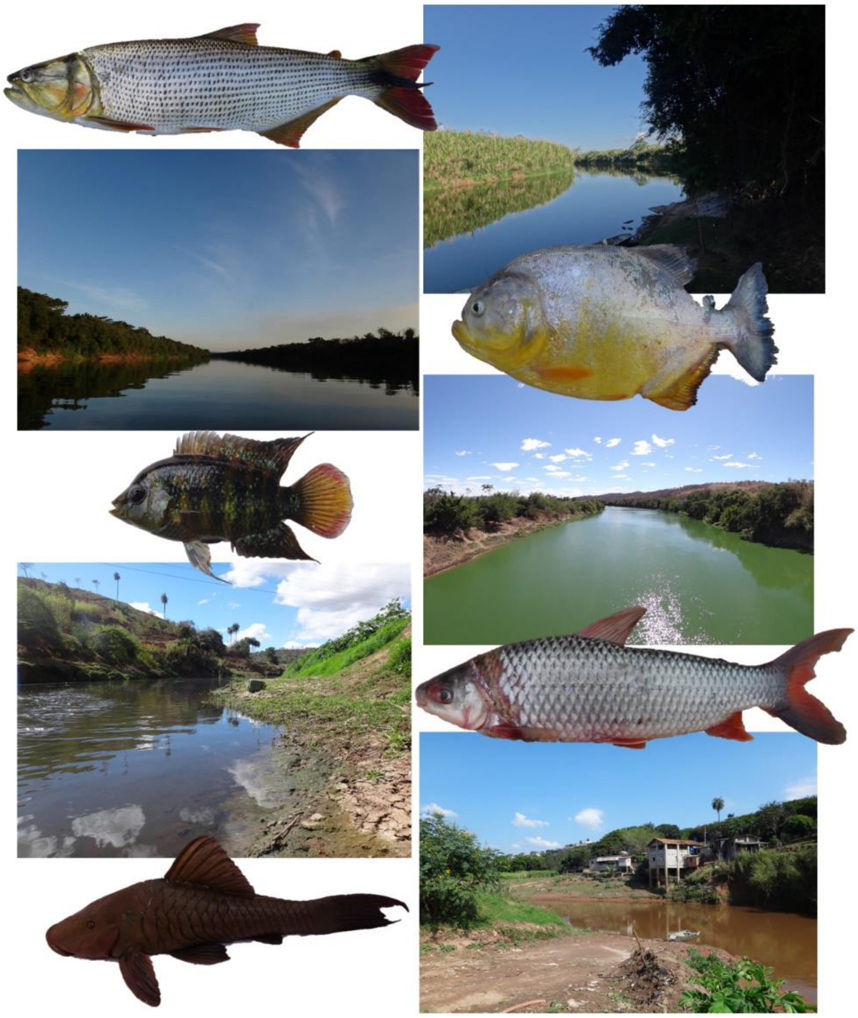
ABSTRACT

The transfer of the capital of Minas Gerais to Belo Horizonte in 1898, resulted in the expansion of a center of urbanization and industrialization along the Rio das Velhas, the largest tributary in extension of the São Francisco basin. Thus, the region that was once known for its abundance of fish, nowadays is in an advanced process of degradation. The main objective of this study was to use the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to evaluate whether the various anthropogenic activities present in the Rio das Velhas basin (especially urban pollution) affect the trophic structure of fish communities. In addition, other objective was to evaluate whether the isotopic composition of fish and other aquatic organisms are good indicators of the presence and intensity of aquatic pollution. In the four chapters that make up this study, the impacts of pollution from the specific level to the community level were assessed. In the first chapter, it was observed that pollution affects the feeding behavior of an exotic species (*Poecilia reticulata*) and an endemic species (*Phalloceros uai*) in a similar way, however the exotic species feeds directly from sewage (and it is found in abundance in polluted places), which may favor the establishment of this species in strongly polluted sites. In the second chapter, it was observed that the nitrogen signature ($\delta^{15}N$) of the periphyton and the cascudos (periphyton consumers) varies temporally and spatially throughout the basin in response to changes in nitrate and ammonia concentrations. And, therefore, both can be used as pollution bioindicators. However, periphyton is more sensitive to changes in nitrate and ammonia concentrations between seasons than fish. In the third chapter, it was observed that pollution promotes changes in the trophic niches of fish communities from different sites of the basin. At polluted points, a large variation in the $\delta^{15}N$ axis was observed, which is probably due to the high concentration of nitrogen (especially nitrate) from anthropic activities. At these points, there was also greater $\delta^{15}N$ enrichment in detritivorous fish, which is not expected for fish that feed on the base of the food web. In the fourth and last chapter, it was observed that autotrophic resources (algae, macrophytes and periphyton) are the main sources of carbon for the fish of Rio das Velhas. However, at points immediately after the Belo Horizonte Metropolitan Region, sewage is an important food source for fish of all trophic guilds. Metrics indicating trophic diversity were also affected by pollution. Although it was observed a reduction in the number of species in the most polluted sites, there was an increase in trophic diversity, and less redundancy in the items consumed by fish. We conclude that stable isotopes are an important tool in assessing the effects of pollution on aquatic communities. In addition, fish, which alter their trophic behavior in response to pollution, proved to be good indicators of water quality and could be used in environmental monitoring programs not only in Rio das Velhas, but in other watersheds.

Keywords: Food web. Bioindicators. Exotic species. Sewage.

SUMÁRIO

PRIMEIRA PARTE



1 INTRODUÇÃO GERAL

O Rio das Velhas é o maior afluente em extensão da Bacia do São Francisco (MACHADO; ALVES; CALLISTO, 2008) e está localizado totalmente em território mineiro, com suas águas abrangendo 51 municípios, entre eles a região metropolitana de Belo Horizonte em seu curso superior. A transferência da capital de Minas Gerais para Belo Horizonte em 1898, teve como consequência a expansão de um polo de urbanização e industrialização nas margens do Rio das Velhas. Com isso, a região que antes era conhecida por sua abundância de peixes (ALVES; POMPEU, 2010), nos dias atuais se encontra em adiantado processo de degradação. As principais atividades poluidoras se encontram no curso superior, que recebe a maior parte do esgoto doméstico e industrial da região metropolitana da capital mineira (ALVES; POMPEU, 2010). Além disso, boa parte do rio está localizada no Quadrilátero Ferrífero, área crítica devido aos inúmeros empreendimentos minerários (MACHADO; ALVES; CALLISTO, 2008). Os efeitos promovidos por essas atividades antrópicas podem ser sentidos por toda a sua extensão, sendo episódios de mortandade de peixes e a baixa qualidade da água conhecida e documentada na literatura (ALVES; POMPEU, 2008).

A partir do ano de 1999, após a criação do Projeto Manuelzão da UFMG, diversos estudos sobre a fauna de peixes vêm sendo desenvolvidos com o objetivo de avaliar e monitorar a qualidade ambiental do rio das Velhas (ALVES; POMPEU, 2005; POMPEU; ALVES; CALLISTO, 2005) e avaliar diferentes aspectos da ictiofauna dessa bacia (JUNQUEIRA et al., 2012; LEAL; JUNQUEIRA; POMPEU, 2011). No ano de 2015 teve início mais um projeto com o objetivo de dar continuidade ao trabalho de monitoramento que já vinha sendo realizado na bacia do Rio das Velhas. Dessa vez, optou-se pela utilização de novas ferramentas, como os isótopos estáveis, que permitiram averiguar aspectos antes não estudados da ictiofauna, como por exemplo, a avaliação da estrutura trófica das comunidades de peixes.

Os isótopos estáveis estão presentes nos ecossistemas e sua distribuição natural reflete, de forma integrada, a história dos processos físicos e metabólicos do ambiente. Os isótopos estáveis de carbono e nitrogênio estão entre os mais usados e podem ser analisados juntos ou separadamente, dependendo do objetivo do estudo. Em conjunto, são amplamente utilizados em estudos analisando teias tróficas principalmente devido a sua capacidade de determinação das fontes de alimento para consumidores em teias alimentares aquáticas e terrestres. A transferência da assinatura isotópica de carbono (^{13}C) ao longo da teia trófica é conservativa podendo ser utilizada para traçar o fluxo de energia em sistemas onde existem vários tipos de alimentos com diferenças nos valores de ^{13}C. O isótopo de nitrogênio (^{15}N), por outro lado, é fracionado consistentemente ao longo da teia trófica, o que possibilita inferências sobre as relações tróficas dos consumidores com a sua dieta (VANDER ZANDEN; CABANA; RASMUSSEN, 1997). Portanto, a partir dos valores de carbono e nitrogênio de cada organismo é possível saber quais os recursos mais utilizados na alimentação desse organismo e inferir qual nível trófico ele ocupa em uma teia trófica. Desta forma, a utilização dos isótopos é extremamente útil na avaliação dos impactos antrópicos (como poluição, uso intensivo do solo, etc.) em ecossistemas aquáticos, pois permite saber como a alimentação dos peixes é afetada diante da degradação ambiental. Além de ser amplamente utilizado em estudos sobre a estrutura trófica, o isótopo de nitrogênio também vem sendo aplicado em trabalhos que traçam a poluição (GARTNER; LAVERY; SMIT, 2002; WANG et al., 2016), pois é capaz de refletir os efeitos da elevada concentração de nitrogênio nos tecidos dos organismos aquáticos.

Considerando a versatilidade desta ferramenta, o principal objetivo deste estudo foi utilizar os isótopos estáveis para avaliar se as diversas atividades antrópicas presentes na bacia do Rio das Velhas (em especial a poluição urbana) afetam a estrutura trófica das comunidades de peixes. Além disso, objetivou-se também avaliar se a composição isotópica dos peixes e de outros organismos aquáticos são bons indicadores de presença e intensidade de poluição aquática. Desta forma, a presente tese foi dividida em quatro capítulos com o objetivo de avaliar os impactos da poluição desde nível específico até à nível de comunidade, respondendo as seguintes perguntas:

1) *Espécies nativas e espécies exóticas são afetadas pela poluição de maneira similar?*

No primeiro capítulo da tese, já publicado na revista **Science of the Total Environment,** nós comparamos a composição isotópica de uma espécie endêmica da bacia do Rio das Velhas (*Phalloceros uai*) com a de uma espécie exótica extremamente abundante na bacia (*Poecilia reticulata*). O objetivo foi avaliar se a poluição promove alterações similares na alimentação e no nicho trófico da espécie exótica e a da espécie nativa.

2) *Amostras de peixes e perifíton podem ser utilizadas para traçar a poluição?*

A partir da composição isotópica de nitrogênio ($\delta\ ^{15}N$) de recursos e consumidores é possível obter informações importantes sobre o grau de poluição dos copos d’água. Isso ocorre porque a composição isotópica de nitrogênio dos organismos que vivem em ambientes aquáticos irá refletir a concentração de nitrogênio (nitrato, amônia, etc.) na água e os processos que ali ocorrem (nitrificação, desnitrificação). Além disso, o $\delta^{15}N$ dos organismos aquáticos também ajuda a determinar quais as principais fontes de poluição, uma vez que determinadas atividades promovem uma diminuição nos valores de $\delta^{15}N$ (como certas indústrias de fertilizantes) e certas atividades promovem um aumento nos valores de $\delta^{15}N$ (como a descarga de esgotos domésticos e pastagens). Portanto, no segundo capítulo da tese, submetido na revista **Ecological Indicators**, nós avaliamos se a composição de nitrogênio do perifíton (biofilme) varia de acordo com os níveis de nitrogênio (concentrações de nitrato e amônia) na bacia. Como os valores de nitrogênio são “acumulados” de um nível trófico para o outro, espera-se que peixes que se alimentam de perifíton também refletirão essas alterações nos valores de nitrogênio. Portanto, o segundo objetivo deste capítulo foi avaliar se a assinatura isotópica dos peixes que consomem esse perifíton (cascudos do gênero *Hypostomus*) também irá variar.

3) A poluição altera o nicho trófico das comunidades de peixes?

Uma vez que se é possível obter informações sobre alimentação e nível trófico ocupado pelos peixes em uma comunidade, torna-se possível também obter informações sobre a estrutura trófica das comunidades aquáticas. Uma das formas de avaliar a estrutura trófica, é a avaliação do nicho isotópico da comunidade, que seria basicamente a área ocupada pelas comunidades em um espaço bi-plot, obtido através dos valores de carbono ($\delta\ ^{13}C$ - eixo x) e nitrogênio ($\delta\ ^{15}N$ - eixo y) de cada peixe.

Portanto, no terceiro capítulo da tese (submetido na revista **Ecosystems**), nós avaliamos se a poluição altera a estrutura trófica das comunidades de peixes, comparando-se os nichos isotópicos de comunidades de locais poluídos com os de locais não poluídos na bacia do Rio das Velhas.

4) A diversidade trófica e as fontes de carbono que sustentam as comunidades de peixes mudam ao longo de um gradiente de poluição?

O quarto capítulo da tese objetivou avaliar se a poluição tem efeito nas fontes de carbono utilizadas pelos peixes (de diferentes guildas) ao longo do gradiente de poluição no Rio das Velhas. Foram considerados 8 pontos amostrais ao longo da calha, sendo que apenas dois (Pontos 1 e 2, antes da Região Metropolitana de Belo Horizonte - RMBH) são menos poluídos, e os demais (Pontos 3 a 8 - após a RMBH) apresentam diferentes níveis de poluição. Além de avaliar quais recursos são preferencialmente consumidos ao longo do gradiente de poluição, também avaliamos se métricas indicadoras de diversidade trófica também são afetadas pela poluição.

REFERÊNCIAS

ALVES, C. B. M.; POMPEU, P. S. A fauna de peixes da bacia do rio das Velhas no final do século XX. In: ALVES, C. B. M.; POMPEU, P. S. (Org.). **Peixes do rio das Velhas:** passado e presente. Belo Horizonte: ARGVMENTVM, 2010, p. 167–189.

ALVES, C. B. M.; POMPEU, P. S. A ictiofauna da bacia do rio das Velhas como indicador da qualidade ambiental. In: LISBOA, A.H.; GOULART, E.M.E.; DINIZ, L.F.M. (Org.). **Projeto Manuelzão:** a história da mobilização que começou em torno de um rio. Belo Horizonte: Instituto Guaicuy, 2008, p. 95-106.

ALVES, C. B. M.; POMPEU, P. S. Historical Changes in the Rio das Velhas Fish Fauna—Brazil. **American Fisheries Society Symposium**, v. 45, n. May 2014, p. 587–602, 2005.

GARTNER, A.; LAVERY, P.; SMIT, AJ. Use of d15N signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. **Marine Ecology Progress Series**, v. 235, n. June, p. 63–73, 2002.

JUNQUEIRA, N. T. et al. Morphological diversity of fish along the rio das Velhas, Minas Gerais, Brazil. **Neotropical Ichthyology**, v. 10, n. 2, p. 417–424, 2012.

LEAL, C. G.; JUNQUEIRA, N. T.; POMPEU, P. S. Morphology and habitat use by fishes of the Rio das Velhas basin in southeastern Brazil. **Environmental Biology of Fishes**, v. 90, n. 2, p. 143–157, 2011.

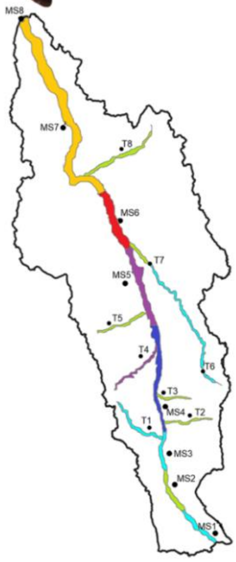
MACHADO, A. T. M.; ALVES, C. B. M.; CALLISTO, M. Projeto Manuelzão: metodologia e resultados. In: LISBOA, A.H.; GOULART, E.M.E.; DINIZ, L.F.M. (Org.). **Projeto Manuelzão:** a história da mobilização que começou em torno de um rio. Belo Horizonte: Instituto Guaicuy, 2008, p. 39–54.

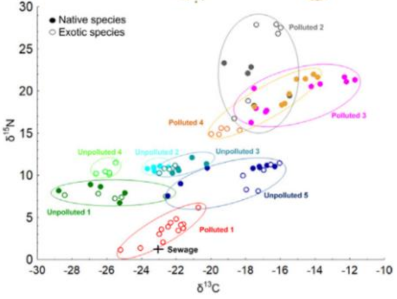
POMPEU, P. S.; ALVES, C. B. M.; CALLISTO, M. The Effects of Urbanization on Biodiversity and Water Quality in the Rio das Velhas Basin, Brazil. **American Fisheries Society Symposium**, v. 47, p. 11–22, 2005.

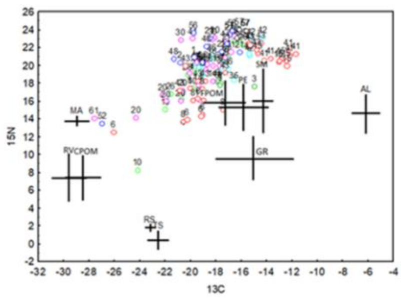
VANDER ZANDEN, M. J.; CABANA, G.; RASMUSSEN, J. B. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ($\delta^{15}N$) and literature dietary data. **Canadian Journal of Fisheries and Aquatic Sciences**, v. 54, n. 5, p. 1142–1158, 1997.

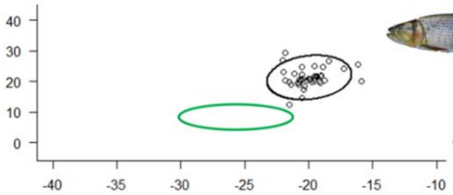
WANG, Y. et al. Selection of effective macroalgal species and tracing nitrogen sources on the different part of Yantai coast, China indicated by macroalgal δ^{15}N values. **Science of The Total Environment**, v. 542, p. 306–314, 2016.

SEGUNDA PARTE – ARTIGOS









ARTIGO I - ***Trophic responses to aquatic pollution of native and exotic livebearer fishes***

Débora Reis de Carvalho, Alexander S. Flecker, Carlos Bernardo Mascarenhas Alves, Jed P. Sparks & Paulo Santos Pompeu

Manuscrito publicado na revista *Science of the total environment*

(Manuscrito redigido na língua e formato indicado para revista)

Science of the Total Environment 681 (2019) 503–515

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Trophic responses to aquatic pollution of native and exotic livebearer fishes

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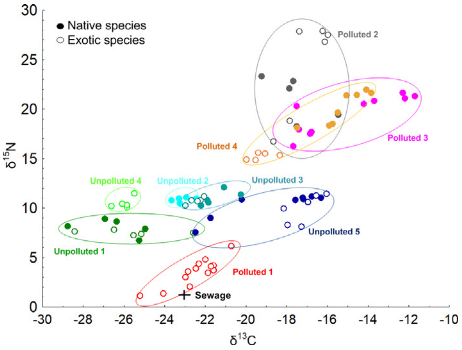
HIGHLIGHTS

- We tested the influence of pollution in the diet of exotic and native fish species.
- We used gut contents and stable isotopes analysis to access the information.
- Both species showed trophic plasticity and changed their trophic niches similarly.
- Exotic species assimilated carbon directly from sewage.
- Sewage consume can facilitate the exotic species establishment at polluted sites.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 March 2019
Received in revised form 25 April 2019
Accepted 7 May 2019
Available online 09 May 2019

Editor: Daniel Wunderlin

Keywords:
Stable isotopes
Gut contents
Carbon
Nitrogen enrichment
Trophic niche
Isotopic niche

ABSTRACT

The objective of this study was to evaluate if aquatic pollution promote diet shifts in two livebearer fishes (Poeciliidae): an exotic species, the guppy (*Poecilia reticulata*), and a native livebearer (*Phalloceros uai*). The study was carried out in a Brazilian basin highly impacted by anthropogenic activities, especially discharge of domestic and industrial sewage from a region with more than five million human inhabitants. To evaluate the trophic ecology of both native and exotic species it was analysed carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes of fish tissue, food resources and, sewage. Moreover, stable isotopes analyses were coupled with gut contents of the two species to provide additional information about fish diet. Exotic guppy abundance was high in the most polluted site, where *P. reticulata* assimilated carbon directly from sewage. The native species was absent in the most polluted site, but presented wider niches than the exotic species in almost all other sites. Gut content analyses indicated high consumption of aquatic insects by both species. However, while the native species consumed a diverse suite of insect taxa, the exotic species consumed mainly Chironomidae larvae. We conclude that aquatic pollution promotes diet shifts in both native and exotic species, with both species changing their trophic niches in a similar way according to the level of degradation of the environment. The ability to directly assimilate sewage, together with its capacity to survive in environments with poor water quality and its reproductive strategy, may favour the establishment of exotic guppies in strongly polluted sites.

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https://doi.org/10.1016/j.scitotenv.2019.05.092
0048-9697/

1. Introduction

The introduction of non-native species, habitat change, climate change, overexploitation and pollution are the five major drivers of biodiversity loss (Mazor et al., 2018). Freshwater ecosystems are often vulnerable to biological invasions (Tricarico et al., 2016), that can result in threats to native fish assemblages and cause biotic homogenization (Rahel, 2002). Environmental quality can influence the establishment of non-native species, since disturbance can act as a precursor to invasions (Allan and Flecker, 1993; Hobbs and Huenneke, 1992). A large number of non-native species have been linked to aquatic environments altered by human activity, especially by urbanization and agriculture (Ruaro et al., 2018). The discharge of pollutants in aquatic environments promotes microbial contamination and changes the concentration of phosphorus, nitrogen, dissolved oxygen and toxic substances such as heavy metals (Holeton et al., 2011; Mouri et al., 2011). Such changes in water quality profoundly alter aquatic communities by favouring resistant species that can inhabit highly polluted environments, often due to increased trophic plasticity, and morphological adaptations.

The ability to change feeding habits is a common trait in invasive fish species (Pettitt-Wade et al., 2015; Tran et al., 2015), and is one of the main traits for successful colonization, establishment, and spread (Peterson and Vieglais, 2001; Pettitt-Wade et al., 2015). Poeciliidae, has been identified as one of the most invasive freshwater fish families worldwide (Magalhães and Jacobi, 2017) and has been linked to multiple negative impacts on native species, including zooplankton, shrimp, fishes, and amphibians (Dudley et al., 2017; Stockwell and Henkanaththegedara, 2011). Poeciliids exploit a broad range of food types, with the majority of the species displaying omnivorous habits, eating a mixture of terrestrial and aquatic invertebrates, detritus, algae, or vascular plant matter (Meffe and Snelson, 1989). In addition, because they are ovoviviparous (internal fertilization and birth to live young), livebearers have an invasive advantage over many other non-native fish families. Furthermore, single pregnant guppies can routinely establish viable populations (Deacon et al., 2011), since female guppies can store sperm, and need only a single mating even for successful reproduction over many months (Magalhães and Jacobi, 2017).

The guppy, *Poecilia reticulata* (Peters, 1859), is one of the best-known members of the family Poeciliidae. This species is endemic to northeastern South America and the Lesser Antilles, and occurs in the coastal rivers of Venezuela, Guyana, Surinam, and Trinidad and Tobago (Rivas et al., 1965). However, the species is now widely distributed in tropical freshwaters as a result of human intervention and is considered to be an indicator of degraded aquatic environments (de Carvalho et al., 2017b). *Poecilia reticulata* had invaded 22 countries across six continents before the 1990s (Courtenay and Welcomme, 1989), and was introduced to Brazil and other river basins for mosquito control due to its high consumption of the aquatic insect larvae (Allan and Flecker, 1993; Warbanski et al., 2017). Currently, *P. reticulata* has been reported in 71 countries (FishBase, 2019) and has established populations in at least 55 (CABI, 2019). Guppies have been relatively well studied, including their life history evolution (e.g. Reznick et al., 2012), genetics (e.g. White and Wilson, 2018), reproductive physiology (e.g. Barrett et al., 2014) and social behaviour (e.g. Chapman et al., 2008). By comparison, many Brazilian native livebearers are poorly studied, including *Phalloceros uai* (Lucinda, 2008). Endemic from one of the largest Brazilian river basins (São Francisco), little or no study has been performed on the biology and ecology of *P. uai*, and as far as we know, the species' description is the only publication that refers to the species (Lucinda, 2008).

The evaluation of animal diets is fundamental for estimating ecological risks of introduced species (Baiser et al., 2010; Pompeu and Godinho, 2001). The establishment of non-native species can: 1) increase interspecific competition, which can result in negative consequences for competing native species, or 2) promote trophic niche divergence, which can facilitate integration of invaders into the community and their coexistence with native species (Tran et al., 2015). Therefore, by comparing the diet of exotic and native species of the same family, it is possible to gain insights for understanding whether an exotic species possesses trophic traits that could facilitate its spread and abundance. Previous studies used mostly gut contents analyses to access the diet habits of livebearer species (e.g. Oliveira and Bennemann, 2005; Silva et al., 2012). Although gut analyses can provide useful taxonomic data on consumed prey items, there are often uncertainties in the identification of some items. Moreover, it is unclear from gut contents whether specific ingested items are assimilated into biomass. For robust and reliable assessment of feeding habits, the complementary analysis of carbon and nitrogen stable isotopes is desirable, since it allows one to infer animal diet by calculating the proportional contribution of sources (dietary items) within a mixture (consumer tissue) (Parnell et al., 2010). Stable isotopes also provide information about the relative assimilation of some resources that are poorly quantified in the analysis of gut contents, as is the case for detritus (Keough et al., 1998), a recurrent food item for guppies.

Therefore, the objective of this study was to evaluate if aquatic pollution promotes shifts in the trophic ecology of livebearer species, and if so whether trophic niche shifts would vary between an exotic (*P. reticulata*) and a native (*P. uai*) species. It was tested the hypothesis that guppies, known for their adaptation to impacted environments (Rolshausen et al., 2015), present greater trophic plasticity than the native species, which favours their establishment in anthropogenically dominated environments. It was expected the exotic species to exhibit broader trophic niches in polluted sites due to differences in resource availability between polluted and unpolluted sites. The native species, in turn, would be expected to exhibit less trophic plasticity and narrow trophic niches. It was also hypothesized less niche overlap between exotic and native species in unpolluted sites, where resources limitation would be predicted to promote niche divergence. It was expected a greater abundance of resources in polluted sites, especially because autotrophic resources are favoured by nutrient enrichment, as nitrogen and phosphorus available from anthropogenic activities.

2. Methodology

2.1. Study area

The study was conducted in the Rio das Velhas Basin, the longest tributary in the São Francisco Basin (Machado et al., 2008). The watershed covers 51 municipalities, including the Metropolitan Region of Belo Horizonte (MRBH), composed by 34 neighboring cities. The transfer of the capital of Minas Gerais to Belo Horizonte in 1898, resulted in the fast expansion of a center of urbanization and industrialization along the Rio das Velhas. Thus, the region that was once known for its abundance of fish (Alves and Pompeu, 2010), is now in an advanced process of degradation. The main polluting activities are located in the upper and middle course, which receives the majority of domestic and industrial sewage of the MRBH (Pompeu et al., 2005).

A total of nine sites were sampled in the main channel (five sites) and tributaries (4 sites) of Rio das Velhas Basin (Fig. 1). The samplings were conducted in dry (July) and wet (October and January) seasons between 2015 and 2016. Selection of the nine sampling sites was done based on environmental conditions (level of degradation) and the presence of pollutants (toxic contamination and anthropogenic activities). The level of degradation of each sample site was obtained from a previous study (Feio et al., 2015). The toxic contamination and anthropogenic activities in the catchments were obtained from the quarterly report for the year 2017 (IGAM-Instituto Mineiro de Gestão das águas, 2017a). Degradation levels (ranging from I to IV) and the presence of toxic contaminants were used to separate the sites between unpolluted (U) and polluted (P) (Table 1).

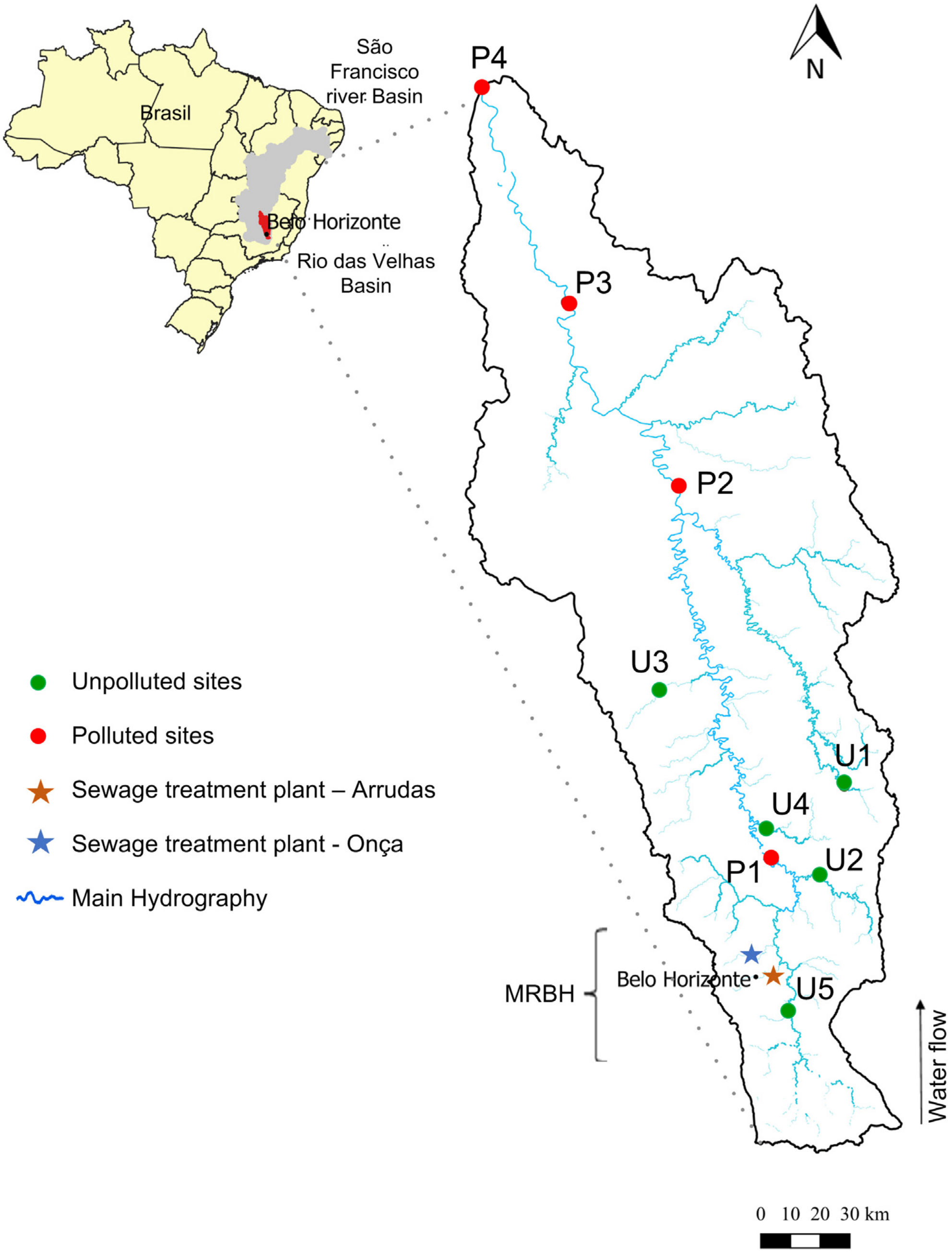

Fig. 1. Sampling area in the Rio das Velhas basin covering 5 unpolluted sites (U1 to U5) and a gradient of pollution that includes 4 sites (P1 to P4) along the main channel of Rio das Velhas. MRBH = Metropolitan Region of Belo Horizonte.

Five sites with degradation level I and without toxic contaminants were classified as "Unpolluted". The Rio Cipó (Unpolluted site 1 – U1) is the most pristine tributary and is considered a reference area in the basin. The Rio Cipó site is largely free from pollution, primarily due to the location of its headwaters in the Serra do Cipó National Park. The remaining tributaries - Rio Jaboticatubas (Unpolluted site 2 - U2), Rio da Onça (Unpolluted site 3 – U3) and Rio Taquaraçu (Unpolluted site 4 – U4) - present intermediate water-quality, with little influence of industry, and with the main impacts caused by small nearby cities (Table 1, Fig. 1). The Unpolluted site 5 (U5) is located in the main channel and also considered unpolluted since it is located upstream of the MRBH.

Four sites with degradation level II and III and with toxic contaminants (ammonium and arsenic) were classified as "Polluted". All polluted sites are located along the main channel and represent a gradient of pollution, since a great amount of sewage from the MRBH reaches the main channel near the Polluted site 1 (P1). Therefore, P1 is considered the most degraded due the direct influence of sewage. Polluted site 2 (P2), Polluted site 3 (P3) and Polluted site 4 (P4) presents a partial improvement in water quality, as the distance from MRBH increases and the main channel receives water from unpolluted tributaries (Table 1, Fig. 1).

Two sewage treatment plants from the MRBH - STPs Arrudas and Onça - were also sampled to obtain complementary measurements of suspended material to obtain the sewage carbon and nitrogen isotopic composition.

Water quality data was accessed through "Instituto Mineiro de Gestão das Águas" website (IGAM-Instituto Mineiro de Gestão das águas, 2017b), which conducts quarterly monitoring in several points of the Rio das Velhas basin. Values of conductivity (µS/cm); dissolved oxygen (mg/l); ammonium (mg/l); nitrate (mg/l) and total phosphorus (mg/l) presented in this study correspond to the means of the values obtained in the IGAM measurements carried out quarterly in the years 2015 and 2016.

2.2. Fish sampling

Poecilia reticulata and *P. uai* were captured with seine nets and mosquito nets (80 cm in diameter, 1 mm mesh) (Table 2). Of the nine sampled sites, the two species were only found in sympatry at five sites (U1, U4, U5, P2 and P4). The exotic species was exclusive to sites P1 and U3, and the native species was exclusive to sites P3 and U2. For stable isotope analysis, it were analysed at least five samples of each fish species at each sampling site whenever possible. Due to the small size of individuals, fish were analysed as a whole after removing digestive tract. All samples were kept frozen until laboratory processing to avoid deterioration of the material. In the laboratory, fish samples were lyophilized for 24 h, ground to a fine and homogeneous powder using a mortar and pestle, and stored in *Eppendorf* tubes.

For diet analyses, it was quantified the gut contents of 10 individuals of each fish species at each sampling site, whenever possible. Individuals designated for gut analyses were fixed in 10% formalin in the field and transferred to 70% alcohol in the laboratory, where gut contents were removed. It was analysed a total of 60 guts of the native species, *P. uai,* and 65 guts of the exotic species, *P. reticulata* (Table 2). The proportion of each food item consumed by each species at each sampling site was estimated using a stereomicroscope and adopting the volumetric method (Hyslop, 1980). Food items were identified under stereomicroscope to the lowest feasible taxonomic unit according to macroinvertebrate identification guides (Costa et al., 2006; Mugnai et al., 2010).

2.3. Resources sampling

The sampling of basal sources was based on the feeding habits of the native (representative of *Phalloceros* genus) and exotic (*P. reticulata*) species documented on literature (Table A.1). Five samples were collected at the nine sites for each of the following resources: periphyton (biofilm), filamentous algae, coarse particulate organic matter (CPOM) and, fine particulate organic matter (FPOM). Samples of raw sewage (suspended material) were obtained from the “Arrudas” and “Onça” Sewage Treatment Plants (STPs) located at the MRBH.

Samples of algae and CPOM were collected haphazardly at all sites where they were present. FPOM samples were collected from sediment deposits by resuspending the sediment and passing the suspended material through a phytoplankton net (0.45 mm). Periphyton was collected by scraping stones and was stored in distilled water. In order to obtain the isotopic signature of the sewage, suspended matter was sampled through a phytoplankton net (0.45 mm mesh) suspended for a period of 3 min in the effluents of the two sewage treatment plants (STPs). After collection, all samples were stored in plastic sample jars and held on ice for one to 5 h prior to storage at a freezer. In the laboratory, liquid samples (periphyton, FPOM and suspended matter) were filtered using a filtration apparatus attached to a vacuum pump using calcined quartz fiber filters (Whatman® QMA quartz filters). All basal resource samples were oven dried at 60 °C for a minimum period of 48 h. Afterwards, they were ground to a fine homogeneous powder using a mortar and pestle and were subsequently stored in *Eppendorf* tubes.

2.4. Data analyses

A total of 46 individuals of *P. uai*, 45 individuals of *P. reticulata*, and 396 basal resources samples were sent to the Center for Nuclear Energy in Agriculture (CENA) at the University of São Paulo (USP), Brazil, for isotopic analysis. About 2–5 mg of dry animal tissue material and approximately 5–10 mg of basal resource samples were used for analysis.

To determine the isotopic ratio, a mass spectrometer system in the Continuous-flow (CF-IRMS) mode was used with a Carlo Erba elemental analyzer (CHN 1110) coupled to a Delta Plus mass spectrometer (Thermo Scientific). Results were expressed as the relative difference of international reference standards, in the delta notation (δ ‰), and calculated using the following formula:

$$\delta X = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 10^3$$

where X is ^{13}C or ^{15}N and R represents the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie and Prosser, 1996).

2.5. Statistical analyses

The statistical analyses applied to each objective are described in the Fig. 2. Differences in δ^{13}C and δ^{15} N of *P. uai* and *P. reticulata* among sites were tested using one-way analysis of variance (ANOVAs), when normality and homoscedasticity assumptions were met. The nonparametric Kruskal-Wallis test was used for data with non-normal distributions. When significant differences ($p < 0.05$) were observed, pairwise comparisons were done using the post-hoc Tukey's test (normal distribution) and Wilcoxon test (non-normal distribution) to identify means that are significantly different from each other.

To evaluate the trophic structure of *P. uai* and *P. reticulata* populations, individuals of both fish species were plotted in *bi-plot* space according to the isotopic values of carbon (x-axis) and nitrogen (y-axis) in each region. Source contributions to *P. uai* and *P. reticulata* diets were estimated for the nine sites based on stable isotope data analysed with Bayesian stable isotope mixing models (Moore and Semmens, 2008; Parnell et al., 2010), specifically using the MixSIAR package in R (Stock and Semmens, 2016a). It was conducted a Markov chain Monte Carlo sampling based on the following parameters: number of chains = 3; chain length = 100,000; burn in = 50,000; thin = 50 and model 4 (Resid*Process) error structure (Stock and Semmens, 2016b). Diagnostic tests (Gelmin–Rubin, Heidelberger–Welch and Geweke) and trace plots were examined for model convergence. The fractionation values used for consumers were 0.4 ± 1.3‰ for carbon and 2.54 ± 1.27‰ for nitrogen (Post, 2002; Vanderklift and Ponsard, 2003).

The isotopic niches of *P. uai* and *P. reticulata* in all sampling sites were quantified based on standard ellipse areas (SEA - expressed in ‰^{2}) through use of the Stable Isotope Bayesian Ellipses package in R - SIBER (Jackson et al., 2011). The standard ellipse area (SEA) represents the core isotopic niche space and is a proxy for the richness and evenness of resources consumed by the population (Bearhop et al., 2004). A small sample size correction (indicated by the subscript letter “c”) was applied to SEA to increase the accuracy of the comparisons, enabling comparisons of niches of populations with different sample sizes (Jackson et al., 2011). The SEAc allows one to calculate the degree of niche overlap (in percentage, where 100% indicates total overlap) and can be used as a quantitative measure of diet similarity among different species (Hill et al., 2015).

3. Results

Native and exotic species were found in both polluted and unpolluted sites (Table 2). In the most polluted site (P1), which exhibits high concentrations of ammonium (5.12 mg/L) and phosphorus (0.55 mg/L), and low concentrations of dissolved oxygen (4.89 mg/L), the exotic species was abundant (N = 3950) and the native species was absent (Tables 1, 2).

Both native and exotic species presented substantial variation in carbon and nitrogen isotopic composition among sites, and displayed enriched δ^{13}C and δ^{15}N values at sites downstream of the discharge of sewage (P2, P3 and P4) (Fig. 3). In the most polluted site (P1), δ^{15}N

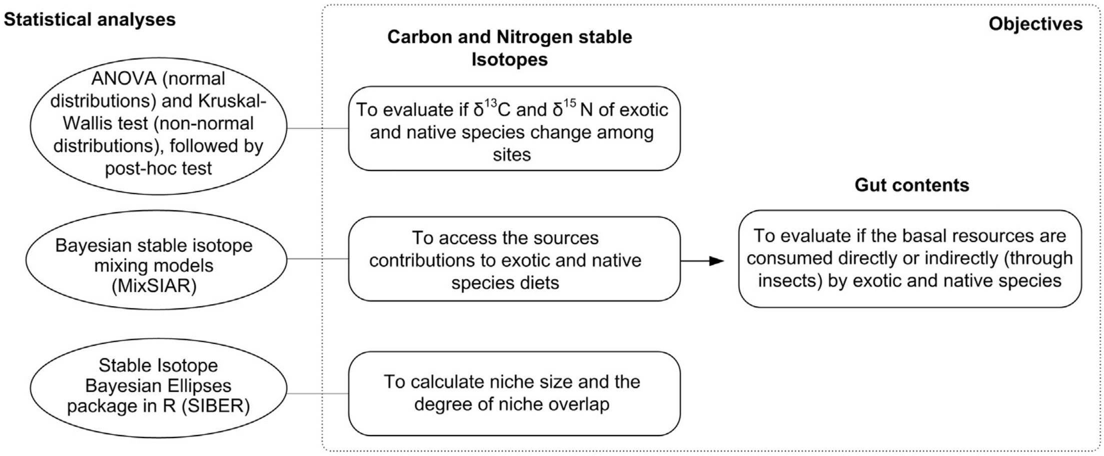

Fig. 2. Statistical analyses and methods (stable isotopes or gut contents) applied to test each objective.

and δ^{13}C values of exotic species were different from all the other sites (with the exception of δ^{13}C values in "U4") (Fig. 3b and d). *Poecilia reticulata* expressed the most depleted δ^{15}N at this site (3.53 ± 1.39‰), as well as all resources sampled in P1 (Fig. 3d, Table A.2).

High variation in δ^{15}N values (between unpolluted and polluted sites) and δ^{13}C values (differing among most sites) was reflected in the wide distribution of both species along the bi-plot space (Fig. 4a). When each sample site was analysed separately, it was possible to see changes by both species in their feeding strategies according to site location (Fig. 4). In unpolluted sites, dominant resources assimilated by both species shifted between periphyton (Fig. 4b, c and d) and algae (Fig. 4e and f). FPOM was also an important source, especially in the most pristine site (U1) (Fig. 4b).

In polluted sites (Fig. 4g, h, i and j), there was a striking difference in resources assimilated at the site close to the sewage discharge (P1, Fig. 4g) compared to sites more distant from this discharge (P2, P3 and P4, Fig. 4h, i and j). In the most polluted site (P1), only the exotic species *P. reticulata* was present, which assimilated mainly carbon direct from sewage. In sites downstream from MRBH, both species assimilated primarily carbon from FPOM and periphyton, and the importance of sewage as an assimilated carbon source was attenuated.

From the gut contents data, both aquatic and terrestrial insects were found to be important food sources for both species (Fig. 5; Table A.3). Aquatic insects were consumed at almost all sites and terrestrial insects were widely consumed by both species especially at P5. The dominant food items consumed by the native

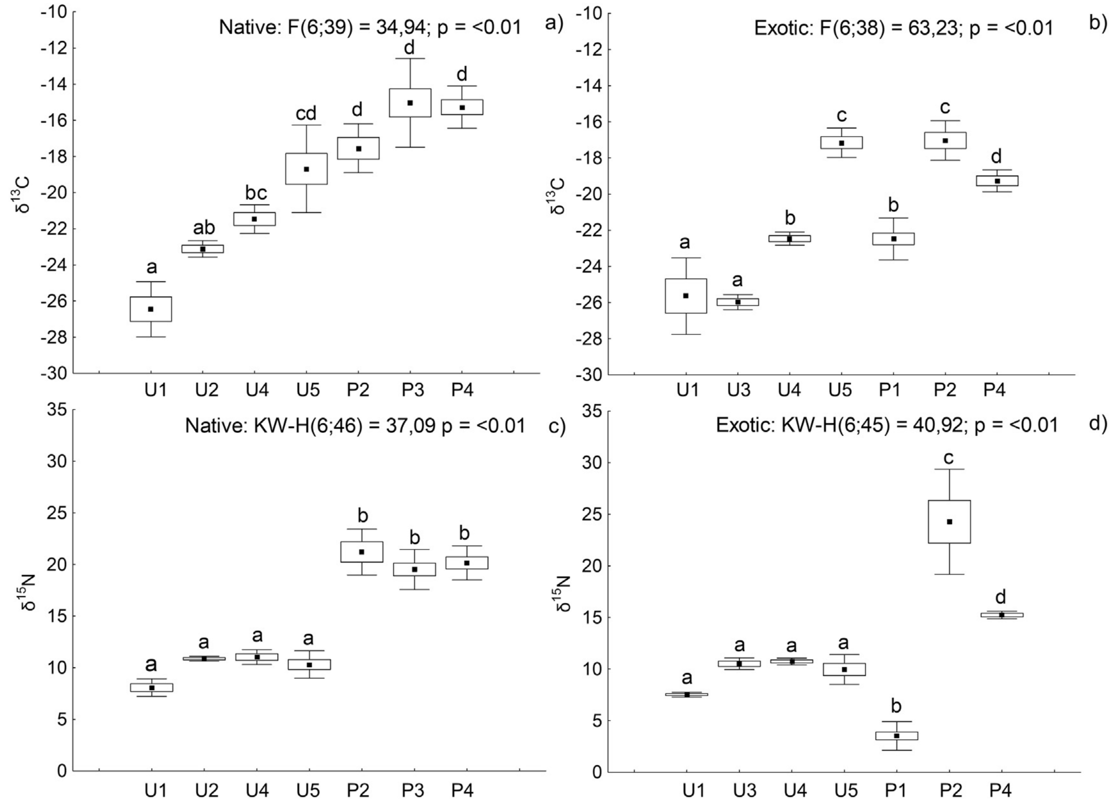

Fig. 3. Variation in δ^{13}C (a and b) and δ^{15}N (c and d) values of the native (*P. uai*) and exotic species (*P. reticulata*) sampled at different sites along the Rio das Velhas Basin. Mean (small box), standard error (bars) and standard deviation (large box). Letters (a–d) above the plots indicate significant differences according to post-hoc test. U = Unpolluted sites, P = Polluted sites.

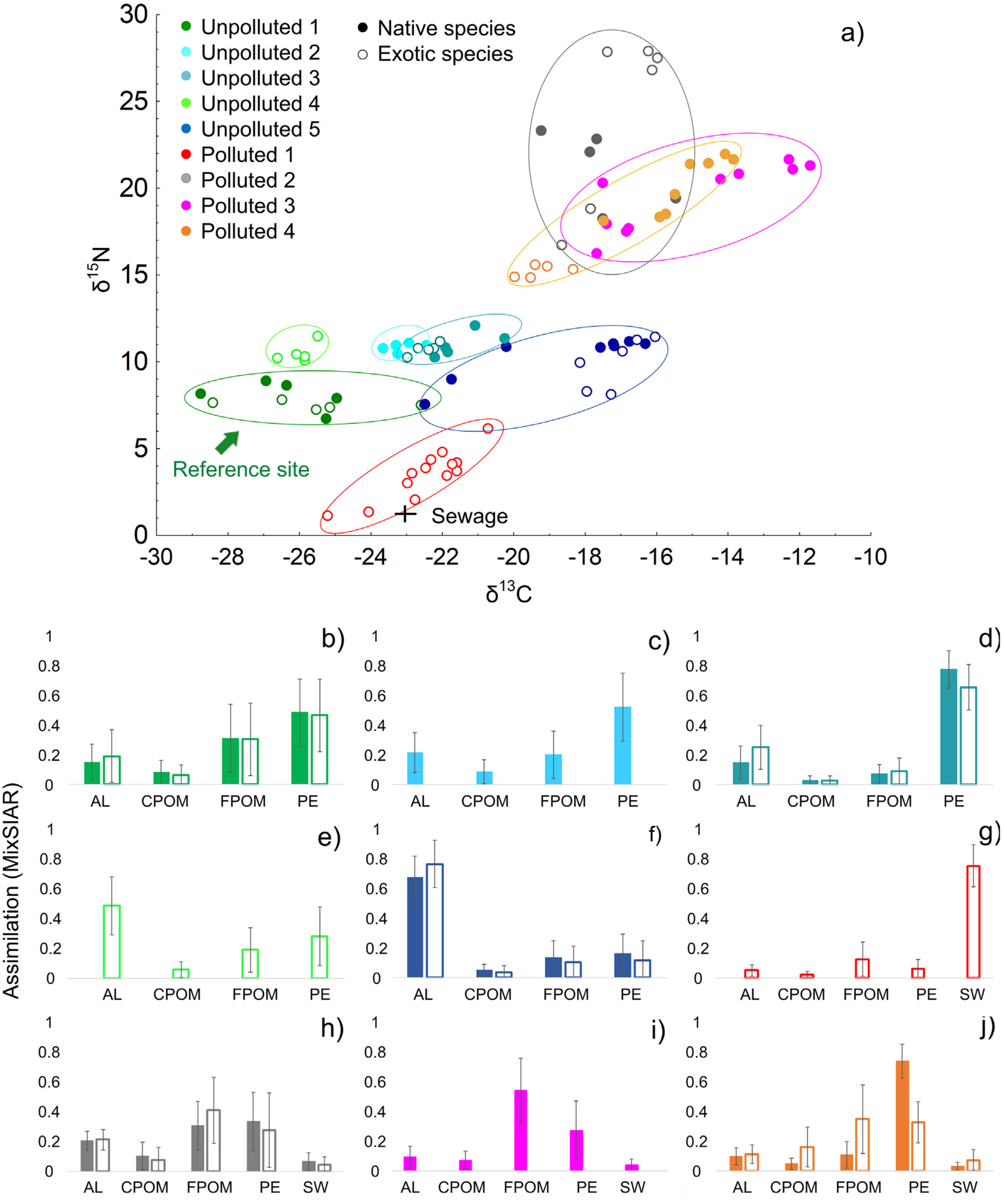

Fig. 4. Distribution of the individuals (points) of native (*P. uai*) and exotic (*P. reticulata*) species in the bi-plot space according its $\delta^{13}C$ (x-axis) and $\delta^{15}N$ (y-axis). The bar graphs represent the resource partitioning results (mean and standard deviation), with the values ranging between 0 and 1 (maximum assimilation). Resources: AL = filamentous algae; CPOM = coarse particulate organic matter; FPOM = fine particulate organic matter; PE = periphyton (biofilm); SW = sewage (with isotopic signatures represented in bi-plot space). Native Species = filled circles and bars; Exotic species = open circles and bars. The reference site in the Rio das Velhas basin (site free from pollution) is indicated with an arrow.

species were aquatic insects (46%), terrestrial insects (17%), detritus/periphyton (17%) and algae (11%). However, the percentage of each resource consumed varied among sites (Table A.3). Exotic species in turn fed mainly on detritus (43%) and aquatic insects (30%), but also varied greatly in the proportion of resources consumed at each site. At the most polluted site (P1), detritus comprised 80% of the species diet, which is consistent with the results of partition analysis, where the exotic species assimilated mainly the carbon from sewage (Fig. 4g). It was observed considerable variation in the taxa of aquatic insects consumed by each species, even when they were found in sympatry. Guppies fed mostly on Diptera larvae, especially from the Chironomidae family. The native species in addition to feeding on larvae of Diptera (Chironomidae), consumed larvae of Ephemeroptera, Plecoptera and

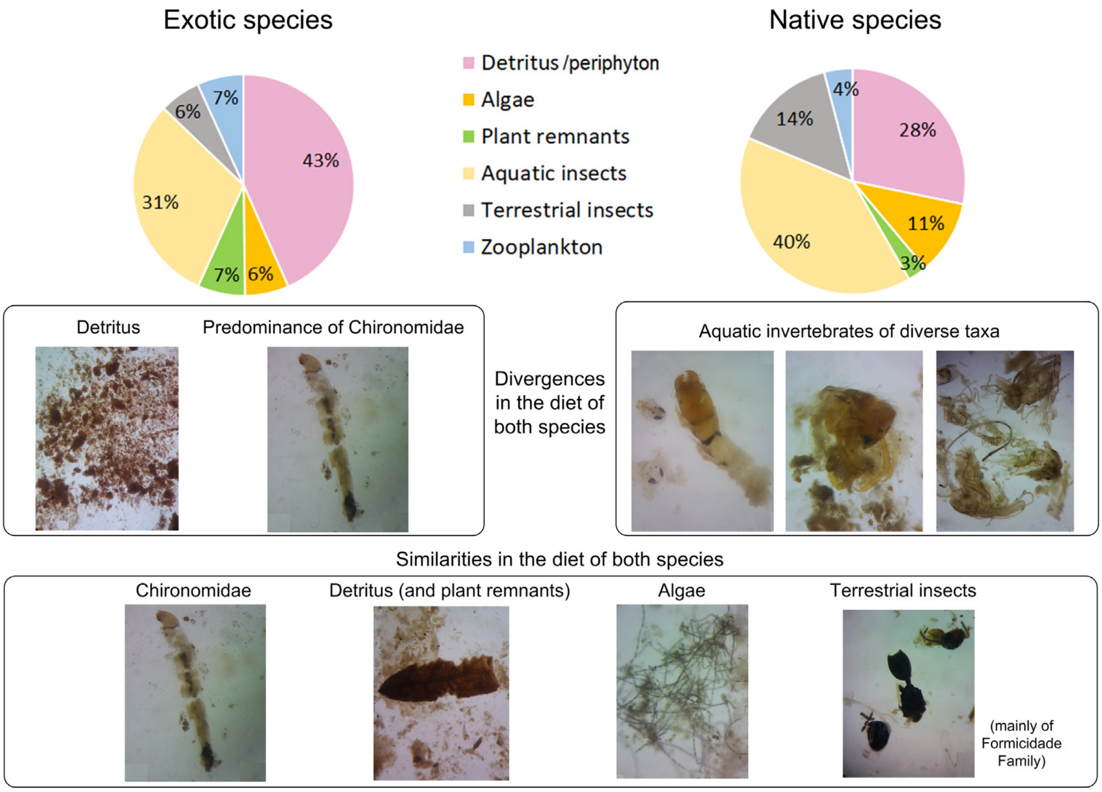

Fig. 5. Schematic drawing with the proportion of each food item found in the guts of the exotic (*P. reticulata*) and native species (*P. uai*), with photos showing the divergences and similarities between the diet of both species.

Trichoptera. Among terrestrial insects, ants (Formicidae) were the most frequently consumed taxon by both species (Fig. 5).

The isotopic niche (SEAc) of the native species was broader than the exotic species at all sites, except for the P2, where the exotic species exhibited a vertically-shaped niche due to large variation in nitrogen isotopic signatures (Fig. 6, Table 3). In general, *P. uai* and *P. reticulata* altered their isotopic niches in a similar manner in response to the presence of sewage in the aquatic environment (Fig. 7). The narrow niches

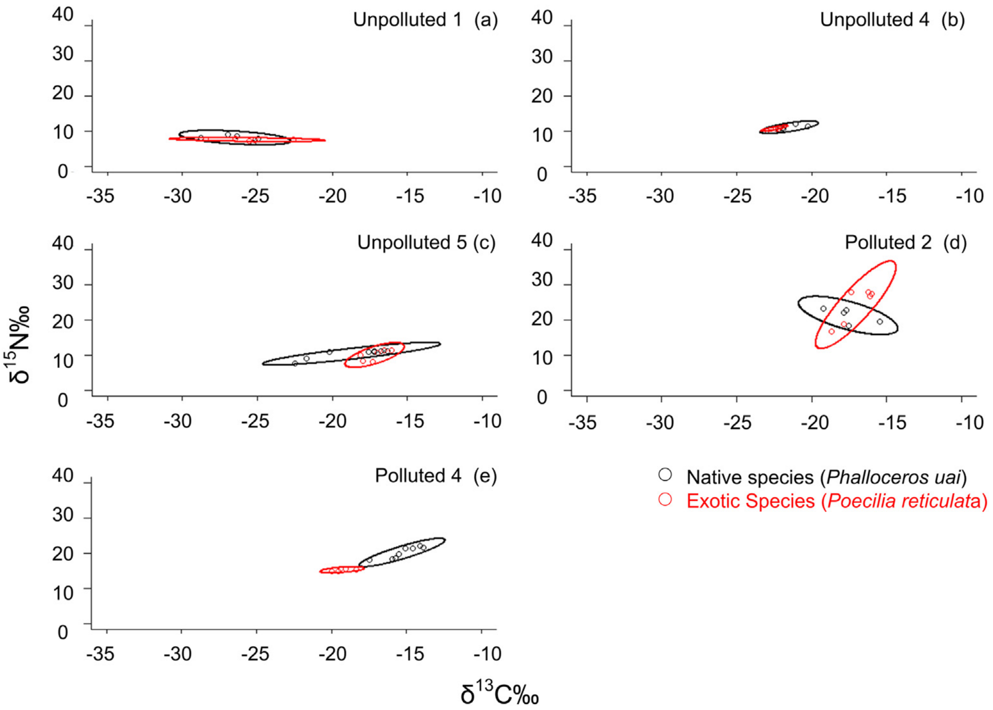

Fig. 6. Isotopic niche of native species (*P. uai*) and exotic species (*P. reticulata*) (evaluated by the ellipse area with 95% confidence interval) in the sites where both species are present at the Rio das Velhas basin.

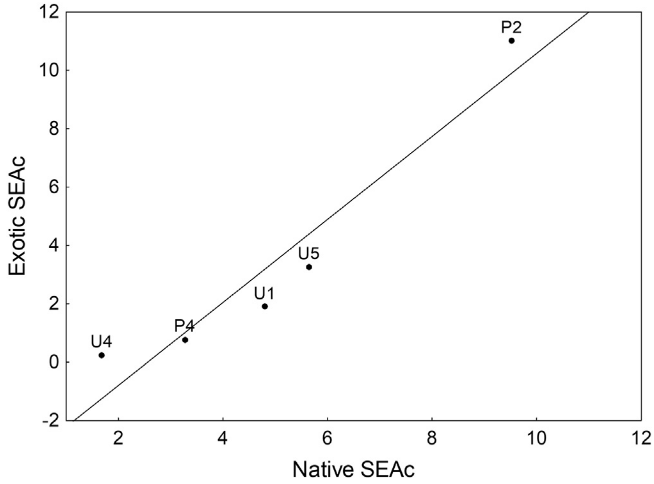

Fig. 7. Relationship of isotopic niche (SEAc) of native species (*P. uai*) and exotic species (*P. reticulata*) in the sites where both species are present at the Rio das Velhas basin. U = Unpolluted sites, P = Polluted sites.

were observed in the tributaries U2 (native species) and U4 (exotic species) (Table 3). In the most pristine site (U1), both species had a broader trophic niche on the horizontal axis (niche with the largest carbon range) and presented the greatest niche overlap (Fig. 6a, Table 3). The smaller niche overlap, in turn, was observed in the polluted site farthest from the MRBH (P4), where the native species also showed a niche with a large carbon range (Fig. 6e, Table 3).

4. Discussion

Previous studies have highlighted the ability of *P. reticulata* to colonize degraded habitats, being frequently associated with polluted environments that have reduced oxygen concentrations and high turbidity (e.g. Casatti et al., 2009; de Carvalho et al., 2017b). The results presented here, reinforce the idea that guppies are able to tolerate environments with poor water quality and also point to their ability to establish in great abundance at impacted sites. In addition, far as is known, this is the first study where the trophic ecology of *P. reticulata* has been compared to an endemic poeciliid species coexisting in a highly polluted basin. The hypothesis that the exotic species exhibits higher trophic plasticity than the native species was not supported, since *P. uai* also displayed considerable variation in diet and larger isotopic niches. However, this is the first study that points to sewage as a direct carbon source for *P. reticulata*, which may be an important mechanism explaining its abundance in polluted environments. There was also no support for the hypothesis that less niche overlap would be observed between species in unpolluted sites. To the contrary, it was found the highest overlap in the most pristine site (U1) and the largest niche divergence occurred at the polluted site closest to the mouth (P4).

4.1. Gut contents

Gut analyses showed considerable variation in the resources consumed by both species, either at unpolluted sites or at unpolluted sites (Fig. 5). Aquatic insects were the most frequent items in gut contents, especially for native species. However, there was a striking difference in insect orders consumed by each species. The exotic species consumed more Chironomidae larvae, considered bioindicators of environments with poor water quality (Al-Shami et al., 2010). In contrast, in addition to Chironomidae larvae, the native species also consumed EPT insects (Ephemeroptera, Plecoptera and Trichoptera), which are considered to be bioindicators of environments with good water quality (Ab Hamid and Md Rawi, 2017). Terrestrial insects were also important in the diet of both species, mostly along the lower course of the Rio das Velhas. The higher consumption of terrestrial insects at P4 may have occurred due to a possible greater availability of this resource at that site, since P4 is located in areas under influence of flood pulses, with an expanded period of close interaction between the aquatic-terrestrial transition zone (Junk et al., 1989).

4.2. Source contributions to *P. uai* and *P. reticulata* diets

Stable isotopes analysis indicated periphyton and algae are the basal resources most assimilated by both species (Fig. 4). This consumption of carbon from these resources may have occurred both directly and indirectly through aquatic and terrestrial insects, since at some sites the enrichment from basal resources to fishes was lower than 2‰ (e.g. U5) and at other sites was higher than 8‰ (e.g. P2) (see Table A.2). Sewage, on the other hand, appears to have been ingested directly by *P. reticulata*, suggested by the fact that 80% of the material found in the gut of *P. reticulata* at P1 was detritus. In addition, the mean enrichment of the nitrogen isotopic signature of *P. reticulata* at P1 (2.39‰) was consistent with the increase of only one trophic level (Post, 2002; Vanderklift and Ponsard, 2003). There are no records of other fish species feeding directly on sewage, probably due to the methods employed, as gut analyses do not identify the exact source of carbon in detritus (Keough et al., 1998). At the same time, insects were common items in the guts of both species, indicating the possibility that carbon from basal resources may have been assimilated indirectly through insect consumption. Therefore, the complementarity of the two analyses (gut contents and stable isotopes) provided a more robust result, highlighting the trophic plasticity of the native and exotic species.

Although the basal resources consumed mostly by both fish species were similar, assimilation varied between polluted and unpolluted sites. Large variation in fish feeding habits over time or space was at least partly the result of changing prey/resources availability (Lawton and Pratchett, 2012; Pérez-Matus et al., 2012). Resource availability, in turn, is related to habitat characteristics and environmental conditions. Sewage treatment plants in the Rio das Velhas Basin treat raw sewage at the secondary level, thus they do not remove all reactive nitrogen and phosphorus. This input of nutrients may have direct effects on primary producers, such as algae, periphyton and macrophytes (Hilton et al., 2006; Kinney and Roman, 1998). Consequently, changes in the availability of resources may provide a “window of opportunity” for the establishment of non-native species (Shea and Chesson, 2002).

Opportunistic consumption of the most abundant food items, especially detritus, seems to be a feeding strategy adopted by *P. reticulata* (Oliveira and Bennemann, 2005; Silva et al., 2012). The results presented in this study reinforce this trait, since sewage/detritus was highly consumed in the most polluted site, as well as larvae of Chironomidae in all sites, a food item found at high densities in the Rio das Velhas basin (Pompeu et al., 2005). Therefore, the ability to inhabit environments with poor water quality, together with feeding plasticity, reproductive strategies and capacity to feed directly from the sewage, can be key factors contributing to the great abundance of *P. reticulata* in polluted environments. Such information is especially important in developing countries where sewage treatment is precarious and much of the untreated sewage is still being dumped into rivers.

In contrast, the native fish species did not appear to utilize sewage as a major food resource, despite greater diet variation among sites compared to the exotic species. Although empirical and theoretical studies of resource use often treat conspecific individuals as ecologically equivalent, it is necessary to keep in mind that individuals of the same species may use different resources (Bolnick et al., 2003). In this respect, *P. uai* seems to present greater intraspecific variation in the use of resources than *P. reticulata*, since individuals showed a larger variation in isotopic signatures within sites, which resulted in wide isotopic niches (Fig. 6).

Table 1
Geographic location and water quality of the sites sampled in the main channel and tributaries of Rio das Velhas. Cond. = Conductivity (μS/cm); D.O. = Dissolved oxygen (mg/l); NO_3 = Nitrate (mg/L); NH_4 = Ammonium (mg/l); P = Total phosphorus (mg/l); Tox. Contam. = Toxic contamination; Deg. Level = degradation level ranging from I to IV (Feio et al., 2015).

Characteristics of sampling sites				Water quality							
Region	Sampling sites	Coordinates	Elevation (m)	Cond.	D.O.	NO_3	NH_4	P	Tox. Contam.	Deg. Level	Main anthropogenic activities
Tributary (Rio Cipó)	Unpolluted 1 (U1)	19°20′01.1″S 43°39′17.0″W 23 K 641315 E 7861707 N	757	18.13	6.7	0.17	0.13	0.03		I	
Tributary (Rio Jaboticatubas)	Unpolluted 2 (U2)	19°27′51.4″S 43°54′18.0″W 23 K 614931 E 7847433 N	651	102.2	7.55	0.5	0.14	0.06		I	
Tributary (Rio da Onça)	Unpolluted 3 (U3)	19°02′48.1″S 44°15′15.9″W 23 K 578454 E 7893841 N	632	312.75	7.85	0.47	0.22	0.05		I	
Tributary (Rio Taquaraçu)	Unpolluted 4 (U4)	19°36′03.1″S 43°44′09.9″W 23 K 632551 E 7832196 N	677	67.55	7.49	0.39	0.2	0.08		I[a]	
Main channel	Unpolluted 5 (U5)	20°01′10.7″S 43°49′45.4″W 23 K 622454 E 7785916 N	729	139.51	7.3	0.99	0.29	0.08		I	
Main channel	Polluted 1 (P1)	19°32′56.7″S 43°53′33.3″W 23 K 616174 E 7838041 N	658	372.5	4.89	1.46	5.12	0.55	Ammonium	III	Discharge of domestic sewage
Main channel	Polluted 2 (P2)	18°25′33.2″S 44°11′10.9″W 23 K 585926 E 7962502 N	552	210.25	7.25	3.51	0.11	0.19	Arsenic	II	Agriculture
Main channel	Polluted 3 (P3)	17°51′55.4″S 44°32′57.4″W 23 K 547752 E 8024649 N	495	173.6	7.78	1.65	0.11	0.15	Arsenic	II	Discharge of domestic sewage and agriculture (sugar cane)
Main channel	Polluted 4 (P4)	17°12′25.9″S 44°48′49.8″W 23 K 519793 E 8097515 N	464	156.31	8.55	1.34	0.1	0.08	Arsenic	II	Discharge of domestic sewage

[a] Rio Taquaraçu was not evaluated by Feio et al., 2015, but was classified as unpolluted due its good water quality and the absence of toxic contaminants.

4.3. Changes on $\delta^{15}N$ and $\delta^{13}C$

The depleted $\delta^{15}N$ of sewage (1.50 ± 0.45‰), although not expected, facilitated the discrimination of resources assimilated by the exotic species at the most polluted site (P1). Most often, sewage is expected to exhibit enriched $\delta^{15}N$ values, sometimes reaching >30‰ (Kendall, 1998) because of isotopic fractionation during nitrification and volatilization in the case of ammonium, or denitrification in the case of nitrate (Nikolenko et al., 2018). However, depleted $\delta^{15}N$ values in algae (Wang et al., 2016), macroinvertebrates and fish (Loomer et al., 2015) also have been observed in aquatic environments under the influence of domestic sewage. At the site P1, sewage seems to be one of the main pollutants, especially due to the high ammonium concentrations in water (Table 1) and the assimilation of sewage by *P. reticulata*. In the case of the Rio das Velhas Basin, depleted $\delta^{15}N$ values probably occur due to the influence of >3000 industries distributed in MRBH (Atlas das Águas, 2009), since inorganic fertilizes are produced by atmospheric N_2 fixation, which presents depleted $\delta^{15}N$ values ranging between 0 and 3‰ (Kendall, 1998; Ulseth and Hershey, 2005). The sites downstream from the MRBH (P2, P3 and P4), in turn, are impacted mostly by domestic sewage from small cities and by agricultural inputs (Table 1). The Ribeirão Jequitibá drains in the city of Sete Lagoas, with >200,000 people and where only 15% of the collected sewage is treated (IGAM-Instituto Mineiro de Gestão das águas, 2013). This tributary reaches the Rio das Velhas main channel before the P2, potentially explaining the high values of $\delta^{15}N$. Agricultural activities, especially pastures, developed in this region may also contribute to nitrogen enrichment in resources and fish tissues (Carvalho et al., 2015; de Carvalho et al., 2017a).

Pastures could also explain the enriched $\delta^{13}C$ of both species at P2, P3 and P4 sites. C_4 grasses from pastures usually display enriched $\delta^{13}C$ values relative to C_3 plants from riparian vegetation, which occurs in accordance with the decarboxylation processes employed by each plant (O'Leary, 1981). In this study, the high contribution of C_4 plants to aquatic environments can be incorporated indirectly through FPOM or terrestrial insects, which were the most assimilated/consumed items in the sites downstream of the MRBH according to stable isotopes and gut contents analysis. Algae, although scarcely assimilated by both species in these sites, also exhibited enriched $\delta^{13}C$ at P2, P3 and P4 (−3.84, −6.19 and −6.85‰, respectively) (Table A.2). Algae generally are quite variable in $\delta^{13}C$ (e.g. McCutchan and Lewis, 2001). However, the reason of enriched $\delta^{13}C$ at P2, P3 and P4 is unknown, and may be related to

Table 2
Total number of individuals of native (*P. uai*) and exotic (*P. reticulata*) species sampled in the Rio das Velhas basin with corresponding numbers of individuals used for stable isotope and gut contents analyses.

Influence of sewage from MRBH	Sites	Characteristics	Total number of individuals sampled		Stable isotopes		Gut contents	
			Native	Exotic	Native	Exotic	Native	Exotic
No	U1	Tributary with good conditions (pristine)	16	10	5	5	10	5
	U2	Tributary with good conditions	27	–	5	–	10	–
	U3	Tributary with good conditions	–	30	–	5	–	10
	U4	Tributary with good conditions	5	27	5	5	[a]	10
	U5	Upstream of sewage discharge of the MRBH	29	26	8	6	10	10
Yes	P1	Immediately downstream of sewage discharge of MRBH	–	3950	–	13	–	10
	P2	Downstream of the sewage discharge of the MRBH	23	19	5	6	10	10
	P3	Downstream of the sewage discharge of the MRBH	30	–	10	–	10	–
	P4	Downstream of the sewage discharge of the MRBH	54	47	8	5	10	10

[a] Native species from U4 had only the stable isotopes analysed due the limited number of individuals sampled.

Table 3
Isotopic niche (standard ellipses - SEAc) and percentage of niche overlap of native species (*P. uai*) and exotic species (*P. reticulata*).

Sites	Niche overlap	Isotopic niche (SEAc)	
		Native	Exotic
U1	0.27	4.8	1.9
U2	–	0.4	–
U3	–	–	0.77
U4	0.15	1.68	0.25
U5	0.25	5.64	3.27
P1	–	–	2.58
P2	0.22	9.52	11.01
P3	–	9.22	–
P4	0.01	3.27	0.78

changes on concentrations of dissolved inorganic carbon (DIC), as well as physiological factors affecting DIC assimilation by the algae.

4.4. Trophic niche

Although both species presented similar variation in the trophic niche according to deteriorated environmental quality, their feeding overlap did not exceed 27% (at the most pristine site – U1). Invasions sometimes result in divergence in trophic niches, partly due to reduced niche widths of invasive species when in sympatry with other species (Tran et al., 2015). Consequently, narrow niches observed for *P. reticulata* may serve to facilitate coexistence in invaded ecosystems. It is important to consider that *P. reticulata* also competes for resources with other native species that have similar feeding habits (and not only with *P. uai*), such as small characids. Therefore, changes in the trophic niche observed may have occurred in response to competition for resources with a suite of other species, as well as in response to the presence of predators (Hawlena et al., 2011). The novelty observed here was that both exotic and native fish species presented similar changes in response to degradation in the environment.

4.5. Effects of diet shifts on aquatic ecosystems

Waste water from domestic sewage is an allochthonous source of organic matter to the food web. Sewage sludge is a high-quality food source that can represent an important vector for transfer of sewage-associated contaminants (e.g., metals, estrogenics) to aquatic consumers (Spies, 1984; deBruyn and Rasmussen, 2002). Therefore, the assimilation of sewage by exotic guppies, *P. reticulata*, can propagate contaminants to higher trophic levels, including humans. Despite fish acting as mobile links between ecosystems by transporting and redistributing nutrients among different zones of the river, guppies were not expected to have high impacts on food webs of sites far from P1, due the small home range and resident habits of *P. reticulata*. In addition, few migratory fish species (and possible predators of *P. reticulata*) are usually collected at the site immediately downstream of the MRBH (Pompeu et al., 2005), which probably decreases the spread of contaminants along the Rio das Velhas Basin. However, it is necessary to emphasize that birds and other consumers of guppies from P1 can also bioaccumulate possible contaminants and, consequently, transfer contaminants to terrestrial environments.

5. Conclusion

We conclude that both native and exotic species present broad trophic plasticity, changing their diet according to the site where they were sampled, likely in response to available resources. Contrary to expectations, exotic guppies displayed narrower trophic niches compared to the native species, which may be an adaptation to facilitate the coexistence in invaded ecosystems, or just by the fact that the native species presents higher interspecific variation in use of resources. However, the exotic species showed high abundance at the most polluted site, as well as a capacity to assimilate carbon directly from sewage.

The use of stable isotopes coupled with gut contents analyses was essential to reach the information about sewage assimilation, since it allowed concluding that the detritus found in the stomach contents of the guppies (and also described in the literature as a recurrent item in gut contents of guppies) is actually sewage-derived organic matter. Further studies including other native and exotic fish species, macroinvertebrates and other aquatic organisms, should be conducted on other highly polluted sites to evaluate the importance of sewage to the entire food web. Analyses of contaminants on fish tissues should be also considered, since sewage sludge can represent an important vector for transfer along the food web. It is also recommended a long-time study to access if the sewage is a constant food item to guppies, since the findings from this study reflect the trophic dynamics of *P. reticulata* and *P. uai* during the dry and wet seasons along only one year.

The findings of this study suggest a key trait (sewage consumption) that likely facilitates the spread and invasiveness of *P. reticulata*. Although guppies have been relatively well studied, this is the first time that sewage has been revealed to be an important food item in highly polluted sites, which can explain high guppy abundance in urban catchments. Although there has been progress in sewage treatment in the Rio das Velhas basin in recent years, there are still areas with raw sewage disposal. Therefore, the results of this study emphasize the need for additional efforts to complement sewage treatment, besides suggesting that the feeding habits of *P. reticulata* can be used in future works monitoring the presence of sewage in the basin. According to current and projected trends, water pollution and species invasion are considered the primary drivers that lead to biodiversity loss in freshwater environments (Mazor et al., 2018). These two impacts are highly correlated, since habitat alteration may enhance invasion success by facilitating increases in the local abundance and regional spread of invaders (Hermoso et al., 2010). As guppy phenotypes respond to changes in their environments (Warbanski et al., 2017), it is important to take into consideration the information presented here for biological control policy and to anticipate potential ecosystem effects.

Acknowledgments

We thank the Agência Peixe Vivo and the Comitê de Bacia Hidrográfica do Rio das Velhas (CBH - Rio das Velhas) for project financial support and the Projeto Manuelzão for the logistical support. This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance code 001 and D.R.C. sandwich doctorate scholarship granted (88881.190508/2018-01). Thanks to Aline Junqueira Grossi (Federal University of Lavras, UFLA) and Luiza Hoehne (Federal University of Minas Gerais, UFMG) for the support on processing of samples. Thanks to the Benthos Ecology Laboratory (UFMG) and the Laboratory of Fish Ecology (UFLA) who assisted in the processing of samples and infrastructure, and to the Centre for Nuclear Energy in Agriculture (CENA) for their support and partnership in the isotopic analysis. Thanks also to the Cornell Limnology group by the comments and suggestions to the manuscript. P.S.P. received a research grant and a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (303548/2017-7) and from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (PPM-00237/13).

Appendix A

Table A.1
Feeding habits of the native (representative of *Phalloceros* genus) and exotic (*P. reticulata*) species documented on literature.

Species	Food items	Classification	Reference
Poecilia reticulata	Algae (79%), organic detritus, diatoms, mosquito larvae parts, protozoan, zooplankton and fish parts (4.5%)	Opportunistic benthopelagic omnivores, whose preference for food fluctuates with season, with a peak in diversity of food types occurring in the rainy season.	(Lawal et al., 2012)
Poecilia reticulata	Mainly benthic algae and invertebrates. Guppies can live and grow on an exclusive diet of green algae, *Ch1orococcum*, but not on the filamentous green, *Oedogonium*.	*Poecilia reticulata* feeds opportunistically with a major component of algal material.	(Dussault and Kramer, 1981)
Phalloceros anisophallos	Fed on detritus, autochthonous algae (diatoms, unicellular and filamentous Chlorophyceae algae), autochthonous animals (aquatic insect larvae: Trichoptera, Chironomidae and other Diptera) and allochthonous animals (terrestrial insects: Hymenoptera). Adults used a larger quantity of algae and detritus, and juveniles used a higher quantity of animal items.	*Phalloceros anisophallos* from Córrego Andorinha displayed an omnivorous diet based mainly on autochthonous resources from the sediment (insect larvae, algae and detritus).	(Mazzoni et al., 2010)
Phalloceros harpagos	Debris and sediment, followed by plant and algae were the most ingested items.	The species shows a high flexibility in the diet.	(Monaco et al., 2014)
Phalloceros harpagos	Algae, non-identified organic material, fish remains (scales), vegetal material, terrestrial insects, aquatic insects and sediment represent the principal items on diet of *P. harpagos*. There was no seasonal variation in diet composition. An important observation from the gut analyses is the presence of remains of individuals from the same species in the guts, suggesting the occurrence of cannibalism.	The species presents omnivorous feeding habit and there was no significantly seasonal variation seasonal on diet composition. The intensity of feeding activity also no varies seasonally, indicating what the species presents uniform feeding through the year, as many quantitative as qualitative terms.	(Souza et al., 2015)

Table A.2
Mean and standard deviation (SD) of δ^{13}C and δ^{15}N of basal resources, and native and exotic guppy species sampled on Rio das Velhas basin.

Site		Unpolluted				Site		Polluted			
		Mean ^{13}C	SD ^{13}C	Mean ^{15}N	SD ^{15}N			Mean ^{13}C	SD ^{13}C	Mean ^{15}N	SD ^{15}N
U1	Algae	-19.68	1.74	5.28	0.18	P1	Algae	-28.59	1.01	-6.17	1.19
	CPOM	-29.83	1.03	2.09	1.09		CPOM	-29.12	0.94	1.81	1.89
	Periphyton	-27.11	1.23	4.82	0.64		Periphyton	-24.42	1.65	2.03	3.90
	FPOM	-25.36	0.64	4.54	0.63		FPOM	-23.77	1.03	1.70	2.65
	P. uai	-26.46	1.52	8.08	0.84		*P. uai*	-	-	-	-
	P. reticulata	-25.64	2.11	7.53	0.23		*P. reticulata*	-22.48	1.16	3.53	1.39
U2	Algae	-21.42	5.24	5.03	1.77	P2	Algae	-3.84	3.36	20.69	4.11
	CPOM	-29.62	1.03	3.52	1.16		CPOM	-28.93	1.53	7.24	4.17
	Periphyton	-24.21	1.63	6.91	1.18		Periphyton	-19.75	3.78	17.65	4.69
	FPOM	-25.23	2.01	5.66	0.47		FPOM	-23.17	1.20	15.65	4.56
	P. uai	-23.12	0.45	10.86	0.23		*P. uai*	-17.55	1.35	21.20	2.22
	P. reticulata	-	-	-	-		*P. reticulata*	-17.03	1.10	24.28	5.09
U3	Algae	-26.74	1.67	6.91	1.09	P3	Algae	-6.19	1.40	14.65	2.24
	CPOM	-30.17	2.05	1.21	1.61		CPOM	-29.15	1.71	6.28	3.18
	Periphyton	-25.97	1.15	6.00	0.47		Periphyton	-18.19	3.87	14.12	2.89
	FPOM	-26.85	0.23	5.09	0.18		FPOM	-18.93	2.87	14.13	3.28
	P. uai	-	-	-	-		*P. uai*	-15.03	2.45	19.52	1.94
	P. reticulata	-25.98	0.42	10.52	0.57		*P. reticulata*	-	-	-	-
U4	Algae	-25.75	0.09	8.70	0.39	P4	Algae	-6.85	0.58	10.50	0.18
	CPOM	-29.51	1.74	3.13	1.85		CPOM	-28.87	1.43	9.09	2.64
	Periphyton	-24.73	1.16	7.69	0.62		Periphyton	-19.64	3.45	14.31	3.70
	FPOM	-25.52	0.43	6.11	0.51		FPOM	-21.91	3.14	13.00	2.99
	P. uai	-21.47	0.79	11.03	0.72		*P. uai*	-15.27	1.17	20.15	1.65
	P. reticulata	-22.47	0.37	10.73	0.34		*P. reticulata*	-19.26	0.61	15.23	0.36
U5	Algae	-21.78	2.16	8.63	0.69						
	CPOM	-30.15	1.07	1.93	3.06						
	Periphyton	-24.09	2.22	6.12	1.10	STP's	Sewage	-23.16	0.40	1.50	0.45
	FPOM	-23.85	1.27	6.35	1.21						
	P. uai	-18.69	2.42	10.31	1.32						
	P. reticulata	-17.15	0.81	9.96	1.44						

Table A.3
Proportion of each food item found in the guts of the native species (*P. uai*) and exotic species (*P. reticulata*) in each sampling site of the Rio das Velhas basin. Bolded number indicates the most consumed items (>30%).

Species	Site	Detritus/periphyton	Algae	Plant remnants	Aquatic insects	Terrestrial insects	Zooplankton
Native species	U1	0.10	**0.36**	0.03	**0.47**	0.00	0.04
	U2	0.15	0.19	0.06	**0.46**	0.07	0.08
	U5	**0.38**	0.01	0.03	**0.42**	0.15	0.01
	P2	0.21	0.14	0.02	**0.47**	0.13	0.03
	P3	0.12	0.03	0.03	**0.51**	0.27	0.03
	P4	0.04	0.01	0.01	**0.45**	**0.40**	0.08
	Mean	0.17	0.12	0.03	0.46	0.17	0.05
Exotic species	U1	0.23	0.03	0.15	**0.59**	0.00	0.00
	U3	0.24	0.14	0.13	**0.38**	0.07	0.05
	U4	**0.95**	0.03	0.02	0.00	0.00	0.00
	U5	**0.62**	0.03	0.03	**0.31**	0.00	0.00
	P1	**0.80**	0.13	0.08	0.00	0.00	0.00
	P2	0.15	0.09	0.05	**0.30**	0.00	**0.41**
	P4	0.05	0.01	0.02	**0.55**	**0.36**	0.02
	Mean	0.43	0.06	0.07	0.30	0.06	0.07

References

Ab Hamid, S., Md Rawi, C.S., 2017. Application of aquatic insects (Ephemeroptera, Plecoptera and Trichoptera) in water quality assessment of Malaysian headwater. Trop. Life Sci. Res. 28, 143–162. https://doi.org/10.21315/tlsr2017.28.2.11.

Allan, J.D., Flecker, A.S., 1993. Biodiversity conservation in running waters. Bioscience 43, 32–43. https://doi.org/10.2307/1312104.

Al-Shami, S., Rawi, C.S.M., Nor, S.A.M., Ahmad, A.H., Ali, A., 2010. Morphological deformities in Chironomus spp. (Diptera: Chironomidae) larvae as a tool for impact assessment of anthropogenic and environmental stresses on three rivers in the Juru River system, Penang, Malaysia. Environ. Entomol. 39, 210–222. https://doi.org/10.1603/EN09109.

Alves, C.B.M., Pompeu, P. dos S., 2010. A fauna de peixes da bacia do rio das Velhas no final do século XX. In: Alves, C.B.M., Pompeu, P.S. (Eds.), Peixes Do Rio Das Velhas: Passado e Presente. ARGVMENTVM, Belo Horizonte, pp. 167–189.

Atlas das Águas, 2009. Impacto ambiental relevante identificado na bacia do rio das Velhas Águas impactadas por esgotos domésticos e efluentes industriais. [WWW Document]. URL. http://www.atlasdasaguas.ufv.br/velhas/impacto_ambiental_relevante_identificado_na_bacia_do_rio_das_velhas.html, Accessed date: 15 January 2019.

Baiser, B., Russell, G.J., Lockwood, J.L., 2010. Connectance determines invasion success via trophic interactions in model food webs. Oikos 119, 1970–1976. https://doi.org/10.1111/j.1600-0706.2010.18557.x.

Barrett, L.T., Evans, J.P., Gasparini, C., 2014. The effects of perceived mating opportunities on patterns of reproductive investment by male guppies. PLoS One 9, e93780. https://doi.org/10.1371/journal.pone.0093780.

Barrie, A., Prosser, S.J., 1996. Mass spectrometry of soils. Mass Spectrometry of Soils. Marcel Dekker, Inc, p. 536.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., Macleod, H., 2004. Determining trophic niche width: a novel approach using stable isotope analysis. J. Anim. Ecol. 73, 1007–1012. https://doi.org/10.1111/j.0021-8790.2004.00861.x.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., Forister, M.L., 2003. The ecology of individuals: incidence and implications of individual specialization. Am. Nat. 161, 1–28. https://doi.org/10.1086/343878.

CABI, 2019. Invasive species compendium-*Poecilia Reticulata* (Guppy) [WWW Document]. URL. CABIhttps://www.cabi.org/isc/datasheet/68208, Accessed date: 24 January 2019.

Carvalho, D.R. de, Castro, D., Callisto, M., Moreira, M.Z., Pompeu, P.S., 2015. Isotopic variation in five species of stream fishes under the influence of different land uses. J. Fish Biol. 87, 559–578. https://doi.org/10.1111/jfb.12734.

de Carvalho, D.R., de Castro, D.M.P., Callisto, M., Moreira, M.Z., Pompeu, P.S., 2017a. 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/s10750-017-3130-6.

de Carvalho, D.R., Leal, C.G., Junqueira, N.T., de Castro, M.A., Fagundes, D.C., Alves, C.B.M., Hughes, R.M., Pompeu, P.S., 2017b. A fish-based multimetric index for Brazilian savanna streams. Ecol. Indic. 77, 386–396. https://doi.org/10.1016/j.ecolind.2017.02.032.

Casatti, L., de Paula Ferreira, C., Carvalho, F.R., 2009. Grass-dominated stream sites exhibit low fish species diversity and dominance by guppies: an assessment of two tropical pasture river basins. Hydrobiologia 632, 273–283. https://doi.org/10.1007/s10750-009-9849-y.

Chapman, B.B., Ward, A.J.W., Krause, J., 2008. Schooling and learning: early social environment predicts social learning ability in the guppy, *Poecilia reticulata*. Anim. Behav. 76, 923–929. https://doi.org/10.1016/j.anbehav.2008.03.022.

Costa, C., Ide, S., Simonka, C.E., 2006. Insetos imaturos. Metamorfose e identificação. Holos. vol. 50 p. 249.

Courtenay, W.R., Welcomme, R.L., 1989. International introductions of inland aquatic species. Copeia 1989, 520–521. https://doi.org/10.2307/1445460.

Deacon, A.E., Ramnarine, I.W., Magurran, A.E., 2011. How reproductive ecology contributes to the spread of a globally invasive fish. PLoS One 6, e24416. https://doi.org/10.1371/journal.pone.0024416.

deBruyn, A.M.H., Rasmussen, J.B., 2002. Quantifying assimilation of sewage-derived organic matter by riverine benthos. Ecol. Appl. 12, 511–520. https://doi.org/10.1890/1051-0761(2002)012[0511:qaosdo]2.0.co;2.

Dudley, B.D., MacKenzie, R.A., Sakihara, T.S., Riney, M.H., Ostertag, R., 2017. Effects of invasion at two trophic levels on diet, body condition, and population size structure of Hawaiian red shrimp. Ecosphere 8, e01682. https://doi.org/10.1002/ecs2.1682.

Dussault, G.V., Kramer, D.L., 1981. Food and feeding behaviour of the guppy, *Poecilia reticulata* (Pisces: poeciliidae). Can. J. Zool. 59, 684–701.

Feio, M.J., Ferreira, W.R., Macedo, D.R., Eller, A.P., Alves, C.B.M., França, J.S., Callisto, M., 2015. Defining and testing targets for the recovery of tropical streams based on macroinvertebrate communities and abiotic conditions. River Res. Appl. 31, 70–84. https://doi.org/10.1002/rra.2716.

FishBase, 2019. *Poecilia reticulata* was reported from 71 countries/islands. WWW Document. URL. FishBasehttp://www.fishbase.se/Country/CountryList.php?ID=3228&GenusName=Poecilia&SpeciesName=reticulata%3E, Accessed date: 24 January 2019.

Hawlena, D., Hughes, K.M., Schmitz, O.J., 2011. Trophic trait plasticity in response to changes in resource availability and predation risk. Funct. Ecol. 25, 1223–1231. https://doi.org/10.1111/j.1365-2435.2011.01891.x.

Hermoso, V., Clavero, M., Blanco-Garrido, F., Prenda, J., 2010. Assessing the ecological status in species-poor systems: a fish-based index for Mediterranean Rivers (Guadiana River, SW Spain). Ecol. Indic. 10, 1152–1161. https://doi.org/10.1016/j.ecolind.2010.03.018.

Hill, J.M., Jones, R.W., Hill, M.P., Weyl, O.L.F., 2015. Comparisons of isotopic niche widths of some invasive and indigenous fauna in a South African river. Freshw. Biol. 60, 893–902. https://doi.org/10.1111/fwb.12542.

Hilton, J., O'Hare, M., Bowes, M.J., Jones, J.I., 2006. How green is my river? A new paradigm of eutrophication in rivers. Sci. Total Environ. 365, 66–83. https://doi.org/10.1016/j.scitotenv.2006.02.055.

Hobbs, R.J., Huenneke, L.F., 1992. Disturbance, diversity, and invasion: implications for conservation. Conserv. Biol. 6, 324–337.

Holeton, C., Chambers, P.A., Grace, L., 2011. Wastewater release and its impacts on Canadian waters. Can. J. Fish. Aquat. Sci. 68, 1836–1859. https://doi.org/10.1139/f2011-096.

Hyslop, E.J., 1980. Stomach contents analysis—a review of methods and their application. J. Fish Biol. 17, 411–429. https://doi.org/10.1111/j.1095-8649.1980.tb02775.x.

IGAM-Instituto Mineiro de Gestão das águas, 2013. Identificação de municípios com condição crítica para a qualidade de água na bacia do rio das Velhas.

IGAM-Instituto Mineiro de Gestão das águas, 2017a. Monitoramento da Qualidade das Águas Superficiais no Estado de Minas Gerais-Relatório Trimestral - 3° Trimestre de 2017 (Belo Horizonte).

IGAM-Instituto Mineiro de Gestão das águas, 2017b. Monitoramento de Qualidade das Águas. WWW Document. Monit. Qual. das Águas URL. http://portalinfohidro.igam.mg.gov.br/monitoramento-de-qualidade-das-aguas, Accessed date: 16 January 2019.

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. J. Anim. Ecol. 80, 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x.

Junk, W.J., Bayley, P.B., Sparks, R.E., 1989. The flood pulse concept in river-floodplain systems. In: DODGE, D.P. (Ed.), Proceedings of the International Large River Symposium (LARS). Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa, pp. 110–127.

Kendall, C., 1998. Tracing Nitrogen Sources and Cycling in Catchments, Isotope Tracers in Catchment Hydrology. Elsevier B.V https://doi.org/10.1016/B978-0-444-81546-0.50023-9.

Keough, J.R., Hagley, C.A., Ruzycki, E., Sierszen, M., 1998. δ 13 C composition of primary producers and role of detritus in a freshwater coastal ecosystem. Limnol. Oceanogr. 43, 734–740. https://doi.org/10.4319/lo.1998.43.4.0734.

Kinney, E., Roman, C., 1998. Response of primary producers to nutrient enrichment in a shallow estuary. Mar. Ecol. Prog. Ser. 163, 89–98. https://doi.org/10.3354/meps163089.

Lawal, M.O., Edokpayi, C.A., Osibona, A.O., 2012. Food and feeding habits of the Guppy, *Poecilia reticulata*, from drainage canal systems in Lagos, southwestern Nigeria. West African J. Appl. Ecol. 20, 1–9.

Lawton, R.J., Pratchett, M.S., 2012. Influence of dietary specialization and resource availability on geographical variation in abundance of butterflyfish. Ecol. Evol. 2, 1347–1361. https://doi.org/10.1002/ece3.253.

Loomer, H.A., Oakes, K.D., Schiff, S.L., Taylor, W.D., Servos, M.R., 2015. Use of stable isotopes to trace municipal wastewater effluents into food webs within a highly developed river system. River Res. Appl. 31, 1093–1100. https://doi.org/10.1002/rra.2826.

Lucinda, P.H.F., 2008. Systematics and biogeography of the genus Phalloceros Eigenmann, 1907 (Cyprinodontiformes: Poeciliidae: Poeciliinae), with the description of twenty-one new species. Neotrop. Ichthyol. 6, 113–158. https://doi.org/10.1590/S1679-62252008000200001.

Machado, A.T.M., Alves, C.B.M., Callisto, M., 2008. Projeto Manuelzão: metodologia e resultados. In: Lisboa, A.H., Goulart, E.M.A., Diniz, L.F.M. (Eds.), Projeto Manuelzão. A História Da Mobilização Que Começou Em Torno de Um Rio. Instituto Guaicuy-SOS Rio das Velhas, Belo Horizonte, pp. 39–54.

Magalhães, A.L.B., Jacobi, C.M., 2017. Colorful invasion in permissive Neotropical ecosystems: establishment of ornamental non-native poeciliids of the genera Poecilia/Xiphophorus (Cyprinodontiformes: Poeciliidae) and management alternatives. Neotrop. Ichthyol. 15, 1–14. https://doi.org/10.1590/1982-0224-20160094.

Mazor, T., Doropoulos, C., Schwarzmueller, F., Gladish, D.W., Kumaran, N., Merkel, K., Di Marco, M., Gagic, V., 2018. Global mismatch of policy and research on drivers of biodiversity loss. Nat. Ecol. Evol. 2, 1071–1074. https://doi.org/10.1038/s41559-018-0563-x.

Mazzoni, R., Araújo, R.R. da S., Santos, G.C.T. dos, Iglesias-Rios, R., 2010. Feeding ecology of Phalloceros anisophallos (Osteichthyes: Cyprinodontiformes) from Andorinha Stream, Ilha Grande, Brazil. Neotrop. Ichthyol. 8, 179–182. https://doi.org/10.1590/S1679-62252010000100021.

McCutchan Jr., J.H., Lewis Jr., W.M., 2001. Seasonal variation in stable isotope ratios of stream algae. Verhandlungen des Int. Verein Limnol. 27, 3304–3307.

Meffe, G.K., Snelson, F.F., 1989. Summary for policymakers. In: Intergovernmental Panel on Climate Change (Ed.), Climate Change 2013- the Physical Science Basis. Cambridge University Press, Cambridge, pp. 1–30 https://doi.org/10.1017/CBO9781107415324.004.

Monaco, I.D.A., Súarez, Y.R., Lima-Junior, S.E., 2014. Influence of environmental integrity on feeding, condition and reproduction of Phalloceros harpagos Lucinda, 2008 in the Tarumã stream micro-basin. Acta Sci. Biol. Sci. 36, 181. https://doi.org/10.4025/actascibiolsci.v36i2.21394.

Moore, J.W., Semmens, B.X., 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett. 11, 470–480. https://doi.org/10.1111/j.1461-0248.2008.01163.x.

Mouri, G., Takizawa, S., Oki, T., 2011. Spatial and temporal variation in nutrient parameters in stream water in a rural-urban catchment, Shikoku, Japan: effects of land cover and human impact. J. Environ. Manag. 92, 1837–1848. https://doi.org/10.1016/j.jenvman.2011.03.005.

Mugnai, R., Nessimian, J.L., Baptista, D.F., 2010. Manual de Identificação de Macroinvertebrados Aquáticos do Estado do Rio de Janeiro. Technical Books, Rio de Janeiro, RJ.

Nikolenko, O., Jurado, A., Borges, A.V., Kn, K., Brouy, S., 2018. Isotopic composition of nitrogen species in groundwater under agricultural areas : a review. Sci. Total Environ. 621, 1415–1432. https://doi.org/10.1016/j.scitotenv.2017.10.086.

O'Leary, M.H., 1981. Carbon isotope fractionation in plants. Phytochemistry 20, 553–567. https://doi.org/10.1016/0031-9422(81)85134-5.

Oliveira, D.C. De, Bennemann, S.T., 2005. Ictiofauna, recursos alimentares e relações com as interferências antrópicas em um riacho urbano no sul. Biota Neotrop 5, 95–107.

Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS One 5, e9672. https://doi.org/10.1371/journal.pone.0009672.

Pérez-Matus, A., Pledger, S., Díaz, F.J., Ferry, L.A., Vásquez, J.A., 2012. Plasticity in feeding selectivity and trophic structure of kelp forest associated fishes from northern Chile. Rev. Chil. Hist. Nat. 85, 29–48. https://doi.org/10.4067/S0716-078X2012000100003.

Peterson, A.T., Vieglais, D.A., 2001. Predicting species invasions using ecological niche modeling : new approaches from bioinformatics attack a pressing problem. Bioscience 51, 363–372. https://doi.org/10.1641/0006-3568(2001)051[0363:PSIUEN]2.0.CO;2.

Pettitt-Wade, H., Wellband, K.W., Heath, D.D., Fisk, A.T., 2015. Niche plasticity in invasive fishes in the Great Lakes. Biol. Invasions 17, 2565–2580. https://doi.org/10.1007/s10530-015-0894-3.

Pompeu, P.D.S., Godinho, A.L., 2001. Mudança na dieta da traíra Hoplias malabaricus (Bloch) (Erythrinidae, Characiformes) em lagoas da bacia do rio Doce devido à introdução de peixes piscívoros. Rev. Bras. Zool. 18, 1219–1225. https://doi.org/10.1590/S0101-81752001000400016.

Pompeu, P.S., Alves, C.B.M., Callisto, M., 2005. The effects of urbanization on biodiversity and water quality in the Rio das Velhas Basin, Brazil. Am. Fish. Soc. Symp. 47, 11–22.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718. https://doi.org/10.2307/3071875.

Rahel, F.J., 2002. Homogenization of freshwater faunas. Annu. Rev. Ecol. Syst. 33, 291–315. https://doi.org/10.1146/annurev.ecolsys.33.010802.150429.

Reznick, D.N., Bassar, R.D., Travis, J., Helen Rodd, F., 2012. Life-history evolution in guppies VIII: the demographics of density regulation in guppies (*Poecilia reticulata*). Evolution (N. Y). 66, 2903–2915. https://doi.org/10.1111/j.1558-5646.2012.01650.x.

Rivas, L.R., Rosen, D.E., Bailey, R.M., 1965. The Poeciliid fishes (Cyrinodontiformes), their structure, zoogeography and systematics. Copeia 1965, 117. https://doi.org/10.2307/1441257.

Rolshausen, G., Phillip, D.A.T., Beckles, D.M., Akbari, A., Ghoshal, S., Hamilton, P.B., Tyler, C.R., Scarlett, A.G., Ramnarine, I., Bentzen, P., Hendry, A.P., 2015. Do stressful conditions make adaptation difficult? Guppies in the oil-polluted environments of southern Trinidad. Evol. Appl. 8, 854–870. https://doi.org/10.1111/eva.12289.

Ruaro, R., Mormul, R.P., Gubiani, É.A., Piana, P.A., Cunico, A.M., da Graça, W.J., 2018. Non-native fish species are related to the loss of ecological integrity in Neotropical streams: a multimetric approach. Hydrobiologia 817, 413–430. https://doi.org/10.1007/s10750-018-3542-y.

Shea, K., Chesson, P., 2002. Community ecology theory as a framework for biological invasions. Trends Ecol. Evol. 17, 170–176. https://doi.org/10.1016/S0169-5347(02)02495-3.

Silva, J.C. da, Delariva, R.L., Bonato, K.O., 2012. Food-resource partitioning among fish species from a first-order stream in northwestern Paraná, Brazil. Neotrop. Ichthyol. 10, 389–399. https://doi.org/10.1590/S1679-62252012005000008.

Souza, M.M. De, Lima-junior, S.E., Súarez, R., 2015. Ecologia trófica de Phalloceros harpagos (Lucinda, 2008) no Córrego Guiraí, Bacia do Alto Rio Paraná–MS. Anais Do Encontro de Iniciação Científica-ENIC.

Spies, R., 1984. Benthic-pelagic coupling in sewage-affected marine ecosystems. Mar. Environ. Res. 13, 195–230. https://doi.org/10.1016/0141-1136(84)90030-8.

Stock, B., Semmens, B., 2016a. MixSIAR GUI User Manual. Version 3.1 1–59. https://doi.org/10.5281/zenodo.47719.

Stock, B.C., Semmens, B.X., 2016b. Unifying error structures in commonly used biotracer mixing models. Ecology 97, 576–582. https://doi.org/10.1002/ecy.1517.

Stockwell, C.A., Henkanaththegedara, S.M., 2011. Evolutionary conservation biology. In: Evans, J.P., Pilastro, A., Schlupp, I. (Eds.), Ecology and Evolution of Poeciliid Fishes. The University of Chicago Press, Chicago, p. 424.

Tran, T.N.Q., Jackson, M.C., Sheath, D., Verreycken, H., Britton, J.R., 2015. Patterns of trophic niche divergence between invasive and native fishes in wild communities are predictable from mesocosm studies. J. Anim. Ecol. 84, 1071–1080. https://doi.org/10.1111/1365-2656.12360.

Tricarico, E., Junqueira, A.O.R., Dudgeon, D., 2016. Alien species in aquatic environments: a selective comparison of coastal and inland waters in tropical and temperate latitudes. Aquat. Conserv. Mar. Freshw. Ecosyst. 26, 872–891. https://doi.org/10.1002/aqc.2711.

Ulseth, A.J., Hershey, A.E., 2005. Natural abundances of stable isotopes trace anthropogenic N and C in an urban stream. J. North Am. Benthol. Soc. 24, 270–289. https://doi.org/10.1899/03-080.1.

Vanderklift, M. a, Ponsard, S., 2003. Sources of variation in consumer-diet delta 15N enrichment: a meta-analysis. Oecologia 136, 169–182. https://doi.org/10.1007/s00442-003-1270-z.

Wang, Y., Liu, D., Richard, P., Di, B., 2016. Selection of effective macroalgal species and tracing nitrogen sources on the different part of Yantai coast, China indicated by macroalgal δ15N values. Sci. Total Environ. 542, 306–314. https://doi.org/10.1016/j.scitotenv.2015.10.059.

Warbanski, M.L., Marques, P., Frauendorf, T.C., Phillip, D.A.T., El-Sabaawi, R.W., 2017. Implications of guppy (*Poecilia reticulata*) life-history phenotype for mosquito control. Ecol. Evol. 7, 3324–3334. https://doi.org/10.1002/ece3.2666.

White, S.J., Wilson, A.J., 2018. Evolutionary genetics of personality in the Trinidadian guppy I: maternal and additive genetic effects across ontogeny. Heredity (Edinb), 1–14 https://doi.org/10.1038/s41437-018-0082-1.

ARTIGO II - *Using δ^{15}N of periphyton and fish to evaluate spatial and seasonal variation of anthropogenic nitrogen inputs in a polluted Brazilian river basin*

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Artigo submetido na revista *Ecological Indicators*

(Manuscrito redigido na língua e formato indicado para revista - número de linhas foi retirado para melhor adequação ao formato da tese)

Abstract

Although the nitrogen isotope ratio ($\delta^{15}N$) of aquatic organisms have been widely used as indicators of the intensity of anthropogenic N loading on watersheds, this approach is still scarce in the Neotropics. Considering the importance of different tools to access aquatic pollution, the present study aims to use the $\delta^{15}N$ of periphyton and its direct consumers (catfishes from *Hypostomus* genus) to evaluate spatial and seasonal variation of anthropogenic nitrogen inputs. The study was carried out in 16 sites distributed along the Rio das Velhas basin, a Brazilian river basin highly impacted by anthropogenic activities, especially discharge of domestic and industrial sewage from a region with more than five million human inhabitants. We correlated the $\delta^{15}N$ of periphyton and *Hypostomus* with the nitrate and ammonium concentrations in water from each site, during dry and wet seasons. The $\delta^{15}N$ of periphyton and *Hypostomus* was correlated, and varied according to nitrate and ammonium concentrations. Nitrate promoted considerable $\delta^{15}N$ enrichment (especially in the dry season), reaching values above 30‰ in periphyton and 36‰ in *Hypostomus*. In contrast, ammonium promoted $\delta^{15}N$ depletion (especially in the wet season), reaching values around -1 ‰ in periphyton and 5 ‰ in *Hypostomus.* However, periphyton was more sensitive to changes in nitrate and ammonium concentrations between seasons than *Hypostomus*. In addition, the $\Delta^{15}N$ (enrichment of primary consumer relative to the primary producer) was higher in polluted sites than in unpolluted sites (especially in the wet season), suggesting that *Hypostomus* may be feeding on resources other than periphyton. We conclude that the $\delta^{15}N$ of periphyton and *Hypostomus* reflect nitrogen concentrations and suggests the occurrence of different nitrogen sources within this basin due to diverse anthropogenic activities. Unfortunately, the mixing and diversity $\delta^{15}N$ sources mean the values of periphyton and *Hypostomus* it is likely best used as an indicator of the intensity of anthropogenic nitrogen loading, but not as tracers of individual nitrogen sources.

Keywords: Nitrogen pollution; Nitrogen enrichment; Bioindicators; *Hypostomus*; Armored catfish; Biofilm.

1 Introduction

The increase of nitrogen compounds from anthropogenic sources into aquatic ecosystems is an issue of worldwide concern, since it can lead to the disruption of aquatic ecosystem services (Compton et al., 2011; Jones et al., 2014; Watanabe et al., 2012). In urban and agricultural areas, the main sources of N are from the intensive application of organic and inorganic fertilizers, inflow from animal manure, sewage discharge (e.g., septic wastewater), and atmospheric precipitation (Nikolenko et al., 2018; Robertson and Vitousek, 2009). In developing countries, urban and industrial waste water receives no or only preliminary treatment before being discharged directly into rivers and streams (Mara, 2013), jeopardizing biodiversity and human health (Burdon et al., 2016; Canobbio et al., 2009;

Haseena et al., 2017). Therefore, the use of different tools to trace the contaminants, is advantageous to develop efficient mitigation strategies.

Analyses of variation in stable N isotope ratios ($^{15}N/^{14}N$) have been applied extensively to obtain information regarding origin, transport and transformation of N compounds, since the isotopic composition of nitrogen species varies depending on their sources and dynamics of N cycling processes (Capone et al., 2008; Kendall, 1998; Nikolenko et al., 2018). Differential isotopic fractionation, resulting in distinctive isotopic ratios, is often useful for estimating both the magnitude and source of nitrogen pollution. However, the interpretation and estimation of fractionation in natural systems is challenging because isotopic enrichment is influenced by shifting dynamics of various microbiological (e.g., denitrification and nitrification) and physicochemical processes (e.g., upward diffusion, sorption, and volatilization) (Kendall, 1998).

Nitrate and ammonium concentrations are highly influenced by the presence of anthropogenic activities. In natural surface waters, the concentration of nitrate and ammonium is low (< 1 mg/L), while high concentrations usually indicate pollution from fertilizers used in agriculture, or industrial, human and animal waste. The $\delta^{15}N$ of both nitrate and ammonium are also influenced by anthropogenic activities, since the $\delta^{15}N\text{-}NO_3^-$ and $\delta^{15}N\text{-}NH_4^+$ varies depending on their sources and dynamics of N cycle processes (Kendall, 1998). Nikolenko and collaborators (2018) recently summarized the factors affecting the $\delta^{15}N\text{-}NO_3^-$ and $\delta^{15}N\text{-}NH_4^+$ in groundwater under agricultural areas. According to these authors, the reported $\delta^{15}N\text{-}NO_3^-$ values for sources of nitrate are: manure/household waste - +5‰ to +35‰, soil organic nitrogen - +3‰ to +8‰, and mineral fertilizers - −8‰ to +7‰. For ammonium sources, the isotopic signature ranges between −13.4 to +2.3‰ for rainwater, +2.4 to +4.1‰ for organic matter, −7.4 to +5.1‰ for mineral fertilizers, +5 to +9‰ for household waste, and +8 to +11‰ for animal manure (Nikolenko et al., 2018).

Considering these changes on $\delta^{15}N$, most of previously global studies used the $\delta^{15}N\text{-}NO_3$ and $\delta^{15}N\text{-}NH_4$ to discriminate between urban, industrial and agricultural sources of pollutants (Anornu et al., 2017; Fenech et al., 2012; Kendall, 1998; Matiatos, 2016; Viers et al., 2012). In the last few decades, the use of $\delta^{15}N$ of primary producer tissue as pollution tracers has increased (Cohen and Fong, 2005, 2004; Costanzo et al., 2001; Savage and Elmgren, 2004; Schubert et al., 2013; Wang et al., 2016), based on the premise that the isotope ratio can reflect N sources in a predictable manner (Cifuentes et al., 1988; Cohen and Fong, 2005). Algae and macrophytes are sensitive to changes in environmental conditions (Gaiser et al., 2006), and have proved to be good indicators for monitoring N sources in river watersheds (Toda et al., 2002). In this context, periphyton can also be applied, since it is a microecosystem biofilm composed of autotrophic and heterotrophic microorganisms including algae, cyanobacteria, heterotrophic bacteria, and fungi (Wu, 2016). Although less common, the $\delta^{15}N$ of fish has also been used as an indicator of the intensity of anthropogenic nitrogen loading to watersheds (Anderson and Cabana, 2006; Lake et al., 2001). However, fishes as indicators may be of more limited utility due to variation among fish species $\delta^{15}N$ due to metabolism and the mobility of many species reflecting different $\delta^{15}N$ baselines.

Although the use of nitrogen isotopes to trace various anthropogenic activities in aquatic environments has been widely used, this approach is still scarce in Brazil. Water pollution in Brazil is not only a major health issue, but an environmental concern, since only 44.9% of the generated sewage and 74.7% of the collected sewage are treated in urban areas (SNIS, 2016). Within Brazil, Rio das Velhas basin stands out as one of the most polluted basins in Brazilian territory (IBGE, 2010). The transfer of the capital of Minas Gerais state to Belo Horizonte in 1898, resulted in the expansion of a center of urbanization and industrialization along the basin, resulting in the third largest Metropolitan Region of Brazil. Thus, the Rio das Velhas, that was once known for its abundance of fish (Alves and Pompeu, 2010), is now in an advanced process of degradation, with frequent episodes of fish kills and low water quality (Alves and Pompeu, 2005; Pompeu et al., 2005).

This study uses the $\delta^{15}N$ of periphyton as an indicator of environmental quality in order to evaluate the effects of human activities along a highly polluted river basin. In addition, we aim to evaluate whether organisms that feed directly on periphyton can be used as an alternative to water quality measurements to trace the presence of pollutants. We chose the armored catfish genus *Hypostomus* to trace nitrogen pollution because periphyton is one of its main food sources (Garavello and Garavello, 2004; Power, 1990; Power et al., 1989). *Hypostomus* species are bottom-dwelling fishes, with jaw specializations that allow them to combine mouth suction and mobile dentition to grasp and explore benthic substrata (Schaefer and Lauder, 1986). In addition, within the Loricariidae, the genus *Hypostomus* is one of the most diversified freshwater catfish groups (Cardoso et al., 2012), and is often resistant to aquatic pollution since they have accessory breathing (Val and de Almeida-Val, 1995), using this strategy when the water conditions are severely hypoxic. Therefore, we analyzed the $\delta^{15}N$ of periphyton and fish species of the *Hypostomus* genus in several sites of Rio das Velhas basin. We tested the hypothesis that $\delta^{15}N$ of periphyton and *Hypostomus* individuals will vary according the nitrate and ammonium concentrations, reflecting different sources of pollution (as summarized in Nikolenko et al., 2018). We expected that: 1) higher values of $\delta^{15}N$ of periphyton will be related to higher nitrate and lower ammonium concentrations; 2) $\delta^{15}N$ of *Hypostomus* will be positively correlated with $\delta^{15}N$ of periphyton, since periphyton is one of its main food resources; and 3) changes in nitrate/ammonium concentrations between dry and wet seasons will be reflected in $\delta^{15}N$ of periphyton and *Hypostomus*.

2 Material and Methods

2.1 Study area

This study was conducted in the Rio das Velhas basin, the longest tributary of São Francisco river basin (Alves and Pompeu, 2010). The Rio das Velhas (RV) is the Rio São Francisco’s second largest tributary by water volume, and is located entirely in the territory of Minas Gerais state, Brazil, covering 51 municipalities along its 801km length (CBH Velhas, 2019) (Figure 1). The climate of the RV basin is tropical humid with dry winters and wet summers. Mean annual precipitation ranges from 1100 to 1600 mm (www.codevasf.gov.br) and temperatures between 18 and 24°C (Feio et al., 2015).

The RV is of significant economic and social importance to Minas Gerais state. However, like many other rivers around the world, it has become heavily polluted in the 20th century (Alves and Pompeu, 2005). The upper course of the RV is located in the Metropolitan Region of Belo Horizonte (MRBH), the third largest urban conurbation in Brazil, with almost 6 million inhabitants (IBGE, 2018), and RV is responsible for most of the MRBH water supply.

The main pollution sources of RV are located in the upper course, which receives the majority of domestic and industrial sewage from MRBH (Alves and Pompeu, 2010), with an estimated discharge of 700 liters of sewage per second into the river. In addition, much of the river is located in the Iron Quadrangle, a critical area because of numerous mining projects (Machado et al., 2008) with mineral exploitation since the 16th century. It is estimated that in the Arrudas and Onça sub-basins alone (located within the upper section), there are 3,125 industries (food, metallurgical, textile, chemical and pharmaceutical industries), of which about 50% have no appropriate treatment for their effluents and solid residues. The middle and lower section of the RV basin are also impacted by anthropogenic activities, such as cattle, aquaculture, and agriculture. Nevertheless, discharge of sewage from small urban centers is still one of the main pollution sources in the middle and lower sections.

2.2 Sampling design

We sampled a total of 16 sites distributed along the RV basin (Figure 1, Table 1). Eight sites along a gradient of pollution in the RV main stem (MS1 to MS8) were sampled once in the wet season (January 2016) and twice in the dry season (July 2015 and July 2016). Eight sites in tributaries - Ribeirão da Mata (T1), Rio Jaboticatubas (T2), Rio Taquaraçu (T3), Rio Jequitibá (T4), Rio da Onça (T5), two sites in Rio Cipó (T6 and T7), and Rio Curimataí (T8) - were sampled once in the wet (October 2015) and once in the dry (July 2015) seasons. The sampling design of the present study followed the sampled design of the Project of Biomonitoring of Rio das Velhas basin (http://cbhvelhas.org.br/wp-content/uploads/2017/12/Biomontoriamento-D%C3%A9cimo-Quarto-Produto-volume-%C3%BAnico-final.pdf).

The sites MS3, MS4, MS5, MS6, MS7, MS8 and the tributaries T1 and T4 are the most polluted localities due to the influence of the urban and industrial wastewater from MRBH, and agriculture/pastures in the middle and lower sections of RV (Table 1). The MS1 and T6 are the least polluted sites because they are located in the headwaters (Table 1). In addition, the MS1 drains a conservation unit (Floresta Estadual do Uaimií) and the T6 drains a National Park (Parque Nacional da

Serra do Cipó). The remaining sites (MS2, T2, T3, T5, T7 and T8) are thought to have good to intermediate water-quality (Table 1).

Water quality data were accessed through the IGAM database (IGAM - Instituto Mineiro de Gestão das águas, 2017), which conducts quarterly monitoring in several points within the RV basin. Values of ammonium (mg/L) and nitrate (mg/L) used in our analysis correspond to measurements carried out during the same months of our samplings in 2015 and 2016.

2.2.1 Periphyton sampling

We collected five samples of periphyton (biofilm) at each site per sampling date. Periphyton was collected by scraping stones with a brush (five rocks per site) and washing with distilled water. After collection, all samples were stored in plastic sample jars and held on ice for one to five hours prior to storage in a freezer. In the laboratory, periphyton samples were filtered through calcined quartz fiber filters (Whatman® WHA quartz filters - 25mm) using a filtration apparatus attached to a vacuum pump. Samples were oven dried at 60°C for 48 hours, ground to a homogeneous powder and subsequently stored in *Eppendorf* tubes. About 5-10 mg of dry material per sample (stored in tin capsules) were used for isotopic analysis.

2.2.2 Fish sampling

Fish were collected with gillnets (20 m long, with 3–16 cm stretch measure mesh), seines (5 m long, 1 mm mesh), cast nets (3 cm stretch measure mesh), and mosquito nets (80 cm in diameter, 1 mm mesh). Gill nets were deployed in the water column for 14 h overnight. Seines were used in shallow areas or littoral zones, mosquito nets were used in near-shore aquatic macrophytes (both shorelines), undercut banks and in riffles, and cast nets were used in habitats too deep to wade. The three latter methods were employed for 1–3 h. Fish from *Hypostomus* genus were not present at all sampling points and seasons (see Table 3). When present, the individuals of *Hypostomus* had a portion of muscle removed for isotopic analysis in the field (the identification to species level was done in the laboratory). All samples were kept frozen until laboratory processing to avoid deterioration of the material. Muscle samples were lyophilized for 24 hours, ground to a fine and homogeneous powder using a mortar and pestle, and stored in *Eppendorf* tubes. About 2-5 mg of dry animal tissue material (stored in tin capsules) were used for isotopic analysis.

2.2.3 Stable isotopes

Samples from 150 *Hypostomus* (encompassing eight species, Table 3) and 176 periphyton samples were sent to the Center for Nuclear Energy in Agriculture (CENA) at University of São Paulo (USP) for isotopic analysis. To determine the isotopic ratio, a mass spectrometer in Continuous-flow (CF-IRMS) mode was used with a Carlo Erba elemental analyzer (CHN 1110) coupled to a Delta Plus mass spectrometer (Thermo Scientific). Results were expressed as the relative difference from the international reference standard PeeDee Belemnite in delta notation (δ ‰), and calculated using the following formula:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$$

where X is ^{15}N and R represents the isotopic ratio $^{15}N/^{14}N$ (Barrie and Prosser, 1996).

2.3 Data analysis

Differences in $\delta^{15}N$ of periphyton and *Hypostomus* among sites and seasons (Dry 1, Wet 2 and Dry 3) were tested using the nonparametric Kruskal-Wallis test due to the non-normal distribution of data and/or differences in the number of samples in each site. Nitrate and ammonium concentrations in stream water and the mean $\delta^{15}N$ values of periphyton and *Hypostomus* were plotted together at RV sampling sites to evaluate whether changes in nitrogen sources were reflected in the primary producers and primary consumers.

To evaluate if the $\delta^{15}N$ of periphyton can be used to monitor nitrogen sources, we tested the relationship between the $\delta^{15}N$ values of periphyton and *Hypostomus* samples and nitrate and ammonium concentrations. To evaluate if organisms that fed directly from periphyton can be used as an alternative to trace the presence of pollutants, we tested the bivariate relationship of $\delta^{15}N$ of *Hypostomus* and nitrate and ammonium concentrations using a non-parametric correlation (Spearman ρ). The relationship between the $\delta^{15}N$ values of periphyton and *Hypostomus* samples were tested for each sampling season using a linear regression.

We also calculated the $\Delta^{15}N$ (the nitrogen enrichment of each fish relative to the periphyton baseline) as the $\delta^{15}N$ of each specimen of *Hypostomus* to the mean $\delta^{15}N$ of periphyton in each site and sampling. Therefore, if *Hypostomus* specimens feed directly from periphyton, it would likely have a $\Delta^{15}N$ ranging between 2.2 - 4.8 ‰ corresponding to an enrichment of one trophic level to aquatic consumers that feed on plant and algal diets (McCutchan et al., 2003, Mill et al., 2007). Therefore, a $\Delta^{15}N$ considerably lower than 2.2‰ and higher 5‰, probably indicates that *Hypostomus* specimens are feeding from other depleted or enriched resources, respectively.

3 Results

Unpolluted sites (T2, T3, T5, T6, T7, T8, MS1 and MS2) exhibited low nitrate (<2 mg/L) and ammonium (<1 mg/L) concentrations at all samplings. The highest nitrate concentrations were observed at sites T4, MS5, MS6, MS7, and MS8, with values ranging between 2.02 to 8.01 mg/L in the dry season. In the wet season, the nitrate concentrations decreased at these sites, with values ranging between 0.62 to 5.36 mg/L. The sites MS3, MS4 and T1 had the highest ammonium concentrations (> 6 mg/L), but also exhibited high variance among the three samplings (Table 2).

The $\delta^{15}N$ of periphyton and *Hypostomus* catfish showed striking differences, depending on location in the basin and season (Table 3, Figure A1). Periphyton $\delta^{15}N$ displayed high variation among sites and between seasons (Table 3, Figure A1), with a strong gradient of ^{15}N enrichment along the RV, especially during the dry season (Figure 2). The most depleted $\delta^{15}N$ values (-5 to 0‰) were observed in periphyton from MS4 in the first dry season and T1 in the wet season, while the most enriched $\delta^{15}N$ values (25 to 30‰) were observed in periphyton samples from MS5 and T4 sampled during the first dry season (Table 3, Figure 2).

The $\delta^{15}N$ of *Hypostomus* also varied among sample sites (Table 3; Figure A1). The most depleted value (4.97‰) was observed at T1 in the wet season, while the most enriched $\delta^{15}N$ value (36.20‰) was observed at T4 in the dry season. *Hypostomus* species presented similar $\delta^{15}N$ value within sites (Table 3), with the exception of *H. commersoni* and *H. macrops* from MS3 (in the second dry season) and MS4 (in the wet season). In both cases, *H. macrops* (MS3 = 20.32‰; MS4 = 25.32‰) was more enriched than *H. commersoni* (MS3 = 5.85‰; MS4 = 17.97‰). *Hypostomus commersoni* also exhibited high individual $\delta^{15}N$ variance at MS4 in the wet (±8.48‰) and dry (±5.10‰) seasons (Table 3).

The $\delta^{15}N$ of periphyton and *Hypostomus* (Table 4) were positively correlated with the nitrate concentrations (i.e., enriched in $\delta^{15}N$ at sites with high nitrate concentrations – see Figure 3) and negatively correlated with ammonium concentrations (i.e., depleted in $\delta^{15}N$ in sites with high ammonium concentrations - see Figure 3) in almost all samplings. It was also observed a high positive relationship between the $\delta^{15}N$ of periphyton and $\delta^{15}N$ of *Hypostomus* (Figure 4), especially in the first dry season (Figure 4a). In the wet season, some species (such as *H. lima, H. commersoni* and *H. macrops*) were comparatively more enriched than the periphyton (Figure 4b).

In the first sampling (dry season), the enrichment of primary consumers compared to primary producers ($\Delta^{15}N$) ranged between 0.08‰ (site MS5) and 6.51‰ (site T4) (Figure 3, Table 5). During the wet season, the $\Delta^{15}N$ was higher than in both dry seasons, ranging between 1.71‰ (site T6) to 17.40‰ (site T4). The highest $\Delta^{15}N$ was observed in sites with high nitrate concentrations (T4, MS4, MS5, MS6, MS7 and MS8) (Figure 3, Table 5). At the third sampling (dry season), the enrichment of primary consumers relative to primary producers decreased, and the highest $\Delta^{15}N$ was observed in the sites with high ammonium concentrations, MS3 (7.40‰) and MS4 (10.92‰) (Figure 3, Table 5).

4 Discussion

Both periphyton and *Hypostomus* seem to be effective indicators of the intensity of anthropogenic nitrogen loads due to their $\delta^{15}N$ variability among sites with different nitrate and ammonium concentrations. The hypothesis that periphyton $\delta^{15}N$ would be correlated with nitrate/ammonium concentrations was supported, as well as the hypothesis that *Hypostomus* and periphyton $\delta^{15}N$ would be correlated. The hypothesis that changes in nitrate/ammonium concentrations between dry and wet seasons would be reflected $\delta^{15}N$ of periphyton and *Hypostomus* was partially supported, since periphyton seems to be more sensitive to changes in nitrogen concentrations between seasons than the studied genus of armored catfishes. *Hypostomus* species presented consistent $\delta^{15}N$ values between seasons and did not vary with changes in nitrogen concentrations. We also observed that nitrate increased $\delta^{15}N$ enrichment of periphyton, while ammonium tended to increase $\delta^{15}N$ depletion, which was, consequently, reflected in $\delta^{15}N$ of primary consumers.

The $\delta^{15}N$ of periphyton is highly influenced by dissolved inorganic nitrogen (DIN) concentrations (Finlay and Kendall, 2007), since periphyton is primarily autotrophic organisms (e.g., algae) and heterotrophic bacteria that assimilate DIN. Usually, the $\delta^{15}N$ of periphyton ranges between −15 to +20‰, with $\delta^{15}N$ values ranging between −1 to +7‰ in unpolluted sites (Finlay and Kendall, 2007). The $\delta^{15}N$ of periphyton in the most pristine site (T6) ranged between 3.7‰ (dry season) to 5.1‰ (wet season), values that we could consider as references for the RV basin. Similar $\delta^{15}N$ values were also observed in other unpolluted sites (MS1, MS2, T2, T3, T5, T7 and T8) (Figure 2, Table 3). Extreme $\delta^{15}N$ values usually indicate the influence of anthropogenic activities (Dailer et al., 2010). In the RV basin, periphyton and *Hypostomus* displayed extreme values at sites with high nitrogen input with enriched $\delta^{15}N$ values related to high nitrate concentrations and depleted values at sites with high ammonium concentrations.

The sites with high ammonium concentrations and depleted $\delta^{15}N$ values were those within the MRBH (T1, MS3 and MS4) and highly influenced by urban and industrial waste water. Wang et al., (2016) also observed a depletion in $\delta^{15}N$ values of algal tissues in areas under sewage influence and with high ammonium concentrations. In their study, the wastewater from the waste water treatment plants (WWTP) includes treated sewage, agriculture and industrial water. Therefore, the negative $\delta^{15}N$ values were attributed to dominant nitrogen loadings from fertilizer usage in agriculture or industrial wastewater, instead of sewage (Wang et al., 2016), since inorganic fertilizers are produced by atmospheric N_2 fixation, presenting a depleted $\delta^{15}N$ of 0±3‰ (Kendall, 1998). This hypothesis seems to apply especially to T1, which is located at an industrial area. Although industrial wastewater may also explain the depleted $\delta^{15}N$ values observed in MS3 and MS4, sites downstream from WWTP may also exhibit depleted $\delta^{15}N$ values due to the $\delta^{15}N$ signature of ammonia as a consequence of a limited nitrification occurring during sewage treatment (Loomer et al., 2015). This hypothesis seems to apply to sites MS3 and MS4, as the $\delta^{15}N$ of suspended matter from two MWWTP located upstream from these sites exhibited depleted $\delta^{15}N$ (1.50 ± 0.45‰) (de Carvalho et al., 2019).

Sites with high nitrate concentrations and enriched $\delta^{15}N$ values, in turn, look to be influenced by urban sewage and cattle farming. For example, T4 receives large amounts of sewage from the city of Sete Lagoas (more than 200,000 inhabitants), which treats only 15% of the collected sewage, and probably contributed to the enriched $\delta^{15}N$ values in primary producers and consumers (Costanzo et al., 2001; Riera et al., 2000; Wang et al., 2016). MS5, MS6, MS7 and MS8 are further from MRBH, but nitrogen carried by RV likely reaches these sites, which could exhibit high $\delta^{15}N$ values due to denitrification (Kendall, 1998; Nikolenko et al., 2018), since sewage of most cities of RV basin is treated only at a secondary level (i.e., do not remove nutrients / contaminants such as nitrogen compounds). These sites are also located in a region where cattle production is between the main land uses, which also can contribute to nitrogen loads and $\delta^{15}N$ enrichment (Dailer et al., 2010). Animal manure and sewage are enriched in ^{15}N due to ammonia volatilization during storage, with enrichment of ^{15}N in residual NH_4^+ (converted into ^{15}N-enriched NO_3^-) (Nikolenko et al., 2018). Consequently, enrichment of $\delta^{15}N$- NO_3^- in manure and sewage (ranging between +5‰ and +35‰) and $\delta^{15}N$- NH_4^+ (ranging between +5‰ and +11‰) is reflected in the primary producers and consumers (Nikolenko et al., 2018). Therefore, although the variance in $\delta^{15}N$ values of periphyton and fish can indicate high nitrogen pollution, identifying nitrogen sources may be complicated where multiple sources are present with distinct signatures and biogeochemical processes that alter isotopic composition (Kendall, 1998).

Sites with high nitrate (MS5, MS6, MS7, MS8 and T4) and ammonium (MS3, MS4 and T4) also displayed high $\Delta^{15}N$, especially in the wet season. An enrichment of approximately 2.2 ± 0.30‰ per trophic level is expected for consumers that feed on plant and algal diets (McCutchan et al., 2003). However, values as high as 4.78 ± 1.3‰ and between 4.2 and 5.7‰ have also been observed in herbivorous fish (Mill et al., 2007) and in muscle tissues of a detritivorous fish (Sacramento et al., 2016), respectively. In the most pristine site (T6), as well as in other unpolluted sites (T2, T3, T5, T7, T8, MS1 and MS2), the $\Delta^{15}N$ values are close to those expected for one trophic level of enrichment. In opposite, at the polluted site T4, $\Delta^{15}N$ was 17.04‰, which could be interpreted as 3-5 trophic levels between periphyton and *Hypostomus* (considering a trophic enrichment between 2.2 and 5.7‰). However, given the natural history of this fish this seems unlikely and the most likely hypothesis is that at polluted sites *Hypostomus* feeds on other resources that are more enriched in $\delta^{15}N$ than periphyton, and do not necessarily indicate a change in trophic position. This hypothesis is reinforced by the fact that highest $\Delta^{15}N$ were observed during the wet seasons, when the intense flow rates and the flood pulse can carry different food sources to aquatic environments (Junk et al., 1989; Winemiller, 2004).

In this study, some *Hypostomus* species showed differences in $\delta^{15}N$ at the same site, such as *H. commersoni* and *H. macrops* at MS3 and MS4. Intrinsic differences in physiology or the consumption of alternative food resources with different $\delta^{15}N$ may explain this variation in consumer enrichment. At both sites, *H. macrops* was more enriched than *H. commersoni* perhaps suggesting the offset is consistent between these two species. *Hypostomus commersoni* also displayed high individual $\delta^{15}N$ variance at MS4 during the wet and dry seasons (Table 3). The time it takes for the isotope to be assimilated and reflected in tissues of primary consumers (turnover rates) can be the main factor influencing the variance in $\delta^{15}N$ of *H. commersoni*. Tropical catfishes, although exhibiting high tissue turnover rates compared to temperate fishes, can take weeks to change their isotopic composition (Mcintyre and Flecker, 2006). Furthermore, turnover rates may vary among individuals of a same species according to body size and life stages (Mcintyre and Flecker, 2006). At MS4 in both seasons, individuals of *H. commersoni* with lower $\delta^{15}N$ were the larger individuals (wet season: one individual with 97.2g and $\delta^{15}N$ = 8.18‰; dry season: individuals with 150.8g and $\delta^{15}N$ = 9.84‰, 152.7g and $\delta^{15}N$ = 9.48‰ and 170.1g and $\delta^{15}N$ = 8.95‰). While smaller individuals were enriched in $\delta^{15}N$ for both seasons (wet season: individuals with 45.86g and $\delta^{15}N$ = 22.39‰ and 31.58g and $\delta^{15}N$ = 23.34‰; dry season: one individual with 38.76g and $\delta^{15}N$ = 19.61‰). As nitrogen turnover in catfish muscle decrease with size (Mcintyre and Flecker, 2006), probably the smaller fishes are better predictors of actual nitrogen concentrations than larger individuals. Therefore, differences in size can explain high $\delta^{15}N$ variance in *H. commersoni*, also indicating the need for caution in the use of primary consumers as indicators, as different metabolism rates can influence the results.

We conclude that $\delta^{15}N$ values of periphyton and *Hypostomus* reflect different N concentrations and suggest different N sources in a basin with diverse anthropogenic activities (industrial and urban waste water, agriculture, and pastures). However, values of periphyton and *Hypostomus* could only be used as indicators of the intensity of anthropogenic nitrogen loading on aquatic systems, but not as tracers of individual nitrogen sources. This result is consistent with Anderson and Cabana (2006), that observed significant relationships between aquatic primary consumer $\delta^{15}N$ and nitrogen loads, but were not able to distinguish unambiguously individual sources of nitrogen. Nevertheless, our results help provide a better understanding of the system than water quality measurements alone can offer, since they integrate different organisms (i.e., periphyton and fish) as bioindicators from a system. In the Ribeirão Jequitibá (site T4), for example, the $\delta^{15}N$ of periphyton and *Hypostomus* provided additional information about the environmental conditions of this tributary. T4 is constantly classified as intermediate water quality in monitoring programs of the RV basin (IGAM - Instituto Mineiro de Gestão das águas, 2017, 2013; Trindade et al., 2016), but showed a sharp $\delta^{15}N$ enrichment in periphyton (dry: 29.69‰ and wet:16.96‰ seasons) and catfish (dry: 36.20‰ and wet: 34.37‰ seasons), thereby suggesting high anthropogenic N inputs and poor water condition (contrasting with the water quality results). This finding highlights the need of additional studies to assess which types of anthropogenic activities influence the $\delta^{15}N$ of primary producers and consumers of Ribeirão Jequitibá (site T4).

We also conclude that periphyton seems to be a better indicator of nitrogen pollution along RV basin, since it varies according to nitrate/ammonium concentrations between seasons. The use of aquatic consumers as indicators likely need to be used with caution because seasonal shifts in the isotopic ratios of food sources may cause time lags between assimilation of N in consumers, resulting in disparate consumer $\delta^{15}N$ values relative to their food sources. Despite such limitations, *Hypostomus*

δ^{15}N can also be used as a proxy/indicator of pollution, especially during the dry season if considering individuals of similar size and metabolism. Due to their ability to survive in polluted areas, the genus *Hypostomus* is a good candidate for programs aiming to follow the effectiveness of recovery efforts. Some characteristics of *Hypostomus* species make them potential bioindicators, such as their resident habits, wide distribution, and high species diversity in freshwater systems. In addition, the diets of primary consumers integrate temporal and spatial variability in the isotopic compositions of resources, which can be advantageous in environmental monitoring studies.

Acknowledgments

We thank the Agência Peixe Vivo and the Comitê de Bacia Hidrográfica do Rio das Velhas (CBH - Rio das Velhas) for the project financial support and the Projeto Manuelzão for the logistical support. This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance code 32004010017P3 and D.R.C. sandwich doctorate scholarship granted (88881.190508/2018-01). Thanks to Aline Junqueira Grossi (Federal University of Lavras, UFLA) and Luiza Hoehne (Federal University of Minas Gerais, UFMG) for the support on processing of samples. Thanks also to the Benthos Ecology Laboratory (UFMG) and the Laboratory of Fish Ecology (UFLA) who assisted in the processing of samples and infrastructure, and to the Centre for Nuclear Energy in Agriculture (CENA) for their support and partnership in the isotopic analysis. P.S.P. received a research grant and a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (303548/2017-7) and from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (PPM-00237/13).

References

Alves, C.B.M., Pompeu, P. dos S., 2010. A fauna de peixes da bacia do rio das Velhas no final do século XX, in: Alves, C.B.M., Pompeu, P.S. (Eds.), Peixes Do Rio Das Velhas: Passado e Presente. ARGVMENTVM, Belo Horizonte, pp. 167–189.

Alves, C.B.M., Pompeu, P.S., 2005. Historical Changes in the Rio das Velhas Fish Fauna—Brazil. American Fisheries Society Symposium 45, 587–602.

Anderson, C., Cabana, G., 2006. Does δ15N in river food webs reflect the intensity and origin of N loads from the watershed? Science of The Total Environment 367, 968–978. https://doi.org/10.1016/j.scitotenv.2006.01.029

Anornu, G., Gibrilla, A., Adomako, D., 2017. Tracking nitrate sources in groundwater and associated health risk for rural communities in the White Volta River basin of Ghana using isotopic approach (δ15N, δ18O-NO3 and 3H). Science of the Total Environment 603–604, 687–698. https://doi.org/10.1016/j.scitotenv.2017.01.219

Barrie, A., Prosser, S.J., 1996. Mass Spectrometry of Soils, in: Mass Spectrometry of Soils. Marcel

Dekker, Inc., p. 536.

Burdon, F.J., Reyes, M., Alder, A.C., Joss, A., Ort, C., Räsänen, K., Jokela, J., Eggen, R.I.L., Stamm, C., 2016. Environmental context and magnitude of disturbance influence trait-mediated community responses to wastewater in streams. Ecology and Evolution 6, 3923–3939. https://doi.org/10.1002/ece3.2165

Canobbio, S., Mezzanotte, V., Sanfilippo, U., Benvenuto, F., 2009. Effect of Multiple Stressors on Water Quality and Macroinvertebrate Assemblages in an Effluent-Dominated Stream. Water, Air, and Soil Pollution 198, 359–371. https://doi.org/10.1007/s11270-008-9851-4

Capone, D.G., Bronk, D.A., Mulholland, M.R., Carpenter, E.J., 2008. Nitrogen in the marine environment, 2nd ed. Elsevier.

Cardoso, Y.P., Almirón, A., Casciotta, J., Aichino, D., Lizarralde, M.S., Montoya-Burgos, J.I., 2012. Origin of species diversity in the catfish genus Hypostomus (Siluriformes: Loricariidae) inhabiting the Paraná river basin, with the description of a new species. Zootaxa 69–83.

CBH Velhas, 2019. A bacia hidrográfica do Rio das Velhas [WWW Document]. Comite da bacia hidrográfica do Rio das Velhas. URL http://cbhvelhas.org.br/a-bacia-hidrografica-do-rio-das-velhas/ (accessed 6.3.19).

Cifuentes, L.A., Sharp, J.H., Fogel, M.L., 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. Limnology and Oceanography 33, 1102–1115.

Cohen, R.A., Fong, P., 2005. Experimental evidence supports the use of δ15N content of the opportunistic green macroalga Enteromorpha intestinalis (Chlorophyta) to determine nitrogen sources to estuaries. Journal of Phycology 41, 287–293. https://doi.org/10.1111/j.1529-8817.2005.04022.x

Cohen, R.A., Fong, P., 2004. Nitrogen uptake and assimilation in Enteromorpha intestinalis (L.) Link (Chlorophyta): using 15N to determine preference during simultaneous pulses of nitrate and ammonium. Journal of Experimental Marine Biology and Ecology 309, 67–77. https://doi.org/10.1016/j.jembe.2004.03.009

Compton, J.E., Harrison, J.A., Dennis, R.L., Greaver, T.L., Hill, B.H., Jordan, S.J., Walker, H., Campbell, H. V., 2011. Ecosystem services altered by human changes in the nitrogen cycle: a new perspective for US decision making. Ecology Letters 14, 804–815. https://doi.org/10.1111/j.1461-0248.2011.01631.x

Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., Loneragan, N.R., Thomas, M., 2001. A New Approach for Detecting and Mapping Sewage Impacts. Marine Pollution Bulletin 42, 149–156. https://doi.org/10.1016/S0025-326X(00)00125-9

Dailer, M.L., Knox, R.S., Smith, J.E., Napier, M., Smith, C.M., 2010. Using δ15N values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawaiʻi, USA. Marine Pollution Bulletin 60, 655–671. https://doi.org/10.1016/j.marpolbul.2009.12.021

de Carvalho, D.R., Flecker, A.S., Alves, C.B.M., Sparks, J.P., Pompeu, P.S., 2019. Trophic responses to aquatic pollution of native and exotic livebearer fishes. Science of The Total Environment 681, 503–515. https://doi.org/10.1016/j.scitotenv.2019.05.092

Feio, M.J., Ferreira, W.R., Macedo, D.R., Eller, A.P., Alves, C.B.M., França, J.S., Callisto, M., 2015. Defining and Testing Targets for the Recovery of Tropical Streams Based on Macroinvertebrate Communities and Abiotic Conditions. River Research and Applications 31, 70–84. https://doi.org/10.1002/rra.2716

Fenech, C., Rock, L., Nolan, K., Tobin, J., Morrissey, A., 2012. The potential for a suite of isotope and chemical markers to differentiate sources of nitrate contamination : A review. Water Research 46, 2023–2041. https://doi.org/10.1016/j.watres.2012.01.044

Finlay, J.C., Kendall, C., 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, in: Finlay, J.C., Kendall, C. (Eds.), Stable Isotope Tracing of Organic Matter Sources and Food Web Interactions in Watersheds. pp. 283–333.

Gaiser, E.E., Childers, D.L., Jones, R.D., Richards, J.H., Scinto, L.J., Trexler, J.C., 2006. Periphyton responses to eutrophication in the Florida Everglades: Cross-system patterns of structural and compositional change. Limnology and Oceanography 51, 617–630. https://doi.org/10.4319/lo.2006.51.1_part_2.0617

Garavello, J.C., Garavello, J.P., 2004. Spatial distribution and interaction of four species of the catfish genus Hypostomus Lacépède with bottom of Rio São Francisco, Canindé do São Francisco, Sergipe, Brazil (Pisces, Loricariidae, Hypostominae). Brazilian Journal of Biology 64, 103–141. https://doi.org/10.1590/S1519-69842004000400006

Haseena, M., Faheem Malik, M., Javed, A., Arshad, S., Asif, N., Zulfiqar, S., Hanif, J., 2017. Water pollution and human health. Environmental Risk Assessment and Remediation 01, 289–297. https://doi.org/10.4066/2529-8046.100020

IBGE, 2018. Estimativas de população [WWW Document]. Instituto Brasileiro de Geografia e Estatística. URL https://www.ibge.gov.br/estatisticas-novoportal/sociais/populacao/9103-estimativas-de-populacao.html?=&t=resultados (accessed 2.26.19).

IBGE, 2010. Indicadores de desenvolvimento sustentável, Brasil 2010.

IGAM - Instituto Mineiro de Gestão das águas, 2017. Monitoramento de Qualidade das Águas [WWW

Document]. Monitoramento de Qualidade das Águas. URL http://portalinfohidro.igam.mg.gov.br/monitoramento-de-qualidade-das-aguas (accessed 1.16.19).

IGAM - Instituto Mineiro de Gestão das águas, 2013. Identificação de municípios com condição crítica para a qualidade de água na bacia do rio das Velhas 49.

Jones, L., Provins, A., Holland, M., Mills, G., Hayes, F., Emmett, B., Hall, J., Sheppard, L., Smith, R., Sutton, M., Hicks, K., Ashmore, M., Haines-young, R., Harper-simmonds, L., 2014. A review and application of the evidence for nitrogen impacts on ecosystem services. Ecosystem Services 7, 76–88.

Junk, W.J., Bayley, P.B., Sparks, R.E., 1989. The flood pulse concept in river-floodplain systems, in: Dodge, D.P. (Ed.), Proceedings of the International Large River Symposium (LARS). Canadian Special Publication of Fisheries and Aquatic Sciences, pp. 110–127.

Kendall, C., 1998. Tracing Nitrogen Sources and Cycling in Catchments, Isotope Tracers in Catchment Hydrology. Elsevier B.V. https://doi.org/10.1016/B978-0-444-81546-0.50023-9

Lake, J.L., McKinney, R.A., Osterman, F.A., Pruell, R.J., Kiddon, J., Ryba, S.A., Libby, A.D., 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. Canadian Journal of Fisheries and Aquatic Sciences 58, 870–878. https://doi.org/10.1139/cjfas-58-5-870

Loomer, H.A., Oakes, K.D., Schiff, S.L., Taylor, W.D., Servos, M.R., 2015. Use of Stable Isotopes to Trace Municipal Wastewater Effluents into Food Webs within a Highly Developed River System. River Research and Applications 31, 1093–1100. https://doi.org/10.1002/rra.2826

Machado, A.T.M., Alves, C.B.M., Callisto, M., 2008. Projeto Manuelzão: metodologia e resultados, in: Lisboa, A.H., Goulart;, E.M.A., Diniz, L.F.M. (Eds.), Projeto Manuelzão. A História Da Mobilização Que Começou Em Torno de Um Rio. Instituto Guaicuy - SOS Rio das Velhas, Belo Horizonte, pp. 39–54.

Mara, D., 2013. Domestic Wastewater Treatment in Developing Countries, Climate Change 2013 - The Physical Science Basis. Routledge, Cambridge. https://doi.org/10.4324/9781849771023

Matiatos, I., 2016. Nitrate source identification in groundwater of multiple land-use areas by combining isotopes and multivariate statistical analysis : A case study of Asopos basin (Central Greece). Science of the Total Environment 541, 802–814. https://doi.org/10.1016/j.scitotenv.2015.09.134

McCutchan, J.H., Lewis, W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102, 378–390.

https://doi.org/10.1034/j.1600-0706.2003.12098.x

Mcintyre, P.B., Flecker, Æ.A.S., 2006. Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters 12–21. https://doi.org/10.1007/s00442-005-0354-3

Mill, A.C., Pinnegar, J.K., Polunin, N.V.C., 2007. Explaining isotope trophic-step fractionation: why herbivorous fish are different. Functional Ecology 21, 1137–1145. https://doi.org/10.1111/j.1365-2435.2007.01330.x

Nikolenko, O., Jurado, A., Borges, A. V, 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.2017.10.086

Pompeu, P.S., Alves, C.B.M., Callisto, M., 2005. The Effects of Urbanization on Biodiversity and Water Quality in the Rio das Velhas Basin , Brazil. American Fisheries Society Symposium 47, 11–22.

Power, M.E., 1990. Resource Enhancement by Indirect Effects of Grazers: Armored Catfish, Algae, and Sediment. Ecology 71, 897–904. https://doi.org/10.2307/1937361

Power, M.E., Dudley, T.L., Cooper, S.D., 1989. Grazing catfish, fishing birds, and attached algae in a Panamanian stream. Environmental Biology of Fishes 26, 285–294. https://doi.org/10.1007/BF00002465

Riera, P., Stal, L.J., Nieuwenhuize, J., 2000. Heavy δ15N in Intertidal Benthic Algae and Invertebrates in the Scheldt Estuary (The Netherlands): Effect of River Nitrogen Inputs. Estuarine, Coastal and Shelf Science 51, 365–372. https://doi.org/10.1006/ecss.2000.0684

Robertson, G.P., Vitousek, P.M., 2009. Nitrogen in Agriculture : Balancing the Cost of an Essential Resource. Annual Review ofEnvironment and Resources 34, 97–125. https://doi.org/10.1146/annurev.environ.032108.105046

Sacramento, P.A., Manetta, G.I., Benedito, E., 2016. Diet-tissue discrimination factors (Δ 13 C and Δ 15 N) and turnover rate in somatic tissues of a neotropical detritivorous fish on C 3 and C 4 diets. Journal of Fish Biology 89, 213–219. https://doi.org/10.1111/jfb.12859

Savage, C., Elmgren, R., 2004. Macroalgal (Fucus vesiculosus) δ N Values Trace Decrease in Sewage Influence. Ecological Applications 14, 517–526.

Schaefer, S.A., Lauder, G. V., 1986. Historical Transformation of Functional Design: Evolutionary Morphology of Feeding Mechanisms in Loricarioid Catfishes. Systematic Zoology 35, 489. https://doi.org/10.2307/2413111

Schubert, P.R., Karez, R., Reusch, T.B.H., Dierking, J., 2013. Isotopic signatures of eelgrass (Zostera marina L.) as bioindicator of anthropogenic nutrient input in the western Baltic Sea. Marine Pollution Bulletin 72, 64–70. https://doi.org/10.1016/j.marpolbul.2013.04.029

SNIS, 2016. Sistema Nacional de informações sobre saneamento: Diagnóstico dos Serviços de Água e Esgotos - 2016. Brasília.

Toda, H., Uemura, Y., Okino, T., Kawanishi, T., Kawashima, H., 2002. Use of nitrogen stable isotope ratio of periphyton for monitoring nitrogen sources in a river system. Water Science and Technology 46, 431–435. https://doi.org/10.2166/wst.2002.0774

Trindade, A.L.C., Almeida, K.C. de B., Barbosa, P.E., Oliveira, S.M.A.C., 2016. Tendências temporais e espaciais da qualidade das águas superficiais da sub-bacia do Rio das Velhas, estado de Minas Gerais. Engenharia Sanitaria e Ambiental 22, 13–24. https://doi.org/10.1590/s1413-41522016131457

Val, A.L., de Almeida-Val, V.M.F., 1995. Fishes of the Amazon and Their Environment, Zoophysiology. Springer Berlin Heidelberg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-79229-8

Viers, J.H., Liptzin, D., Rosenstock, T.S., Jensen, V.B., Hollander, A.D., McNally, A., King, A.M., Kourakos, G., Lopez, E.M., Mora, N.D. La, Hung, A.F.-, Dzurella, K.N., Canada, H., Laybourne, S., McKenney, C., Darby, J., Quinn, J.F., Harter, T., 2012. Nitrogen Sources and Loading to Groundwater.

Wang, Y., Liu, D., Richard, P., Di, B., 2016. Selection of effective macroalgal species and tracing nitrogen sources on the different part of Yantai coast, China indicated by macroalgal δ15N values. Science of The Total Environment 542, 306–314. https://doi.org/10.1016/j.scitotenv.2015.10.059

Watanabe, M., Ortega, E., Bergier, I., Silva, J., 2012. Nitrogen cycle and ecosystem services in the Brazilian La Plata Basin : anthropogenic influence and climate change. Brazilian Archives of Biology and Technology 72, 691–708.

Winemiller, K.O., 2004. Floodplain River Food Webs: Generalizations and Implications for Fisheries Management, in: Welcomme, R.L., Petr, T. (Eds.), Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries. RAP Publication 2004/16, pp. 285–309.

Wu, Y., 2016. Periphyton: Functions and Application in Environmental Remediation. Elsevier.

Table 1 Geographic location and description of each site sampled in the main stem and tributaries of Rio das Velhas basin according the IGAM - Instituto Mineiro de Gestão das águas (2017c, 2017b).

	Site	Coordinates	Elevation (m)	City	Description
Main stem	MS1	23K 649606 E 7753356 N	1010	Ouro Preto	Site located in the headwater of Rio das Velhas. Good water quality and absence of toxic contaminants.
	MS2	23K 622454 E 7785916 N	729	Nova Lima	Site with small influence of MRBH. Medium water quality and medium toxic contaminants.
	MS3	23K 618796 E 7806723 N	674	Santa Luzia	Site highly influenced by the MRBH with high sewage discharge. Bad water quality and medium toxic contaminants.
	MS4	23K 616174 E 7838041 N	658	Lagoa Santa	Site highly influenced by the MRBH. High entrance of sewage. Bad water quality and medium toxic contaminants.
	MS5	23K 589298 E 7920498 N	567	Curvelo	Polluted site under influence of MRBH. Bad water quality and high toxic contaminants.
	MS6	23K 585926 E 7962502 N	552	Corinto	Polluted site. Presents a partial improvement in the water quality as the distance from MRBH increases. Medium water quality and high toxic contaminants.
	MS7	23K 547752 E 8024649 N	495	Lassance	Polluted site. Presents a partial improvement in the water quality as the distance from MRBH increases. Medium water quality and high toxic contaminants.
	MS8	23K 519793 E 8097515 N	464	Várzea da Palma	Polluted site. Presents a partial improvement in the water quality as the distance from MRBH increases. Medium water quality and high toxic contaminants.

Tributaries	T1	23K 605473 E 7823991 N	684	Pedro Leopoldo	Polluted tributary. Receives a large amount of sewage, both from nearby cities and from several industries in the region (especially cement industries). Bad water quality and high toxic contaminants.
	T2	23K 632551 E 7832196 N	677	Taquaraçu de Minas	Tributary in good conditions of conservation. Good water quality and low toxic contaminants.
	T3	23K 614931 E 7847433 N	651	Jaboticatubas	Tributary in good conditions of conservation. Good water quality and low toxic contaminants.
	T4	23K 599696 E 7871820 N	621	Jequitibá	Polluted tributary. Receives untreated sewage from a big city (Sete Lagoas). Medium water quality and medium toxic contaminants.
	T5	23K 578454 E 7893841 N	632	Cordisburgo	Tributary in intermediate conditions of conservation. Medium water quality and low toxic contaminants.
	T6	23K 641315 E 7861707 N	757	Santana do Riacho	The most pristine tributary due to the location of its headwaters in the Serra do Cipó National Park. Presents good water quality and absence of toxic contaminants. Reference area in the basin.
	T7	23K 605789 E 7933699 N	567	Presidente Juscelino	Tributary in intermediate conditions of conservation. Medium water quality and low toxic contaminants.
	T8	23K 586829 E 8010438 N	518	Augusto de Lima	Tributary in intermediate conditions of conservation. Medium water quality and low toxic contaminants.

Table 2 Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations (mg/L) at 16 sites sampled in the Rio das Velhas basin during three sampling periods in the years 2015 and 2016.

Sites		1 – Dry (2015)		2 – Wet (2015 tributaries and 2016 main stem)		3 – Dry (2016)	
		NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
Main stem	MS1	0.88	<0.1	0.18	<0.1	0.13	<0.1
	MS2	1.05	0.34	0.62	0.21	0.99	0.29
	MS3	1.36	9.8	1.16	1.75	2.38	11.8
	MS4	0.59	0.53	0.71	0.75	0.94	12.0
	MS5	5.33	1.75	2.06	<0.1	5.24	3.22
	MS6	4.52	<0.1	1.75	<0.1	8.01	<0.1
	MS7	2.7	<0.1	0.68	<0.1	4.17	<0.1
	MS8	2.02	<0.1	0.62	0.1	4.16	<0.1
Tributaries	T1	0.82	1.24	0.47	6.56	-	-
	T2	0.27	<0.1	0.17	0.77	-	-
	T3	0.57	0.16	0.22	0.11	-	-
	T4	7.48	0.11	5.36	<0.1	-	-
	T5	0.5	0.43	0.47	0.14	-	-
	T6	0.18	0.18	<0.1	0.13	-	-
	T7	0.16	<0.1	0.24	0.2	-	-
	T8	0.25	<0.1	0.27	0.11	-	-

Table 3 Number of samples (N) and mean and standard deviation of $\delta^{15}N$ values of periphyton and *Hypostomus* species sampled at 16 sites in the Rio das Velhas Basin during dry and wet seasons. $p<0.05$ indicates a significative variance among $\delta^{15}N$ values of different samplings (Dry 1, Wet 2 and Dry 2). Letters (a, b and c) indicate significant differences according to post-hoc test. NA= not applicable (due to the small number of samples)

Sites	Samples	**Dry 1** (2015)				**Wet 2** (2015 tributaries and 2016 main stem)				**Dry 3** (2016)				***p***
		N	**Mean**	**SD**		**N**	**Mean**	**SD**		**N**	**Mean**	**SD**		
MS1	**Mean *Hypostomus***	**0**	-	-		**0**	-	-		**0**	-	-		NA
	Periphyton	**5**	4.18	0.52	a	**5**	4.60	0.43	a	**5**	4.44	0.34	a	0.23
MS2	**Mean *Hypostomus***	**7**	10.25	0.56	a	**5**	8.27	1.59	b	**6**	8.80	0.76	b	**<0.01**
	H. francisci	0	-	-		3	8.56	1.76		0	-	-		
	H. lima	7	10.25	0.56		2	7.84	1.81		6	8.80	0.76		
	Periphyton	**5**	7.07	0.22	a	**5**	5.82	1.10	ab	**5**	5.46	1.10	b	**0.03**
MS3	**Mean *Hypostomus***	**0**	-	-		**0**	-	-		**6**	8.27	5.98		NA
	H. commersoni	0	-	-		0	-	-		5	5.85	1.09		
	H. macrops	0	-	-		0	-	-		1	20.32	-		
	Periphyton	**5**	0.12	1.68	a	**5**	5.51	0.97	b	**5**	0.87	0.51	a	**<0.01**
MS4	**Mean *Hypostomus***	**0**	-	-		**7**	22.17	6.33	a	**4**	11.97	5.10	a	0.06
	H. commersoni	0	-	-		3	17.97	8.48		4	11.97	5.10		
	H. macrops	0	-	-		4	25.32	1.12		0	-	-		
	Periphyton	**5**	-1.88	0.55	a	**5**	6.92	1.35	b	**5**	1.05	0.91	ab	**<0.01**
MS5	**Mean *Hypostomus***	**5**	28.18	1.22	a	**5**	26.50	1.76	a	**3**	26.91	0.52	a	0.25
	H. commersoni	1	26.39	0.00		1	26.75	-		0	-	-		
	H. francisci	0	-	-		3	25.50	0.97		1	27.47	-		
	H. macrops	4	28.62	0.81		1	29.23	0.00		2	26.63	0.25		
	Periphyton	**5**	28.09	1.73	a	**5**	18.28	1.80	b	**5**	23.39	0.53	ab	**<0.01**
MS6	**Mean Hypostomus**	**6**	24.71	1.21	a	**5**	24.09	0.68	a	**3**	25.00	0.63	a	0.41
	H. commersoni	1	23.59	0.00		0	-	-		0	-	-		
	H. francisci	5	24.93	1.20		5	24.09	0.68		3	25.00	0.63		
	Periphyton	**5**	21.69	1.25	a	**5**	11.62	0.87	b	**5**	19.65	1.92	ab	**<0.01**
MS7	**Mean *Hypostomus***	**6**	21.72	0.98	ab	**5**	23.01	0.76	a	**4**	18.74	1.34	b	**<0.01**
	H. francisci	3	22.26	0.63		3	23.34	0.81		0	-	-		
	H. lima	3	21.18	1.04		0	-	-		4	18.74	1.34		
	H. margaritifer	0	-	-		2	22.51	0.41		0	-	-		
	Periphyton	**5**	17.63	1.99	a	**5**	11.68	0.81	b	**5**	13.04	0.53	ab	**<0.01**
MS8	**Mean *Hypostomus***	**4**	20.80	0.58	a	**5**	21.41	2.29	a	**0**	-	-		0.14
	H. francisci	4	20.80	0.58		5	21.41	2.29		0	-	-		
	Periphyton	**5**	19.07	0.69	a	**5**	12.15	1.96	b	**5**	11.71	0.99	b	**<0.01**
T1	**Mean *Hypostomus***	**3**	7.16	0.36	a	**7**	4.97	0.77	b					**0.02**
	H. commersoni	1	6.99	0.00		7	4.97	0.77						
	H. lima	2	7.24	0.46		0	-	-						
	Periphyton	**3**	2.31	0.76	a	**5**	-1.06	0.56	b					**0.02**
T2	**Mean *Hypostomus***	**5**	11.36	0.26	a	**5**	11.75	0.52	a					0.17

	H. auroguttatus	0	-	-		5	11.75	0.52				
	H. francisci	5	11.36	0.26		0	-	-				
	Periphyton	**1**	7.60	0.00		**5**	7.71	0.69				NA
T3	**Mean *Hypostomus***	**0**	-	-		**8**	11.06	0.44				NA
	H. auroguttatus	0	-	-		3	10.60	0.23				
	H. francisci	0	-	-		5	11.34	0.25				
	Periphyton	**2**	5.37	0.11		**5**	7.52	0.66				NA
T4	**Mean *Hypostomus***	**5**	36.20	0.20	a	**4**	34.37	2.78	a			0.22
	H. lima	5	36.20	0.20		4	34.37	2.78				
	Periphyton	**4**	29.69	1.78	a	**5**	16.96	1.28	b			**0.01**
T5	**Mean *Hypostomus***	**5**	10.59	1.18	a	**5**	10.20	0.55	a			0.92
	H. francisci	4	10.65	1.36		3	9.85	0.36				
	H. lima	1	10.36	0.00		2	10.73	0.12				
	Periphyton	**2**	6.52	0.23		**5**	5.79	0.36				NA
T6	**Mean *Hypostomus***	**0**	-	-		**2**	6.76	1.47				NA
	H. francisci	0	-	-		1	7.80	-				
	H. sp.5	0	-	-		1	5.72	-				
	Periphyton	**1**	3.66	0.00		**5**	5.05	0.33				NA
T7	**Mean *Hypostomus***	**4**	9.09	0.93		**2**	9.10	0.81				NA
	H. francisci	1	9.46	0.00		2	9.10	0.81				
	H. margaritifer	3	8.97	1.11		0	-	-				
	Periphyton	**1**	6.41	0.00		**5**	5.60	0.18				NA
T8	**Mean *Hypostomus***	**5**	11.66	0.42	a	**4**	11.72	0.41	a			0.81
	H. francisci	3	11.59	0.57		1	11.25	-				
	H. macrops	0	-	-		1	11.77	-				
	H. sp.3	2	11.76	0.02		0	-	-				
	H. sp.5	0	-	-		2	11.93	0.43				
	Periphyton	**2**	7.39	0.15		**5**	6.42	0.10				NA

Table 4 Correlation between $\delta^{15}N$ of periphyton and *Hypostomus* and nitrate (NO_3^-) and ammonium (NH_4^+) concentrations in different samplings (Dry 1, Wet 2, and Dry 3)

	Samplings	**N compounds**	**N**	**Spearman R**	**t(N-2)**	***p***
$\delta^{15}N$ Periphyton	Dry 1	NO_3^-	56	0.77	8.74	<0.01
		NH_4^+	56	-0.36	-2.79	0.01
	Wet 2	NO_3^-	80	0.57	6.17	<0.01
		NH_4^+	80	-0.60	-6.56	<0.01
	Dry 3	NO_3^-	40	0.73	6.51	<0.01
		NH_4^+	40	-0.49	-3.47	<0.01
$\delta^{15}N$ *Hypostomus*	Dry 1	NO_3^-	55	0.82	10.34	<0.01
		NH_4^+	55	-0.08	-0.58	0.57
	Wet 2	NO_3^-	69	0.69	7.70	<0.01
		NH_4^+	69	-0.66	-7.16	<0.01
	Dry 3	NO_3^-	26	0.57	3.39	<0.01
		NH_4^+	26	-0.37	-1.93	0.07

Table 5 Mean enrichment between the $\delta^{15}N$ of *Hypostomus* to the mean $\delta^{15}N$ of periphyton ($\Delta^{15}N$) and standard deviation (SD) per site and samplings

Sites	1 – Dry (2015)			2 – Wet (2015 tributaries and 2016 main stem)			3 – Dry (2016)		
	Mean and SD $\Delta^{15}N$			Mean and SD $\Delta^{15}N$			Mean and SD $\Delta^{15}N$		
MS1	-		-	-		-	-		-
MS2	3.18	±	0.56	2.45	±	1.59	3.34	±	0.76
MS3	-		-	-		-	7.40	±	5.98
MS4	-		-	15.26	±	6.33	10.92	±	5.10
MS5	0.08	±	1.22	8.22	±	1.76	3.51	±	0.52
MS6	3.02	±	1.21	12.47	±	0.68	5.35	±	0.63
MS7	4.09	±	0.98	11.32	±	0.76	5.70	±	1.34
MS8	1.73	±	0.58	9.26	±	2.29	-		-
T1	4.84	±	0.36	6.03	±	0.77	-		-
T2	3.76	±	0.26	4.04	±	0.52	-		-
T3	-		-	3.54	±	0.44	-		-
T4	6.51	±	0.20	17.40	±	2.78	-		-
T5	4.07	±	1.18	4.41	±	0.55	-		-
T6	-		-	1.71	±	1.47	-		-
T7	2.68	±	0.93	3.51	±	0.81	-		-
T8	4.26	±	0.42	5.30	±	0.41	-		-

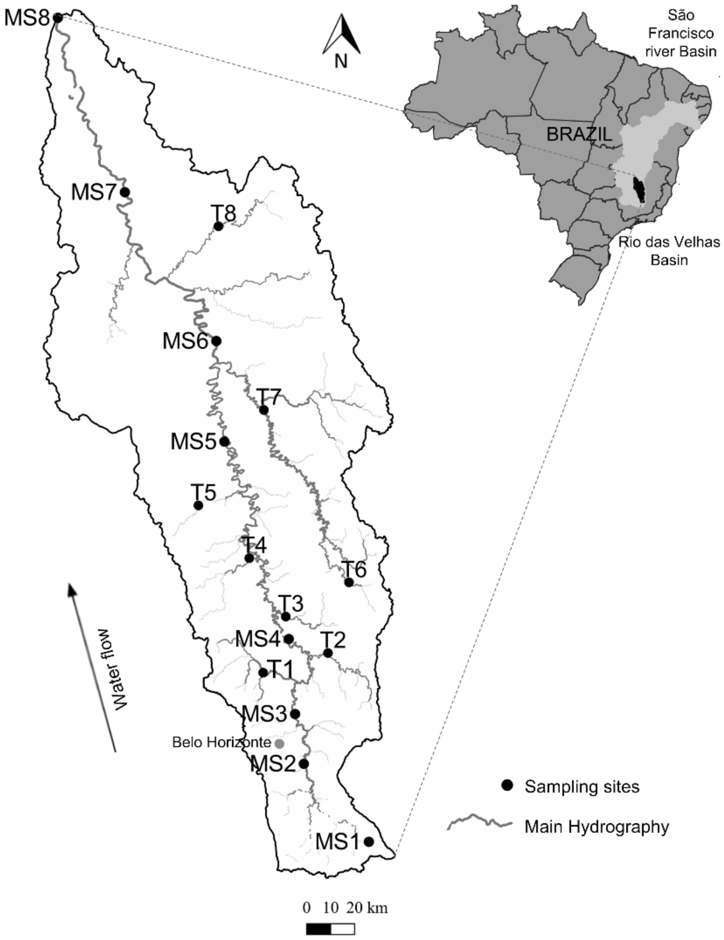

Figure 1 Sampling area in the Rio das Velhas basin, covering 8 sites on the main tributaries (T1 to T8) and 8 sites along the main stem (MS1 to MS8)

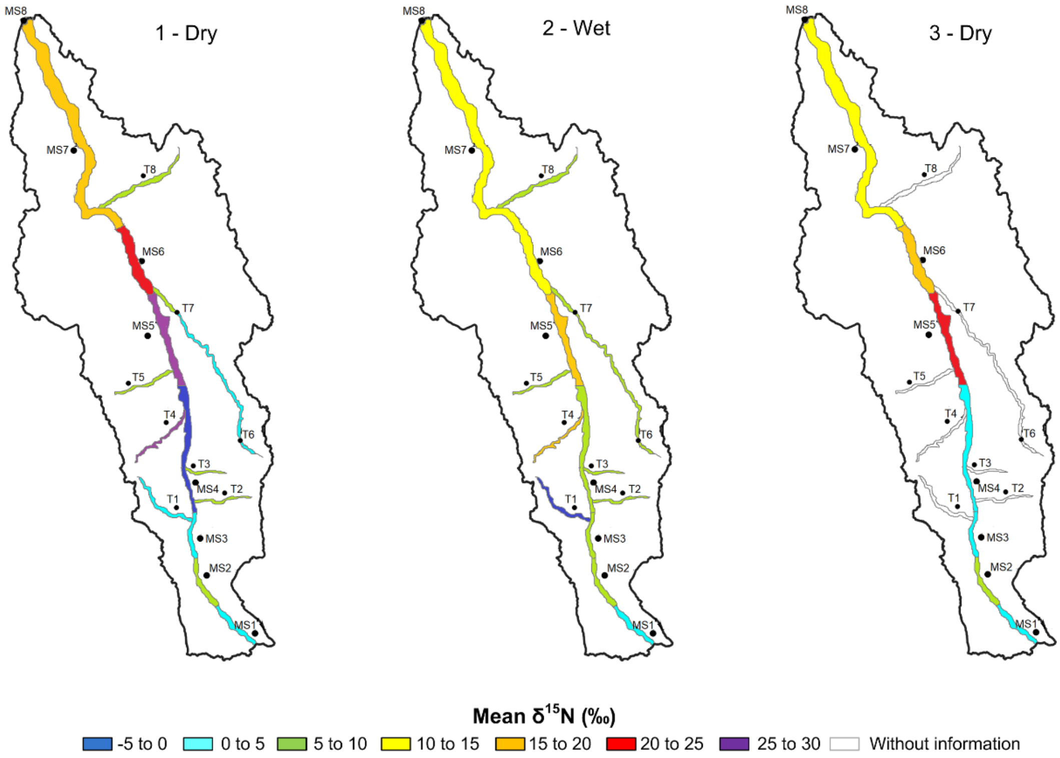

Figure 2 Schematic drawing representing a possible pattern of $\delta^{15}N$ enrichment of periphyton in the river network based on 16 sites sampled in different seasons at Rio das Velhas Basin

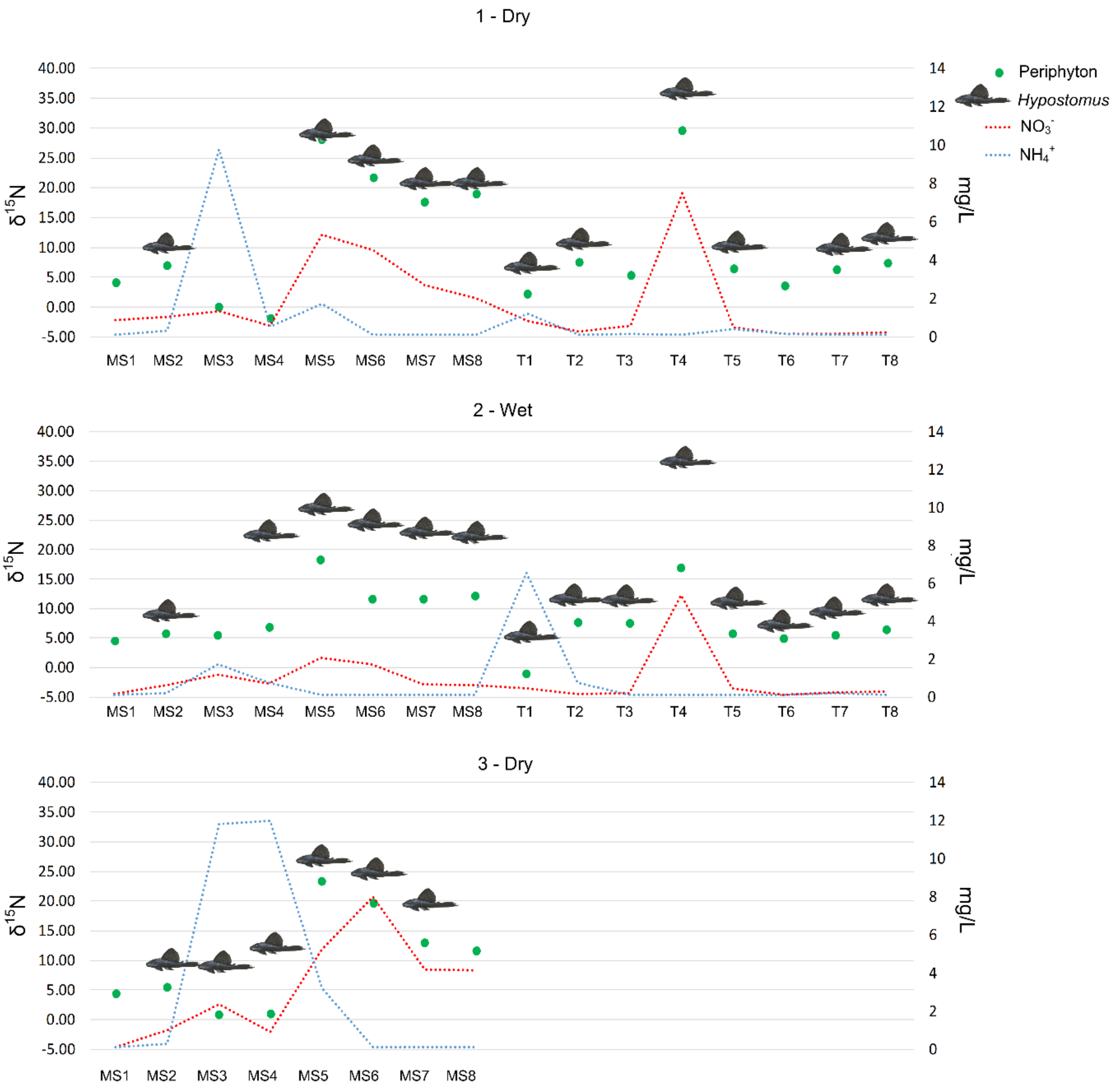

Figure 3 Nitrate (NO_3^- – red dashed line) and ammonium (NH_4^+ – blue dashed line) concentrations (mg/L) and mean $\delta^{15}N$ of periphyton and *Hypostomus* at the 16 sites sampled in the Rio das Velhas basin during 3 sampling periods

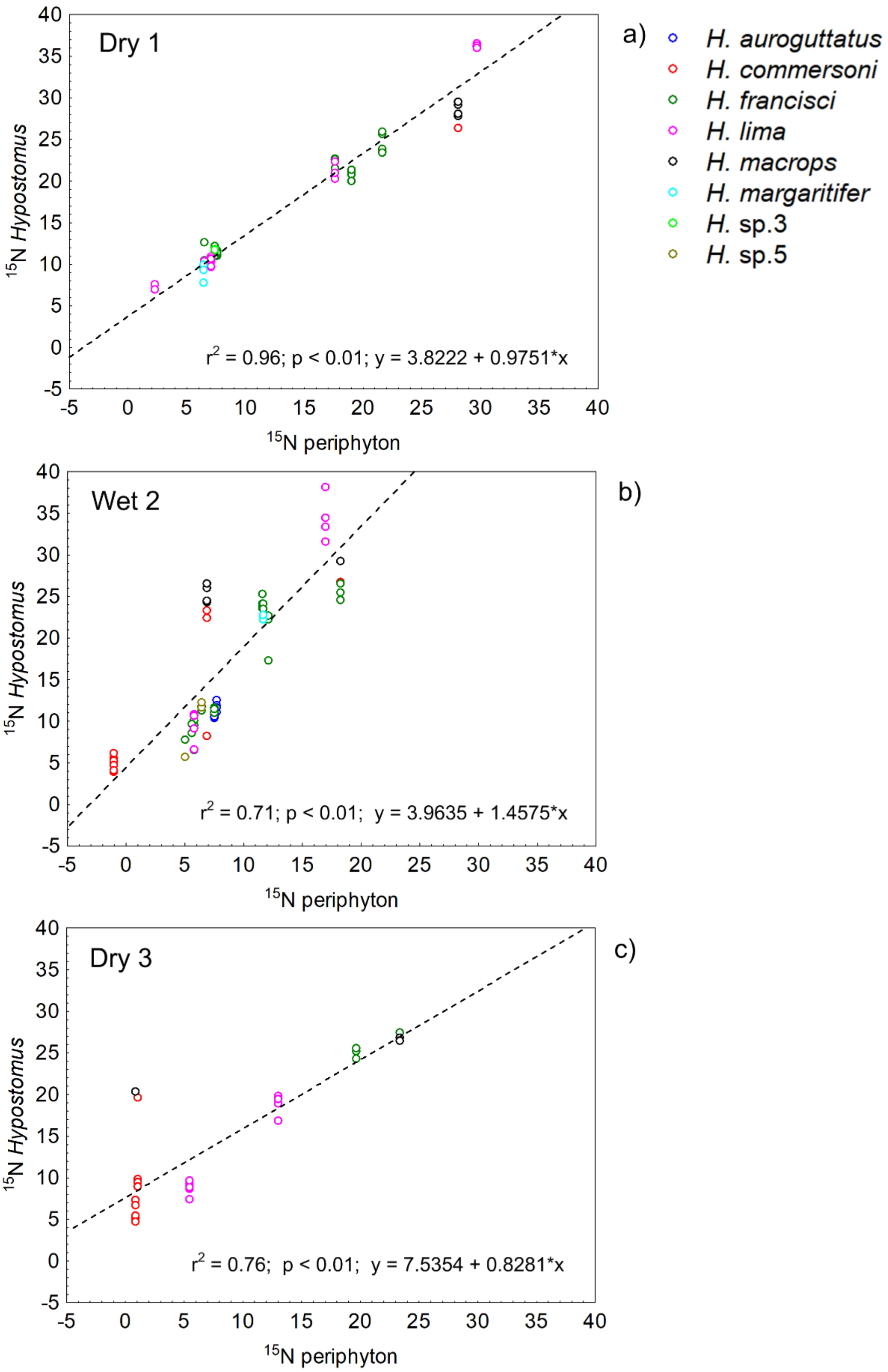

Figure 4 Correlation between δ^{15}N values of periphyton and *Hypostomus* individuals sampled at the 16 sites sampled at the Rio das Velhas Basin in Dry 1 (a), Wet 2 (b) and Dry 3 (c) samplings

Supplementary material

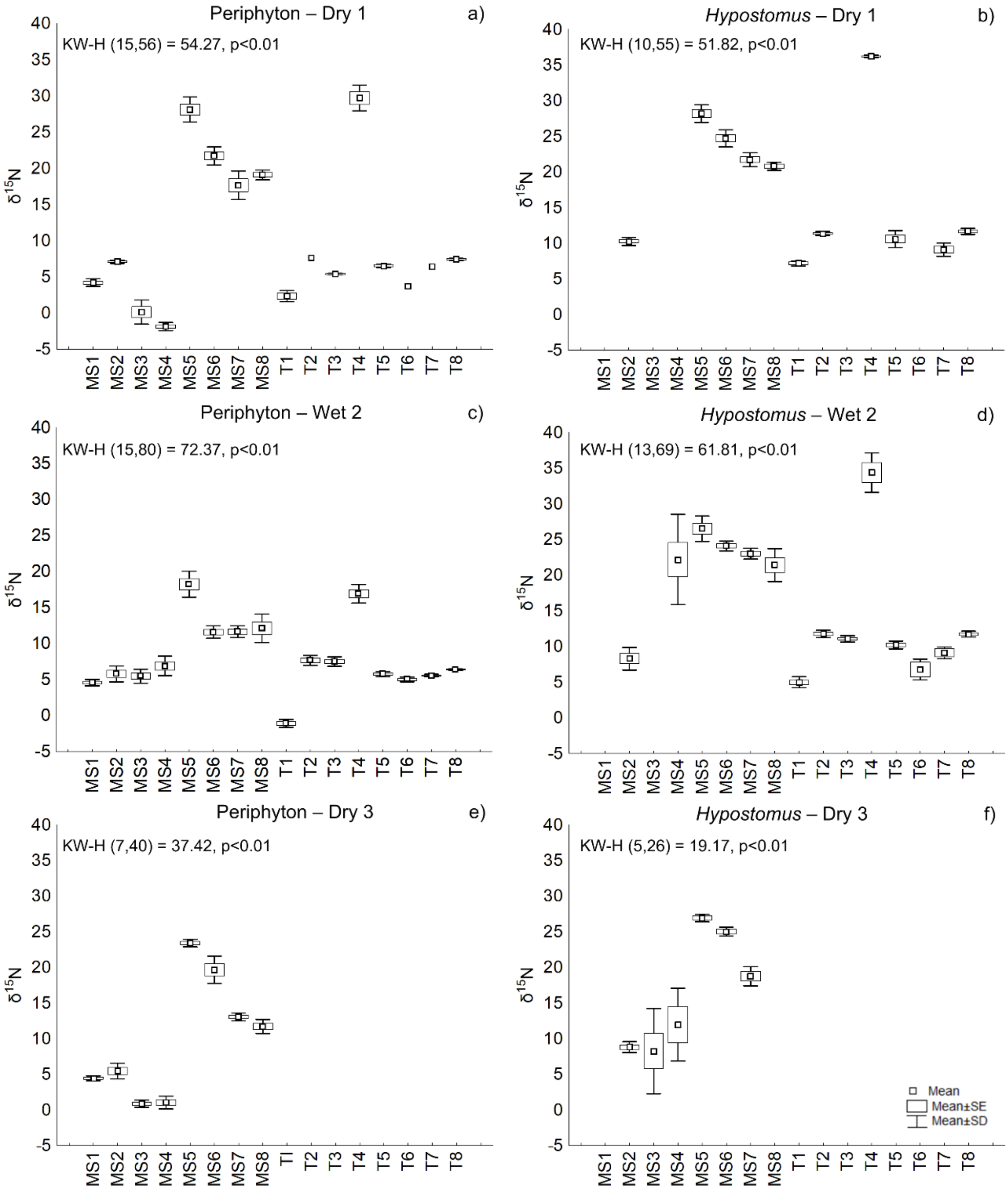

Figure A1 Variance in $\delta^{15}N$ of periphyton (a, c, e) and *Hypostomus* (b, d, f) among sampling sites and seasons - Dry 1 (a and b), Wet 2 (c and d) and Dry 3 (e and f).

ARTIGO III - ***Nitrogen pollution promotes changes in the niche space of fish communities***

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Manuscrito submetido na revista *Ecosystems*

(Manuscrito redigido na língua e formato indicado para revista - número de linhas foi retirado para melhor adequação ao formato da tese)

Abstract

Anthropogenic nitrogen has increased to benefit food production and global development. However, one implication of this increase has been to raise concentrations of nitrogen in aquatic ecosystems around the world. The objective of this study was to evaluate whether nitrogen pollution promotes changes in the niche space of fish communities. We examined 16 sites along a Brazilian river basin highly impacted by anthropogenic activities, especially discharge of domestic and industrial sewage from a region with more than five million inhabitants. We analysed the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope rations of fish species, autotrophic sources (periphyton), allochthonous sources (CPOM) and particulate organic matter (FPOM). To estimate the magnitude of nitrogen pollution, we used the nitrate and ammonium concentrations of each site. Sampling was conducted in dry and wet seasons to evaluate the influence of seasonality on niche space. Nitrogen pollution tended to increase niche space and seasonality influenced only the niche space of fish communities from polluted sites. In addition, unpolluted sites exhibited a typical trophic structure, whereas in nitrogen polluted sites isotopic analyses suggested unrealistic trophic positioning (e.g., detritivorous fishes at the top of the food web). This study suggests that changes in niche space reflect both changes in the baseline isotopic values and the trophic behaviour of fishes. Under conditions of high pollution, other factors not necessarily reflecting trophic behaviour appear to influence the isotopic estimate of niche space. For example, there are likely multiple isotopically distinct sources that have not been sampled creating a challenging isotopic baseline.

Keywords: Stable isotopes, Nitrate, Ammonium, Trophic niche, Food web, Carbon.

1 Introduction

Stable isotope analysis has become an increasingly important tool in the study of food webs. A common application is to estimate the niche space based on ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios as a representation of the total extent of trophic diversity within a food web (Layman and others 2007a). More recently, the niche space concept has also been used as a framework to examine trophic changes across anthropogenic disturbances, such as the influence of changing land use (Castro and others 2016; de Carvalho and others 2017), aquatic ecosystem fragmentation (Layman and others 2007b), resource competition between sympatric species (Alonso and others 2019), and to compare the trophic behaviour of native and exotic species (Philippsen and others 2015; de Carvalho and others 2019b). Our primary interest in this study was to evaluate if this framework is useful to access the effects of nitrogen pollution on the community scale, specifically on the food webs of fish communities.

Since the industrial revolution, the rate of anthropogenic creation of reactive nitrogen has exceeded the rate of natural processes, which has substantially altered the global nitrogen cycle (Vitousek and others 1997; Galloway and Cowling 2002). While the increasing amount of anthropogenic nitrogen has driven increases in food production and global development, all nitrogen created by human activities is eventually released into the environment (Galloway and Cowling 2002), reaching ground and surface waters (Vitousek and others 1997; Fenech and others 2012). Wastewaters from farming, aquaculture, urban and industrial effluents, and runoff are the major anthropogenic point sources of nitrogen to aquatic ecosystems (Camargo and Alonso 2006). Further, nonpoint sources such as animal manure, inorganic N fertilizers, and cultivation of N_2-fixing crop species (see more in Camargo and Alonso 2006), are also important sources of reactive nitrogen to aquatic environments.

As a result, concentrations of reactive nitrogen in aquatic ecosystems are increasing globally, causing water quality degradation and jeopardizing ecosystem sustainability, biodiversity and human health (Vitousek and others 1997; Rabalais 2002; Jones and others 2014). Besides threatening biodiversity due the toxicity of inorganic nitrogenous compounds (Camargo and others 2005), nitrogen enrichment of aquatic ecosystems can also lead to eutrophication and promote productivity and biomass of autotrophic organisms (Vitousek and others 1997; Smith and others 1999; Smith 2003). Consequently, changes in the availability of basal resources can influence the feeding behaviour of aquatic consumers (Carvalho and others 2015; de Carvalho and others 2019a) and promote changes in the aquatic food web (Santoro and others 2014; de Carvalho and others 2017).

Stable isotopes have become a common approach for study of trophic niches, providing a representation of the trophic ecology of organisms (Bearhop and others 2004; Layman and others 2007a; Newsome and others 2007; Flaherty and Ben-David 2010) and increasing our understanding of pollution effects on freshwater organisms (DeBruyn and Rasmussen 2002; Anderson and Cabana 2005, 2006; de Carvalho and others 2019b). The $\delta^{13}C$ has been effective tracing organic carbon sources and energy flow in aquatic ecosystems, for example, detecting the increases in the importance of autochthonous resources that sustain food webs of degraded environments, as well as the assimilation of sewage-derived organic matter by food web consumers (DeBruyn and Rasmussen 2002; de Carvalho and others 2019b). The $\delta^{15}N$, in turn, provides an integrative measure of food chain length, food web fluxes, and nitrogen cycling (Finlay and Kendall 2007). Over the last few decades, $\delta^{15}N$ has been intensively applied to access the influence of nitrogen inputs on primary producers (Savage and Elmgren 2004; Schubert and others 2013; Wang and others 2016) and consumers (Riera 1998; Ulseth and Hershey 2005; Anderson and Cabana 2006). Different anthropogenic nitrogen sources often have different $\delta^{15}N$, thus potentially allowing for their identification from $\delta^{15}N$ values observed in aquatic organisms. For example, nitrogen derived from animal manure and wastewater usually promotes $\delta^{15}N$ enrichment, while industrial wastewater and synthetic fertilizers are generally depleted in ^{15}N (Kendall 1998; Nikolenko and others 2018). Therefore, the use of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can be an important tool to detect the influence of nitrogen pollution in food webs.

Considering possible changes in the availability of basal resources and in the $\delta^{15}N$ composition of primary producers at the base of the food web (reflecting the incorporation of enriched or depleted nitrogen compounds), it is likely changes will occur in food webs of aquatic communities in nitrogen polluted sites. The objective of this study was to evaluate whether these changes would be reflected in the niche space of fish communities. We also evaluated whether seasonality influenced niche space, considering that riverine food webs can exhibit considerable temporal variation (Winemiller 2004). We tested four alternative hypotheses on pollution-driven changes in isotopic niche space described in the Figure 1, using a large network (16 sites sampled in both the dry and wet seasons, totalling 1863 samples of fishes and basal resources) in a basin located in the third largest urban area in Brazil.

2 Material and Methods

Study area

All measurements were made in the Rio das Velhas basin, the largest tributary of the São Francisco River basin (Alves and Pompeu 2010), located entirely in the territory of Minas Gerais State of Brazil, covering 51 municipalities along its 801 km length (Figure 2). The basin, with a drainage area of 29.173 km^2, has an estimated average annual flow rate of 300 m^3/s and an average width of 38.3 m. The upper course of the Rio das Velhas is located in the Metropolitan Region of Belo Horizonte (MRBH), the third largest urban area in Brazil with almost 6 million inhabitants (IBGE 2018), and Rio das Velhas is responsible for most of the MRBH water supply, presenting significant economic and social importance.

The primary pollution sources are located in the upper reaches, which receives the majority of domestic and industrial sewage from MRBH (Alves and Pompeu 2010), with an estimated discharge of 700 liters of sewage per second to the river. In the Arrudas and Onça sub-basins (located in the upper section), there are at least 3,125 industries (food, metallurgical, textile, chemical and pharmaceutical industries), of which about 50% have no appropriate treatment for their effluents and solid residues. In addition, much of the river is located in the Iron Quadrangle, a critical area because of numerous mining projects (Machado and others 2008). The middle and lower section of Rio das Velhas basin are also impacted by anthropogenic activities, including agriculture and industry. However, discharge of domestic sewage from small cities is still likely the primary pollution source in the middle and lower sections.

Sampling design

We sampled a total of 16 sites distributed along the main stem (MS1 to MS8) and main tributaries (T1 to T8) of the Rio das Velhas basin (Figure 2, Table A1). Sampling occurred in 2015 and 2016, once in the dry (July) and once in the wet (tributaries in October and main stem in January) seasons. With the exception of the site MS1 (located in the Rio das Velhas headwater) and MS2 (located before MRBH), all sites along the main stem are influenced by anthropogenic activities. Among the tributaries, T1 and T4 are the most polluted. T1 is located in the Ribeirão da Mata and receives a large amount of sewage, both from nearby cities and from several regional industries, especially cement. The site T4 is located in the Ribeirão Jequitibá, which drains a city with more than 200.000 inhabitants (Sete Lagoas) and treats less than 15% of its domestic sewage, with the remaining untreated sewage being discharged directly into the river. Site T6 (located in Rio Cipó) is the most pristine tributary of Rio das Velhas basin, considered as a reference area mainly due to the existence of the Serra do Cipó National Park in its headwater. The remaining tributaries (T2, T3, T5, T7 and T8) are under good to intermediate environmental conditions (see supplementary material, Table A1).

Concentrations of nitrate (NO_3^- mg/L) and ammonium (NH_4^+ mg/L) of each site were accessed through the IGAM website (IGAM - Instituto Mineiro de Gestão das águas 2017), which conducts quarterly monitoring in several points of the Rio das Velhas basin. The sites were separated into three groups according to dissolved inorganic nitrogen inputs - 1) Low NO_3^- and NH_4^+, 2) High NO_3^-, and 3) High NO_3^- and NH_4^+ - using the mean nitrate and ammonium concentrations from eight measurements carried out quarterly during the years 2015 and 2016 (Table 1). The site T6 (located at Rio Cipó) was used as a reference site in the basin.

Sampling

At each site we collected five samples for stable isotope analyses of: (a) each fish species (wherever possible), (b) periphyton (biofilm), (c) Coarse particulate organic matter (CPOM), and fine particulate organic matter (FPOM). Storage and processing of resource samples occurred according to specific methodologies detailed below. For isotopic analysis, we collected at least 2-5 mg of dry material from animal tissue and 5-10 mg of other organic samples. After laboratory processing, samples were stored in *Eppendorf* tubes and forwarded to the Center for Nuclear Energy in Agriculture at the University of São Paulo for isotope analysis.

To determine the isotopic ratio, a mass spectrometer in Continuous-flow (CF-IRMS) mode was used with a Carlo Erba elemental analyzer (CHN 1110) coupled to a Delta Plus mass spectrometer (Thermo Scientific). Results were expressed as the relative difference from the international reference standard PeeDee Belemnite in delta notation (δ ‰), and calculated using the following formula:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N and R represents the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie and Prosser 1996).

Fish

Fish were collected with gillnets (20 m long, with 3–16 cm stretch measure mesh), seines (5 m long, 1 mm mesh), cast nets (3 cm stretch measure mesh), and mosquito nets (80 cm in diameter, 1 mm mesh). Gill nets were deployed in the water column for 14 h overnight. Seines were used in shallow areas or littoral zones, mosquito nets were used in near-shore aquatic macrophytes (both shorelines), undercut banks and in riffles, and cast nets were used in habitats too deep to wade. The three latter methods were employed for 1–3 h. In the field, after a preliminary identification, specimens had a muscle sample removed for isotopic analysis. All samples were kept frozen until laboratory processing to avoid deterioration of the material. In the laboratory, specimen identifications were confirmed, and the fish samples were lyophilized for 24 hours, and then ground to a fine homogeneous powder using a mortar and pestle.

Only resident fish species were considered, since migratory species may not reflect the condition of the site where they were sampled. All fish species were classified into trophic guilds (detritivorous, algivorous, herbivorous, omnivorous, invertivorous/insectivorous, and piscivorous/parasites) according literature sources (see supplementary material, Table A2).

Basal Resources: Periphyton, CPOM, FPOM

Periphyton (or biofilm) was collected by scraping stones using a small brush and was stored in distilled water. Samples of CPOM were collected haphazardly on the riverbed of all sites. FPOM samples were collected from benthic sediments and from the water column. FPOM from sediment was obtained by resuspending the sediment and passing the suspended material through a phytoplankton net (45 µm mesh). FPOM in suspension (i.e., seston) was obtained by placing a phytoplankton net (45 µm mesh) in a fixed position within the water column for a period of three minutes. After collection, all samples were stored in plastic sample jars and held on ice for one to five hours prior to storage in a freezer. In the laboratory, periphyton and FPOM samples were filtered through quartz fiber filters (Whatman® QMA quartz filters) using a filtration apparatus attached to a vacuum pump. All basal resource samples were oven dried at 60°C for 48 hours. Afterwards, samples were ground to a fine homogeneous powder using a mortar and pestle.

Data analyses

We analyzed a total of 1863 samples for stable C and N isotopes, including 1300 individuals of 72 fish species (Table A2), 160 CPOM samples, 136 periphyton samples, and 267 FPOM samples.

Niche space

To evaluate if nitrogen pollution promotes changes in niche space, we quantified the isotopic niche (Standard ellipse area - SEA) of fish communities at all 16 sites during the dry and wet seasons. The isotopic niches of fish communities were quantified based on standard ellipse areas (SEA - expressed in ‰) through use of the Stable Isotope Bayesian Ellipses package in R – SIBER (Jackson and others 2011). The standard ellipse area (SEA) represents the core isotopic niche space and is a proxy for the richness and evenness of resources consumed by the fish assemblage (Bearhop and others 2004). A small sample size correction (indicated by the subscript letter “c”) was applied to SEA to increase the accuracy of the comparisons, enabling comparisons of niches of communities with different sample sizes (Jackson and others 2011).

Differences in SEAc among sites grouped by nitrate and ammonium concentrations were evaluated using the nonparametric Kruskal-Wallis test due to the non-normal distribution of data and differences in the number of sites in each group (Low NO_3^- and NH_4^+, High NO_3^-, and High NO_3^- and NH_4^+). For graphical representation, the values of SEAc of site T6 were used as a reference.

Basal resources

Differences in the $\delta^{13}C$ and $\delta^{15}N$ of periphyton, CPOM and FPOM among sites grouped according to the nitrate and ammonium concentrations were tested using the nonparametric Kruskal-Wallis test due to the non-normal distribution of data and differences in the number of sites in each group (Low NO_3^- and NH_4^+, High NO_3^-, and High NO_3^- and NH_4^+). For graphical representation, the mean $\delta^{13}C$ and $\delta^{15}N$ of samples from the site T6 were used as a reference. To evaluate if the isotopic niche of fish communities varied with changes in the baseline isotopic signatures, we tested the relationship between $\delta^{13}C$ and $\delta^{15}N$ values of fishes and $\delta^{13}C$ and $\delta^{15}N$ values of basal resources using a non-parametric correlation (Spearman ρ), with a significance level of $p<0.05$.

Trophic behaviour

To evaluate if nitrogen pollution promotes changes in trophic behaviour (i.e., diet) of fishes, we tested if the delta ^{15}N ($\Delta^{15}N$) changed among the sites grouped according nitrate and ammonium concentrations. The $\Delta^{15}N$ corresponds to the nitrogen enrichment of each fish compared to the baseline (periphyton, CPOM and FPOM). It is expected a mean ^{15}N enrichment of approximately 2.2 ± 0.30‰ per trophic level (McCutchan and others 2003), although values ranging between 3–5‰ are also considered (Peterson and Fry 1987; Post 2002). Therefore, a larger $\Delta^{15}N$ indicates a higher trophic level.

The variance of $\Delta^{15}N$ among sites grouped according to nitrate and ammonium concentrations was tested separately for each guild (at each site and season), since fishes from different guilds can occupy different trophic positions and consequently present different $\Delta^{15}N$. For piscivorous/parasites individuals, $\Delta^{15}N$ was calculated with each basal resource as well as using potential prey as baseline. As potential prey we considered small-sized fishes abundant at all sites: *Astyanax eigenmanniorum, Astyanax fasciatus, Astyanax intermedius, Astyanax lacustris, Astyanax rivularis, Astyanax taeniatus, Lepidocharax burnsi, Phalloceros uai, Piabarchus stramineus, Piabina argentea* and *Poecilia reticulata.*

We expected a small $\Delta^{15}N$ for detritivores and herbivorous/algivorous fishes, as they are primary consumers that feed directly on detritus or primary producers (as periphyton, algae and macrophytes). Invertivores and omnivores should be more enriched than primary consumers because they have a diet that includes invertebrates. Piscivorous and parasitic fish, in turn, feed on other fish species and should occupy the highest trophic level, consequently displaying the largest $\Delta^{15}N$.

The differences in $\Delta^{15}N$ among the three groups were tested using the nonparametric Kruskal-Wallis test due the non-normal distribution of data and differences in the number of sites in each group (Low NO_3^- and NH_4^+, High NO_3^-, and High NO_3^- and NH_4^+). For graphical representation, the mean $\Delta^{15}N$ of fishes from the site T6 was used as a reference. Herbivorous/algivorous fishes were not included in these analyses because they were found only at few sites (with a small number of individuals).

In addition, all individuals (classified into trophic guilds) were plotted in *bi-plot* space according to the isotopic values of carbon (x-axis) and nitrogen (y-axis) to evaluate the trophic structure and to represent the food web of the reference site (site T6), sites with low NO_3^- and NH_4^+ (represented by the site T3), sites with high NO_3^- (represented by the site T4) and sites with high NO_3^- and NH_4^+ concentrations (represented by the site MS4), per season. The food web structure of the remaining sites is presented in the supplementary material (Figure A2, Figure A3, Figure A4).

3 Results

Niche space

The shape of isotopic niches of fish communities differed among sites and between seasons (Figure 3, Figure 4). In general, unpolluted sites exhibited isotopic niche estimates similar to the reference site in both the dry and wet seasons. Sites with high NO_3^- stood out by presenting fish communities enriched in both $\delta^{13}C$ and $\delta^{15}N$, regardless the season. The differences in isotopic niche were generally less pronounced at sites with both high NO_3^- and NH_4^+, but this was not always the case and some exhibited wider niches (as MS4).

Seasons exhibited smaller differences in the isotopic niches (SEAc) of fish communities in unpolluted sites (Figure 5). Conversely, sites with high NO_3^- or both high NO_3^- and NH_4^+ exhibited greater variance in SEAc and had pronounced differences between seasons (Figure 5). Although differences in isotopic niches were not the same for all sites with high nitrogen inputs, polluted sites exhibited higher SEAc (Figure 5).

Basal resources

Periphyton, FPOM and CPOM had greater $\delta^{15}N$ values at sites with high NO_3^- concentrations, especially in the dry season (Figure 6a, Figure 6b, Figure 6c). Fishes from these sites also displayed enriched $\delta^{15}N$ (Figure 3, Figure 4). At sites with both high NO_3^- and NH_4^+, periphyton and FPOM exhibited depleted $\delta^{15}N$ in both seasons (Figure 6a, Figure 6b). Some fishes from these sites fishes also exhibited depleted $\delta^{15}N$ values (Figure 3, Figure 4). At sites with low NO_3^- and NH_4^+, the $\delta^{15}N$ of all basal resources was similar to the reference site (see solid and dashed lines in the Figures 6a, Figure 6b, and Figure 6c), and exhibited smaller variance. The $\delta^{15}N$ of fishes was strongly correlated with $\delta^{15}N$ of periphyton (Spearman R = 0.85, t(N-2) = 58.49, $p < 0.01$), CPOM (Spearman R = 0.71, t(N-2) = 36.00, $p < 0.01$) and FPOM (Spearman R = 0.84, t(N-2) = 55.44, $p < 0.01$) (see supplementary material, Figure A1).

The $\delta^{13}C$ of basal sources also differed among sites (Figure 6d, Figure 6e, Figure 6f). CPOM was the resource with the most consistent $\delta^{13}C$ values, and was similar among the three groups in the wet season. Periphyton and FPOM, in turn, exhibited enriched $\delta^{13}C$ in nitrogen polluted sites, especially in the dry season (Figure 6d, Figure 6e). However, despite the $\delta^{13}C$ of fishes being correlated with the $\delta^{13}C$ of periphyton (Spearman R = 0.53, t(N-2) = 22.76, $p < 0.01$), CPOM (Spearman R = 0.12, t(N-2) = 4.19, $p < 0.01$) and FPOM (Spearman R = 0.34, t(N-2) = 12.89, $p < 0.01$), the relationship was not as strong as observed for $\delta^{15}N$ (See Figure A1 in the supplementary material).

Trophic behaviour

In the reference site (T6), the $\Delta^{15}N$ of all trophic guilds exhibited values consistent with the natural history of the fish (Figure 7, Figure 8). The $\Delta^{15}N$ of fishes from sites with low NO_3^- and NH_4^+ concentrations were those more similar to the reference site, and also exhibited less variation among individuals and between seasons (Figure 7, Figure 8). Food webs of these unpolluted sites showed a typical trophic structure, with piscivorous fishes at the highest trophic level, invertivores and omnivores at intermediate trophic levels, and detritivores, herbivores and algivores occupying low trophic positions as primary consumers (Figure 9, Figure A2). At the most pristine site, the $\delta^{15}N$ of piscivorous fish did not exceed 15‰, the invertivorous and omnivorous fishes occupied the middle portions of the food web, and detritivorous fishes the bottom (Figure 9a). Generally, in sites with low NO_3^- and NH_4^+ concentrations (Figure 9b, Figure A2), the maximum $\delta^{15}N$ observed was similar. However, some sites with low NO_3^- and NH_4^+ concentrations (e.g., T2 and T7) contained individuals with enriched $\delta^{15}N$ (Figure A2).

In nitrogen polluted sites, the $\Delta^{15}N$ for all trophic guilds increased, especially in the wet season. High variance was also observed in $\Delta^{15}N$, with some fishes having a $\Delta^{15}N$ larger than 20‰ and others exhibiting negative $\Delta^{15}N$ (Figure 7, Figure 8). Further, in many of the nitrogen polluted sites, the trophic structure of the food web was not as expected. At T4, MS3, MS4, MS5 and MS6, for example, detritivorous fishes were more enriched in $\delta^{15}N$ compared to all other guilds, including piscivorous/parasites (see the food web of the site T4 and MS4 in the Figure 9c and Figure 9d, respectively).

4 Discussion

The shape of isotopic niches (and its position in bi-plot space) of fish communities was different in polluted and unpolluted sites. This study set out to determine if differences in niche space would reflect both changes in baseline isotopic signatures and the trophic behaviour of fishes and this was generally supported. We observed that the isotopic ratio of fish changes according to the changes in $\delta^{15}N$ and $\delta^{13}C$ of basal resources, and that the $\Delta^{15}N$ of trophic guilds changes according to the NO_3^- and NH_4^+ concentrations found at a given site. Seasonality also influenced niche space, especially in the wet season and in highly polluted sites. However, our observation of unlikely trophic positions of fish in some sites suggests that other mechanisms may be operating in this system.

Niche space

One hypothesis explaining differences in niche space among fish communities from polluted and unpolluted sites is a change in fish trophic behaviour, especially in response to resources availability. Human activities can modify hydrology and sediments in aquatic ecosystems, which in some cases contrast significantly with more natural systems (i.e. pristine or forested). For example, at anthropogenically altered sites it is expected a further inflow of sediments (organic and inorganic) from different sources as: run-off from rural, agricultural and forested land, channel banks, sewage treatment stations, and others (Taylor and others 2008). In addition, the seasons and river flow characteristics are related with the type and amount of material being supplied and carried along the river. Moreover, with heavy rains, wastes from dump sites are often washed into water bodies (Adeyemo 2003). Therefore, possible inputs of resources with different $\delta^{13}C$ and $\delta^{15}N$ and more intense flow rates in the wet season could explain the larger changes in the niche space of fish communities from polluted than unpolluted sites.

Larger isotopic niches in polluted sites also could be the result of other factors including differences in sample sizes among sites and fish species composition among sites (i.e., higher abundance of generalist species in polluted sites). Here, we compared the niche space of sites with differences in the assemblage composition and numbers of individuals (sample size). However, ellipses (SEAc) were unbiased with respect to sample size, and their estimation via Bayesian inference allows robust comparison to be made among data sets comprising different sample sizes, allowing direct comparison of isotopic niches across communities (Jackson and others 2011). Moreover, the broadest trophic niches were observed in polluted sites MS4 and T4, which had similar numbers of species and individuals compared to the most pristine site (T6) (Table A2).

Polluted ecosystems usually exhibit more generalist species (Santoro and others 2014), which use a wide diversity of resources. Often, there is a tendency for more generalist populations to be more ecologically variable and exhibit higher niche variation (Bolnick and others 2007). However, in our study, even the specialist species (e.g. those from *Hypostomus* genus) exhibited broader niches in polluted sites. In addition, the most generalist species (e.g. *Astyanax* genus, *Poecilia reticulata, Phalloceros uai*) were evenly distributed along the sampled sites (Table A2), and exhibited broad niches only in polluted sites (Alonso and others 2019; de Carvalho and others 2019b).

Basal resources

Differences in $\delta^{15}N$ of resources were generally observed in the $\delta^{15}N$ of fishes, supporting the hypothesis that changes in baseline isotopic signatures reflect the niche space of fish communities. However, $\delta^{15}N$ of fishes and resources differ at sites with different ammonium and nitrate concentrations. Fishes and resources from sites with high NO_3^- exhibited enriched $\delta^{15}N$, while those from sites with both high NO_3^- and NH_4^+ exhibited mostly depleted $\delta^{15}N$. The major difference among these two groups of sites was the type of anthropogenic influence. Sites with high NO_3^- were primarily influenced by domestic sewage from small cities and agriculture, while sites with high NO_3^- and NH_4^+ were highly influenced by the MRBH, the third largest urban area in Brazil with almost 6 million inhabitants (IBGE 2018) and more than 3000 industries (Atlas das Águas 2009).

Most often, sewage is expected to exhibit enriched $\delta^{15}N$ values, sometimes reaching >30‰ (Kendall, 1998) because of isotopic fractionation during nitrification and volatilization in the case of ammonium, or denitrification in the case of nitrate (Nikolenko and others 2018). However, depleted $\delta^{15}N$ values in algae (Wang and others 2016), macroinvertebrates and fish (Loomer and others 2015; Alonso and others 2019; de Carvalho and others 2019b) also have been observed in aquatic environments influenced by domestic sewage. Sites downstream sewage treatment plants (STP's) may exhibit depleted $\delta^{15}N$ values because the $\delta^{15}N$ signature of ammonia leaving the plant may not be enriched due to limited nitrification occurring during sewage treatment (Loomer and others 2015). This influence may apply to sites MS3 and MS4 because of the $\delta^{15}N$ of suspended matter from two STP's located upstream of these sites exhibited depleted $\delta^{15}N$ (1.50 ± 0.45‰) (de Carvalho and others 2019b), which can be reflected in the $\delta^{15}N$ of the resources. In addition, the direct assimilation of sewage depleted in $\delta^{15}N$ was found in an exotic guppy sampled at the site MS4 (de Carvalho and others 2019b). Therefore, the observation of fishes with both depleted and enriched $\delta^{15}N$ in MS4 may be due to one part of the consumer community using sewage sludge ($\delta^{15}N$ depleted) as a resource, and the other part consuming resources more enriched in $\delta^{15}N$.

The $\delta^{13}C$ of resources also varied among sites with different nitrogen concentrations. In sites where resources were more $\delta^{13}C$ enriched (sites with high NO_3^-), fish were also more enriched in $\delta^{13}C$, and the niche space was shifted to the right on the x-axis (see Figure 3 and 4). The influence of pasture, and consequently increased inputs of C_4 grass litter to the river (incorporated indirectly through FPOM), could explain the enriched $\delta^{13}C$ in FPOM at sites with high NO_3^-. C_4 grasses from pastures display enriched $\delta^{13}C$ values relative to C_3 plants from riparian vegetation (O'Leary 1981). Periphyton, also exhibited enriched $\delta^{13}C$ at these sites. Algae, one of the biofilm components, generally is quite variable in $\delta^{13}C$ (e.g. McCutchan Jr. and Lewis Jr., 2001). However, the reason for enriched $\delta^{13}C$ at Rio das Velhas is unknown, since several factors can be related to the variability in algal $\delta^{13}C$, as the range concentrations of dissolved inorganic carbon (DIC), nutrients (as phosphorous), light exposure, turbulence, and others (France 1995; Hill and others 2008).

Trophic behavior

We observed differences in niche space and the $\Delta^{15}N$ of most fishes from nitrogen polluted sites, particularly in the wet season. An average ^{15}N enrichment of approximately 2.2 ± 0.30‰ per trophic level is commonly utilized in river isotope web studies (McCutchan and others 2003), although values ranging between 3–5‰ have also been observed (Peterson and Fry 1987; Post 2002). In contrast to the reference site (and other sites with low NO_3^- and NH_4^+), nitrogen polluted sites exhibited large increases in $\Delta^{15}N$, sometimes reaching differences of 20‰ between the fish tissue and the basal resources. A $\Delta^{15}N$ of 20‰ corresponds to at least 6 trophic levels between fish and basal resources, suggesting a long food chain. However, the most likely explanation is that at polluted sites fishes from all guilds fed on other unsampled resources that were more enriched in $\delta^{15}N$ than periphyton, CPOM and FPOM, and the increases in $\Delta^{15}N$ does not necessarily indicate a change in trophic position. This interpretation is reinforced by the fact that the highest $\Delta^{15}N$ were observed during the wet seasons when higher river flows and flood pulses can carry different food sources to aquatic environments (Junk and others 1989; Winemiller 2004). The negative $\Delta^{15}N$ observed in some nitrogen polluted sites indicates that fishes did not consume FPOM, CPOM and periphyton, reinforcing the idea of the existence of unsampled resources.

We also observed increases in the $\Delta^{15}N$ variance of all trophic guilds associated with increases in nitrogen concentrations. Unpolluted sites in this study may have a small number of potential nitrogen sources with unique $\delta^{15}N$ values and the opposite may be true in polluted sites. Therefore, it is possible that within a polluted site different fishes could consume resources with a wider variety of isotopic baselines. In addition, changes in the turnover rate among different fish species can influence the results, since turnover rates are related to ontogenetic stages and metabolic rates of each fish and can take weeks to years (Hesslein and others 1993; Cabana and Rasmussen 1996; Mcintyre and Flecker 2006). Therefore, the presence of more unique resources with different isotopic signatures, together with the differences in turnover rates, may explain the high $\Delta^{15}N$ variance and changes in food web structure of polluted sites.

Nitrogen pollution can be responsible for changes in the architecture of biodiversity between ecosystems and lead to less robust trophic structures (Santoro and others 2014). We observed that food webs of sites with low NO_3^- and NH_4^+ are traditionally organized [typical food web: piscivorous (high trophic level) -> invertivorous and omnivorous (intermediate trophic levels) -> detritivorous/herbivorous/algivorous (primary consumers)] and vary less between seasons (more stable than sites with high nitrogen concentrations). In the most polluted sites, $\delta^{15}N$ of detritivorous fishes were more enriched than fishes from other guilds (including piscivores), in both the dry and wet seasons. This pattern was not expected, since in theory, piscivorous fishes should occupy the top of the food web. The reason why $\delta^{15}N$ enrichment of piscivorous fishes was lower than detritivores is not clear. In addition, in a polluted site, two piscivorous individuals of the same species exhibited completely different $\delta^{15}N$ enrichment (see blue points in the Figure 9c). We sampled only resident fish so it is unlikely piscivorous fishes were not feeding on fish from the same site where they were sampled. We also discard the possibility that the individuals were at different stages of development, and exhibiting different diets (Winemiller 1989; Pompeu and Godinho 2001), because both individuals were of the same size (24.2 cm/140.80 g and 24.0 cm/143.25 g, respectively). Therefore, we surmise that the two fish were feeding on different baselines because of varied pollution inputs.

5 Conclusions

We conclude that nitrogen pollution promotes an increase in niche space and higher variability in $\Delta^{15}N$. In addition, seasonality influences the niche space of fish communities and this effect is larger in nitrogen polluted sites. The findings of this study suggest that differences in niche space reflect both changes in baseline isotopic signatures and the trophic behaviour of fishes. However, within polluted sites the interpretation of trophic information is potentially confounded by multiple pollution-derived isotopic backgrounds.

Studies using stable isotopes to evaluate the niche space of fish communities living in highly polluted sites are scarce, and our study presents an important approach for understanding the effects of pollution on aquatic ecosystems. However, caution is required in the use of stable isotopes to analyse niche space and trophic structure of aquatic communities highly influenced by nitrogen pollution, since under high nitrate concentrations $\Delta^{15}N$ may not be a good proxy for trophism.

Acknowledgments

We thank the Agência Peixe Vivo and the Comitê de Bacia Hidrográfica do Rio das Velhas CBH - Rio das Velhas for project financial support and the Projeto Manuelzão for the logistical support. This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance code 001 and D.R.C. sandwich doctorate scholarship granted (88881.190508/2018-01). Thanks to Aline Junqueira Grossi (Federal University of Lavras, UFLA) and Luiza Hoehne (Federal University of Minas Gerais, UFMG) for the support on processing of samples. Thanks to the Benthos Ecology Laboratory (UFMG) and the Laboratory of Fish Ecology (UFLA) who assisted in the processing of samples and infrastructure, and to the Centre for Nuclear Energy in Agriculture for their support and partnership in the isotopic analysis. P.S.P. received a research grant and a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (303548/2017-7) and from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (PPM-00237/13).

References

Adeyemo OK. 2003. Consequences of Pollution and Degradation of Nigerian Aquatic Environment on Fisheries Resources. The Environmentalist 23:297–306. http://link.springer.com/10.1023/B:ENVR.0000031357.89548.fb

Alonso MB, de Carvalho DR, Alves CBM, Moreira MZ, Pompeu PS. 2019. Changes in trophic characteristics of two fish species of Astyanax (Teleostei: Characidae) in response to aquatic pollution. Zoologia 36:1–12. https://zoologia.pensoft.net/article/30445/

Alves CBM, Pompeu P dos S. 2010. A fauna de peixes da bacia do rio das Velhas no final do século XX. In: Alves CBM, Pompeu PS, editors. Peixes do rio das Velhas: passado e presente. Belo Horizonte: ARGVMENTVM. pp 167–89.

Anderson C, Cabana G. 2005. δ ^{15} N in riverine food webs: effects of N inputs from agricultural watersheds. Canadian Journal of Fisheries and Aquatic Sciences 62:333–40. http://www.nrcresearchpress.com/doi/abs/10.1139/f04-191

Anderson C, Cabana G. 2006. Does δ15N in river food webs reflect the intensity and origin of N loads from the watershed? Science of The Total Environment 367:968–78. https://linkinghub.elsevier.com/retrieve/pii/S004896970600115X

Atlas das Águas. 2009. Impacto ambiental relevante identificado na bacia do rio das Velhas Águas impactadas por esgotos domésticos e efluentes industriais. http://www.atlasdasaguas.ufv.br/velhas/impacto_ambiental_relevante_identificado_na_bacia_do_rio_das_velhas.html. Last accessed 15/01/2019

Barrie A, Prosser SJ. 1996. Mass Spectrometry of Soils. In: Mass Spectrometry of Soils. Marcel Dekker, Inc. p 536.

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology 73:1007–12. http://doi.wiley.com/10.1111/j.0021-8790.2004.00861.x

Bolnick DI, Svanback R, Araujo MS, Persson L. 2007. Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. Proceedings of the National Academy of Sciences 104:10075–9. http://www.pnas.org/cgi/doi/10.1073/pnas.0703743104

Cabana G, Rasmussen JB. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences of the United States of America 93:10844–7. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=38243&tool=pmcentrez&rendertype=abstract

Camargo JA, Alonso Á. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environment International 32:831–49. http://linkinghub.elsevier.com/retrieve/pii/S0160412006000602

Camargo JA, Alonso A, Salamanca A. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 58:1255–67. https://linkinghub.elsevier.com/retrieve/pii/S0045653504009993

Carvalho DR, Castro D, Callisto M, Moreira MZ, Pompeu PS. 2015. Isotopic variation in five species of stream fishes under the influence of different land uses. Journal of Fish Biology 87:559–78. http://doi.wiley.com/10.1111/jfb.12734

de Carvalho DR, de Castro DMP, Callisto M, Chaves AJ de M, Moreira MZ, Pompeu PS. 2019a. Stable isotopes and stomach content analyses indicate omnivorous habits and opportunistic feeding behavior of an invasive fish. Aquatic Ecology 3. http://link.springer.com/10.1007/s10452-019-09695-3

de Carvalho DR, de Castro DMP, Callisto M, Moreira MZ, Pompeu PS. 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. http://link.springer.com/10.1007/s10750-017-3130-6

de Carvalho DR, Flecker AS, Alves CBM, Sparks JP, Pompeu PS. 2019b. Trophic responses to aquatic pollution of native and exotic livebearer fishes. Science of The Total Environment 681:503–15. https://doi.org/10.1016/j.scitotenv.2019.05.092

Castro DMP, De Carvalho DR, Pompeu PDS, Moreira MZ, Nardoto GB, Callisto M. 2016. Land use influences niche size and the assimilation of resources by benthic macroinvertebrates in tropical headwater streams. PLoS ONE. http://www.scopus.com/inward/record.url?eid=2-s2.0-84961126310&partnerID=MN8TOARS

DeBruyn AMH, Rasmussen JB. 2002. Quantifying assimilation of sewage-derived organic matter by riverine benthos. Ecological Applications 12:511–20.

Fenech C, Rock L, Nolan K, Tobin J, Morrissey A. 2012. The potential for a suite of isotope and chemical markers to differentiate sources of nitrate contamination : A review. Water Research 46:2023–41. http://dx.doi.org/10.1016/j.watres.2012.01.044

Finlay JC, Kendall C. 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. In: Finlay JC, Kendall C, editors. Stable isotope tracing of organic matter sources and food web interactions in watersheds. Second. pp 283–333.

Flaherty EA, Ben-David M. 2010. Overlap and partitioning of the ecological and isotopic niches. Oikos 119:1409–16. http://doi.wiley.com/10.1111/j.1600-0706.2010.18259.x

France RL. 1995. Carbon-13 enrichment in benthic compared to planktonic algae : foodweb implications. Marine Ecology Progress Series 124:307–12.

Galloway JN, Cowling EB. 2002. Reactive nitrogen and the word: 200 years of change. Ambio 31:64–71. http://www.jstor.org/stable/4315217

Hamilton SK, Lewis WM. 1992. Stable carbon and nitrogen isotopes in algae and detritus from the

Orinoco River floodplain, Venezuela. Geochimica et Cosmochimica Acta 56:4237–46.

Hesslein RH, Hallard KA, Ramlal P. 1993. Replacement of Sulfur, Carbon, and Nitrogen in Tissue of Growing Broad Whitefish (Coregonus nasus) in Response to a Change in Diet Traced by δ 34 S, δ 13 C, and δ 15 N. Canadian Journal of Fisheries and Aquatic Sciences 50:2071–6. http://www.nrcresearchpress.com/doi/10.1139/f93-230

Hill WR, Fanta SE, Roberts BJ. 2008. 13C dynamics in benthic algae: Effects of light, phosphorus, and biomass development. Limnology and Oceanography 53:1217–26. http://doi.wiley.com/10.4319/lo.2008.53.4.1217

IBGE. 2018. Estimativas de população. Instituto Brasileiro de Geografia e Estatística. https://www.ibge.gov.br/estatisticas-novoportal/sociais/populacao/9103-estimativas-de-populacao.html?=&t=resultados. Last accessed 26/02/2019

IGAM - Instituto Mineiro de Gestão das águas. 2017. Monitoramento de Qualidade das Águas. Monitoramento de Qualidade das Águas. http://portalinfohidro.igam.mg.gov.br/monitoramento-de-qualidade-das-aguas. Last accessed 16/01/2019

Jackson AL, Inger R, Parnell AC, Bearhop S. 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80:595–602. http://www.ncbi.nlm.nih.gov/pubmed/21401589. Last accessed 19/03/2014

Jones L, Provins A, Holland M, Mills G, Hayes F, Emmett B, Hall J, Sheppard L, Smith R, Sutton M, Hicks K, Ashmore M, Haines-young R, Harper-simmonds L. 2014. A review and application of the evidence for nitrogen impacts on ecosystem services. Ecosystem Services 7:76–88.

Junk WJ, Bayley PB, Sparks RE. 1989. The flood pulse concept in river-floodplain systems. In: Dodge DP, editor. Proceedings of the International Large River Symposium (LARS). Canadian Special Publication of Fisheries and Aquatic Sciences. pp 110–27.

Kendall C. 1998. Tracing Nitrogen Sources and Cycling in Catchments. (Kendall C, McDonnell JJ, editors.). Elsevier B.V. http://linkinghub.elsevier.com/retrieve/pii/B9780444815460500239

Layman CA, Arrington DA, Montaña CG, Post DM. 2007a. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42–8.

Layman CA, Quattrochi JP, Peyer CM, Allgeier JE. 2007b. Niche width collapse in a resilient top predator following ecosystem fragmentation. Ecology Letters 10:937–44. http://doi.wiley.com/10.1111/j.1461-0248.2007.01087.x

Loomer HA, Oakes KD, Schiff SL, Taylor WD, Servos MR. 2015. Use of Stable Isotopes to Trace Municipal Wastewater Effluents into Food Webs within a Highly Developed River System. River Research and Applications 31:1093–100. http://doi.wiley.com/10.1002/rra.2826

Machado ATM, Alves CBM, Callisto M. 2008. Projeto Manuelzão: metodologia e resultados. In: Lisboa AH, Goulart; EMA, Diniz LFM, editors. Projeto Manuelzão. A história da mobilização que começou em torno de um rio. Belo Horizonte: Instituto Guaicuy - SOS Rio das Velhas. pp 39–54.

McCutchan JH, Lewis WM, Kendall C, McGrath CC. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–90. http://doi.wiley.com/10.1034/j.1600-0706.2003.12098.x

McCutchan Jr. JH, Lewis Jr. WM. 2001. Seasonal variation in stable isotope ratios of stream algae. Verhandlungen des Internationalen Verein Limnologie 27:3304–7.

Mcintyre PB, Flecker ÆAS. 2006. Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. :12–21.

Newsome SD, Rio CM del, Bearhop S, Phillips DL. 2007. A niche for isotopic ecology. Frontiers in Ecology and the Environment 5:429–36.

Nikolenko O, Jurado A, Borges A V, Knöller K, Brouyère S. 2018. Isotopic composition of nitrogen species in groundwater under agricultural areas: A review. Science of The Total Environment 621:1415–32. https://linkinghub.elsevier.com/retrieve/pii/S0048969717327894

O'Leary MH. 1981. Carbon isotope fractionation in plants. Phytochemistry 20:553–67.

http://linkinghub.elsevier.com/retrieve/pii/0031942281851345

Peterson BJ, Fry B. 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics 18:293–320. http://www.annualreviews.org/doi/10.1146/annurev.es.18.110187.001453

Philippsen JS, Hauser M, Benedito E. 2015. Isotopic niches of sympatric native and exotic fish species in a Neotropical floodplain. Anais da Academia Brasileira de Ciências 87:825–33. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0001-37652015000200825&lng=en&tlng=en

Pompeu PDS, Godinho AL. 2001. Mudança na dieta da traíra Hoplias malabaricus (Bloch) (Erythrinidae, Characiformes) em lagoas da bacia do rio Doce devido à introdução de peixes piscívoros. Revista Brasileira de Zoologia 18:1219–25. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-81752001000400016&lng=pt&tlng=pt

Post DM. 2002. Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. Ecology 83:703. http://www.esajournals.org/doi/abs/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Rabalais NN. 2002. Nitrogen in Aquatic Ecosystems. AMBIO: A Journal of the Human Environment 31:102–12. http://www.ncbi.nlm.nih.gov/pubmed/12077998

Riera P. 1998. δ15N of organic matter sources and benthic invertebrates along an estuarine gradient in Marennes-Oléron Bay (France):implications for the study of trophic structure. Marine Ecology Progress Series 166:143–50. http://www.int-res.com/abstracts/meps/v166/p143-150/

Santoro R, Bentivoglio F, Carlino P, Calizza E, Costantini ML, Rossi L. 2014. Sensitivity of food webs to nitrogen pollution: A study of three transitional water ecosystems embedded in agricultural landscapes. Transitional Waters Bulletin 8:84–97.

Savage C, Elmgren R. 2004. Macroalgal (Fucus vesiculosus) δ N Values Trace Decrease in Sewage

Influence. Ecological Applications 14:517–26.

Schubert PR, Karez R, Reusch TBH, Dierking J. 2013. Isotopic signatures of eelgrass (Zostera marina L.) as bioindicator of anthropogenic nutrient input in the western Baltic Sea. Marine Pollution Bulletin 72:64–70. https://linkinghub.elsevier.com/retrieve/pii/S0025326X13002257

Seckbach J, Kaplan IR. 1973. Growth pattern and13C12C isotope fractionation of Cyanidium caldarium and hot spring algal mats. Chemical Geology 12:161–9.

Smith VH. 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. Environmental Science and Pollution Research 10:126–39. http://dx.doi.org/10.1065/espr2002.12.142

Smith VH, Tilman GD, Nekola JC. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100:179–96. https://linkinghub.elsevier.com/retrieve/pii/S0269749199000913

Taylor KG, Owens PN, Batalla RJ, Garcia C. 2008. Sediment and contaminant sources and transfers in river basins. In: Owens PN, editor. Sustainable Management of Sediment Resources. Vol. 4. pp 83–135. http://www.sciencedirect.com/science/article/pii/S1872199008800037

Ulseth AJ, Hershey AE. 2005. Natural abundances of stable isotopes trace anthropogenic N and C in an urban stream. Journal of the North American Benthological Society 24:270–89. https://www.journals.uchicago.edu/doi/10.1899/03-080.1

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilma DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications 7:737–50. https://linkinghub.elsevier.com/retrieve/pii/S1240130797877382

Wang Y, Liu D, Richard P, Di B. 2016. Selection of effective macroalgal species and tracing nitrogen sources on the different part of Yantai coast, China indicated by macroalgal δ15N values. Science of The Total Environment 542:306–14.

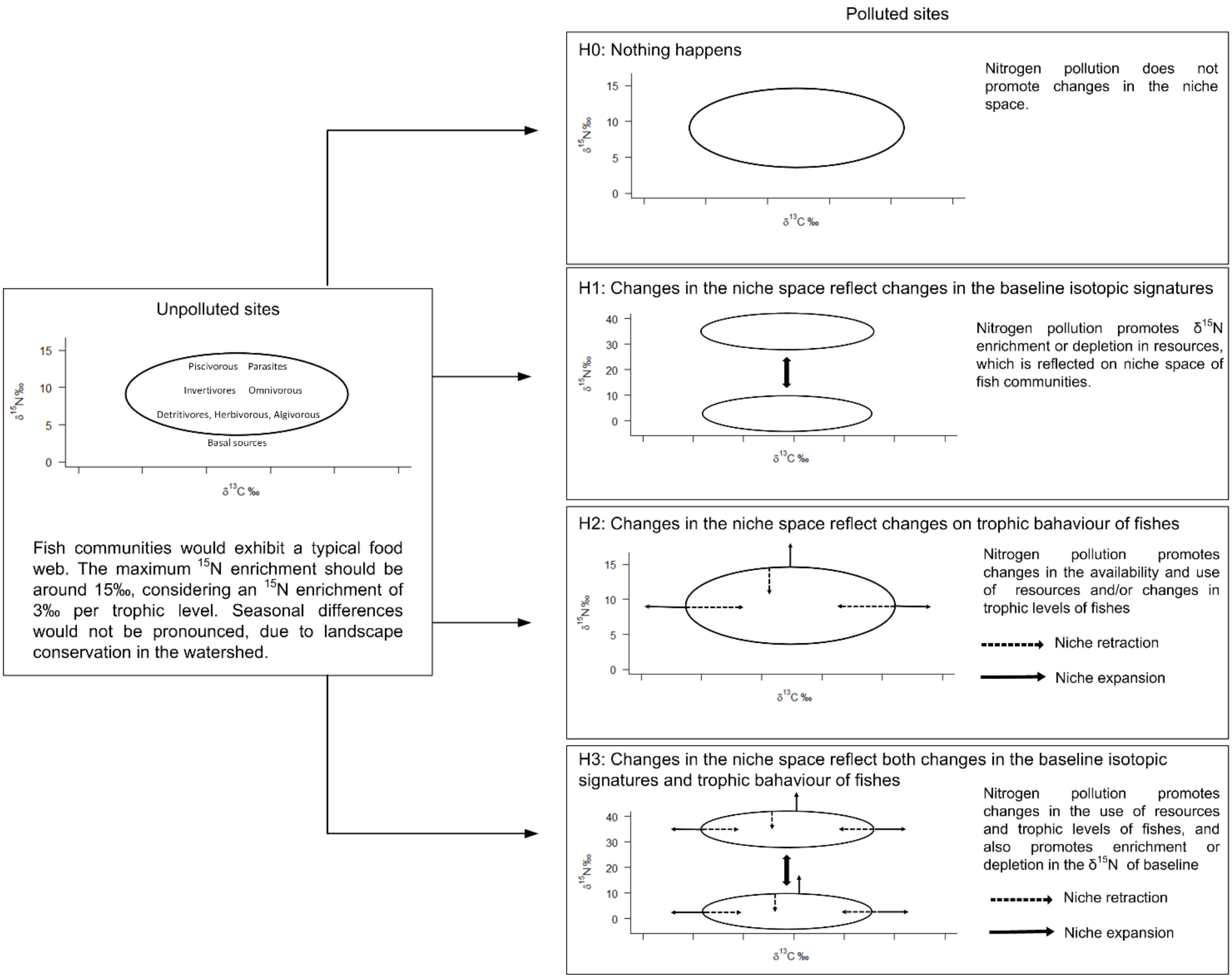
https://linkinghub.elsevier.com/retrieve/pii/S0048969715308718

Winemiller KO. 1989. Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan ilanos. Environmental Biology of Fishes 26:177–99.

Winemiller KO. 2004. Floodplain River Food Webs: Generalizations and Implications for Fisheries Management. In: Welcomme RL, Petr T, editors. Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries. Vol. 2. RAP Publication 2004/16. pp 285–309.

Table 1. Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations of each site sampled in the Rio das Velhas basin during dry and wet seasons. The mean of nitrate and ammonium concentrations in eight samplings carried out quarterly in the years 2015 and 2016 (IGAM - Instituto Mineiro de Gestão das águas 2017) were used to separate the sampling sites in three groups: 1) Low NO_3^- and NH_4^+; 2) High NO_3^-; and 3) High NO_3^- and NH_4^+

Sites		NO_3^-			NH_4^+		
		Mean	Dry	Wet	Mean	Dry	Wet
Reference	T6	0.17	0.18	0.1	0.13	0.18	0.13
Low NO_3^- and NH_4^+	T2	0.39	0.27	0.17	0.20	0.1	0.77
	T3	0.50	0.57	0.22	0.14	0.16	0.11
	T5	0.47	0.5	0.47	0.22	0.43	0.14
	T7	0.53	0.16	0.24	0.13	0.1	0.2
	T8	0.45	0.25	0.27	0.12	0.1	0.11
	MS1	0.33	0.13	0.18	0.10	0.1	0.1
	MS2	0.99	0.99	0.62	0.29	0.29	0.21
High NO_3^-	T4	6.63	7.48	5.36	0.33	0.11	0.1
	MS5	5.00	5.24	2.06	0.75	3.22	0.1
	MS6	3.51	8.01	1.75	0.11	0.1	0.1
	MS7	1.65	4.17	0.68	0.11	0.1	0.1
	MS8	1.34	4.16	0.62	0.10	0.1	0.1
High NO_3^- and NH_4^+	T1	1.40	0.82	0.47	4.32	1.24	6.56
	MS3	1.44	2.38	1.16	6.06	11.8	1.75
	MS4	1.46	0.94	0.71	5.12	12	0.75



Nitrogen pollution does not promote changes in the niche space.

Nitrogen pollution promotes $\delta^{15}N$ enrichment or depletion in resources, which is reflected on niche space of fish communities.

Nitrogen pollution promotes changes in the availability and use of resources and/or changes in trophic levels of fishes

Nitrogen pollution promotes changes in the use of resources and trophic levels of fishes, and also promotes enrichment or depletion in the $\delta^{15}N$ of baseline

Fish communities would exhibit a typical food web. The maximum ^{15}N enrichment should be around 15‰, considering an ^{15}N enrichment of 3‰ per trophic level. Seasonal differences would not be pronounced, due to landscape conservation in the watershed.

Figure 1. Tested hypotheses and the expected niche space of fish communties from unpolluted sites and nitrogen polluted sites. Typical food web = piscivorous/parasites occuping the highest trophic level, invertivores and omnivorous occuping an intermediate trophic level and detritivores, herbivorous and algivorous occuping the first trophic level

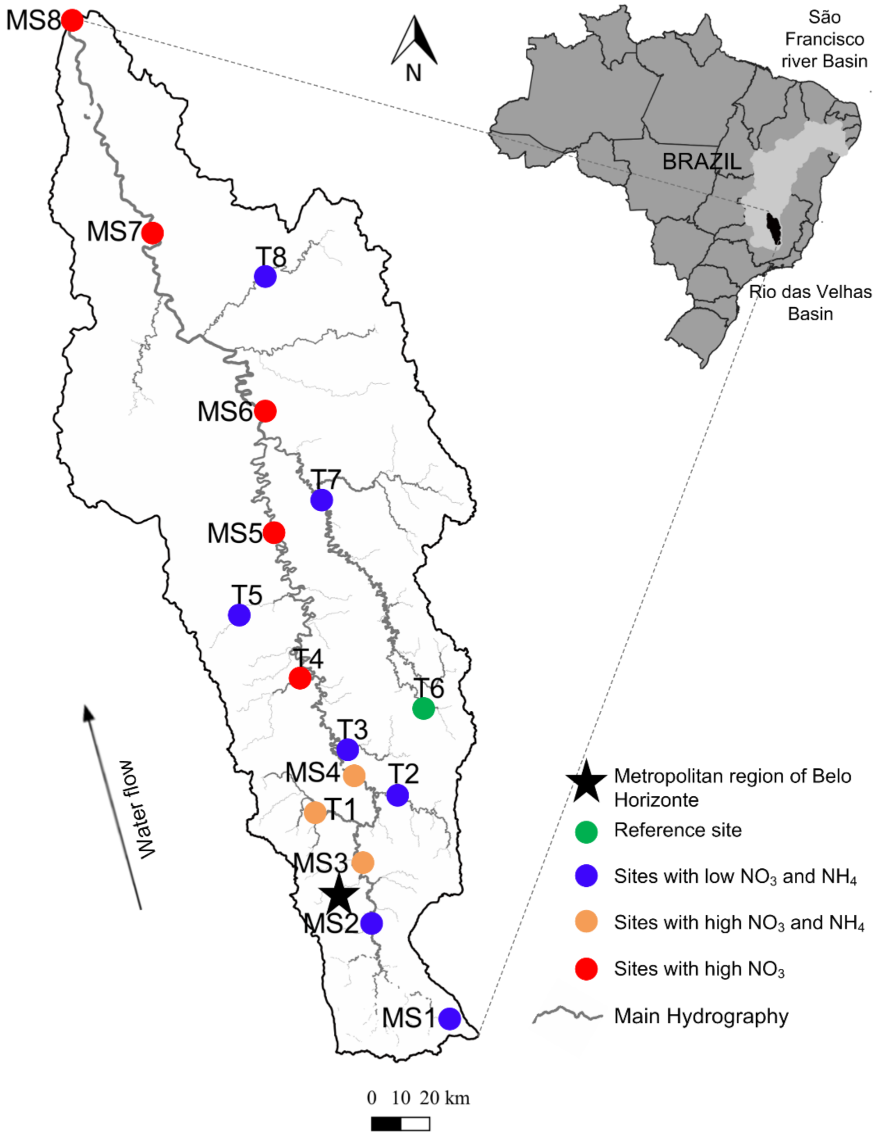


Figure 2. Sampling area covering 16 sites at tributaries and main stem of the Rio das Velhas basin, the largest tributary of the São Francisco River basin

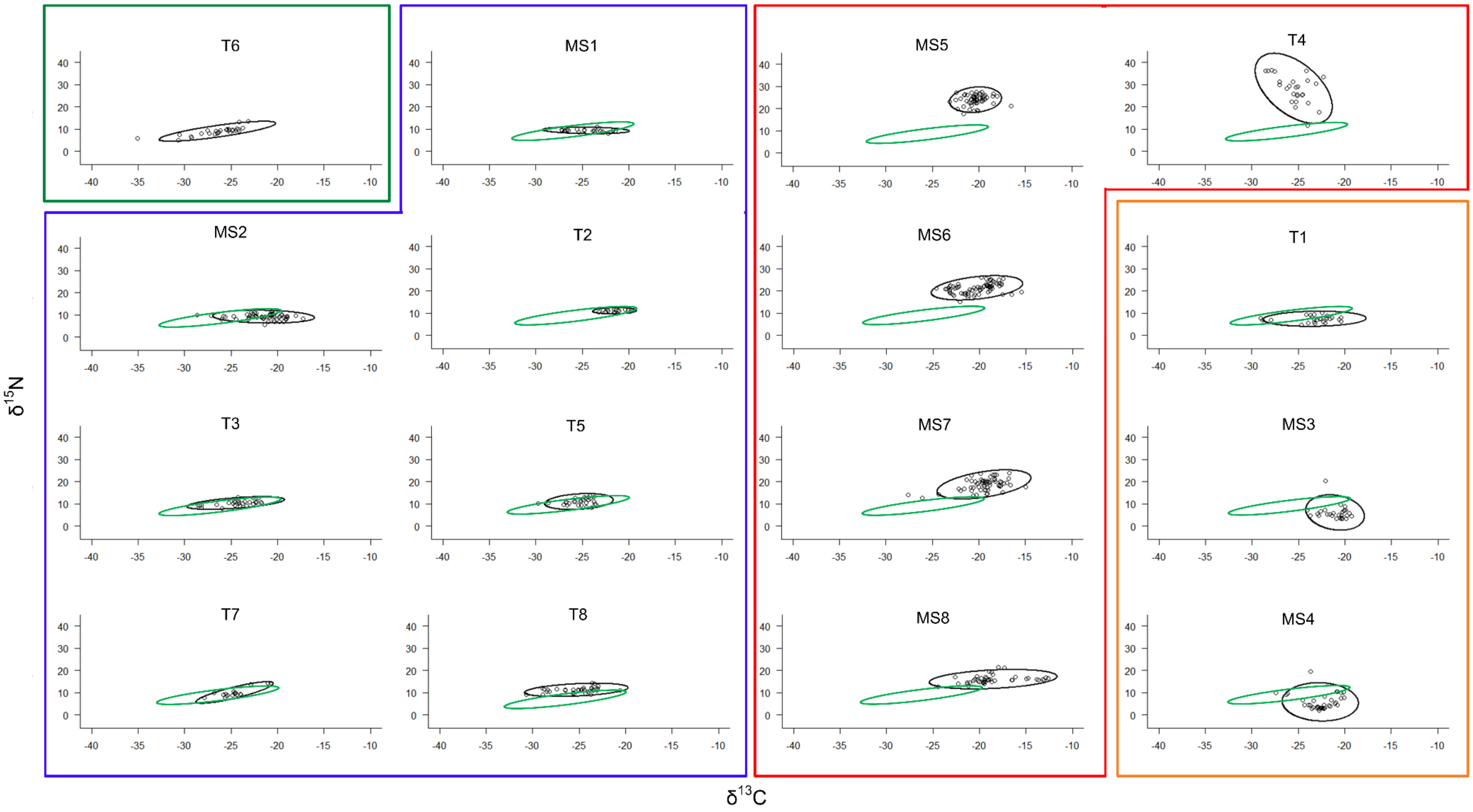


Figure 3. Isotopic niche of fish communities (evaluated by the standard ellipse area – SEA, with 95% confidence interval) in the 16 sites sampled in the dry season at the tributaries (T1 to T8) and main stem (MS1 to MS8) of the Rio das Velhas basin. Green ellipses represent the isotopic niche of the reference site (T6) in the dry season. Reference site = green box, sites with low NO_3^- and NH_4^+ = blue box, sites with high NO_3^- = red box, sites with high NO_3^- and NH_4^+ = orange box

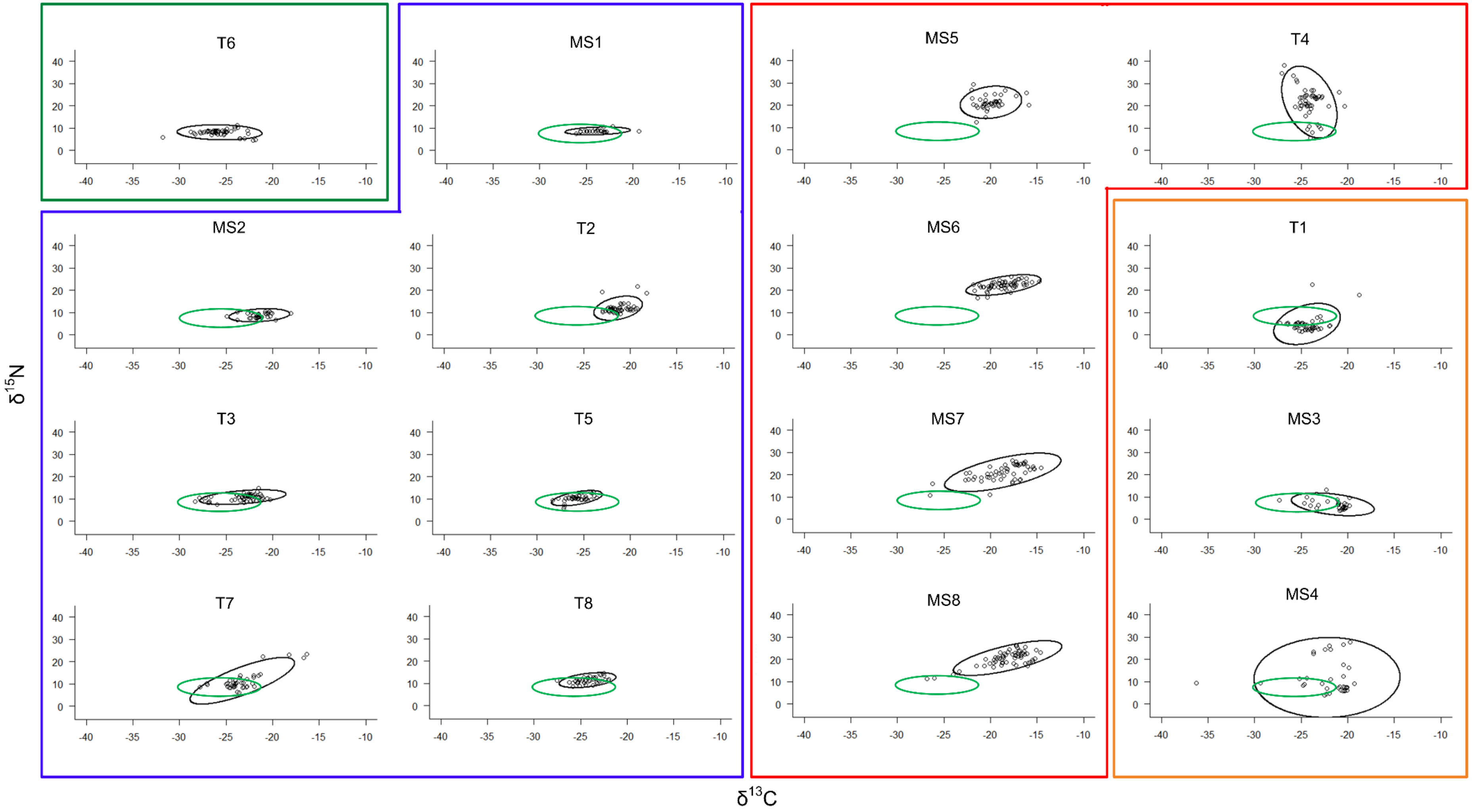


Figure 4. Isotopic niche of fish communities (evaluated by the standard ellipse area – SEA, with 95% confidence interval) in the 16 sites sampled in the wet season at the tributaries (T1 to T8) and main stem (MS1 to MS8) of the Rio das Velhas basin. Green ellipses represent the isotopic niche of the reference site (T6) in the wet season. Reference site = green box, sites with low NO_3^- and NH_4^+ = blue box, sites with high NO_3^- = red box, sites with high NO_3^- and NH_4^+ = orange box

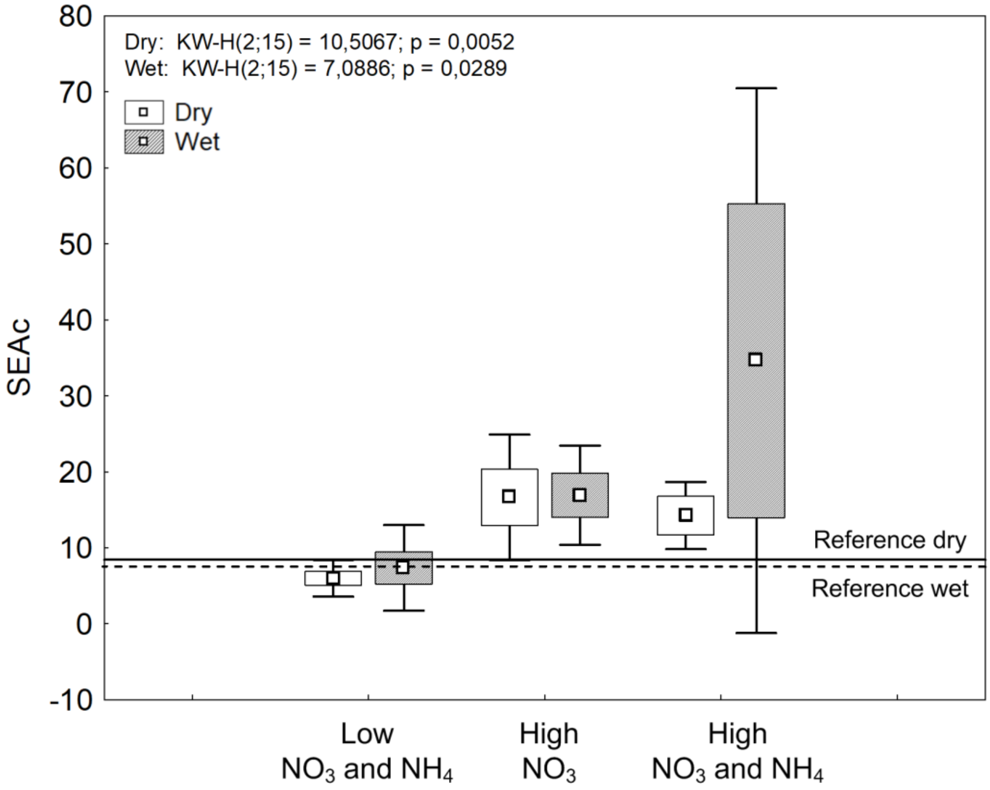


Figure 5. Mean and standard deviation of the isotopic niche (SEAc) of fish communities sampled in dry and wet seasons at sites with low NO_3^- and NH_4^+, high NO_3^- and high NO_3^- and NH_4^+ concentrations in the Rio das Velhas basin. Values of SEAc of the reference site (T6) are indicated with dashed (wet season) and solid (dry season) lines

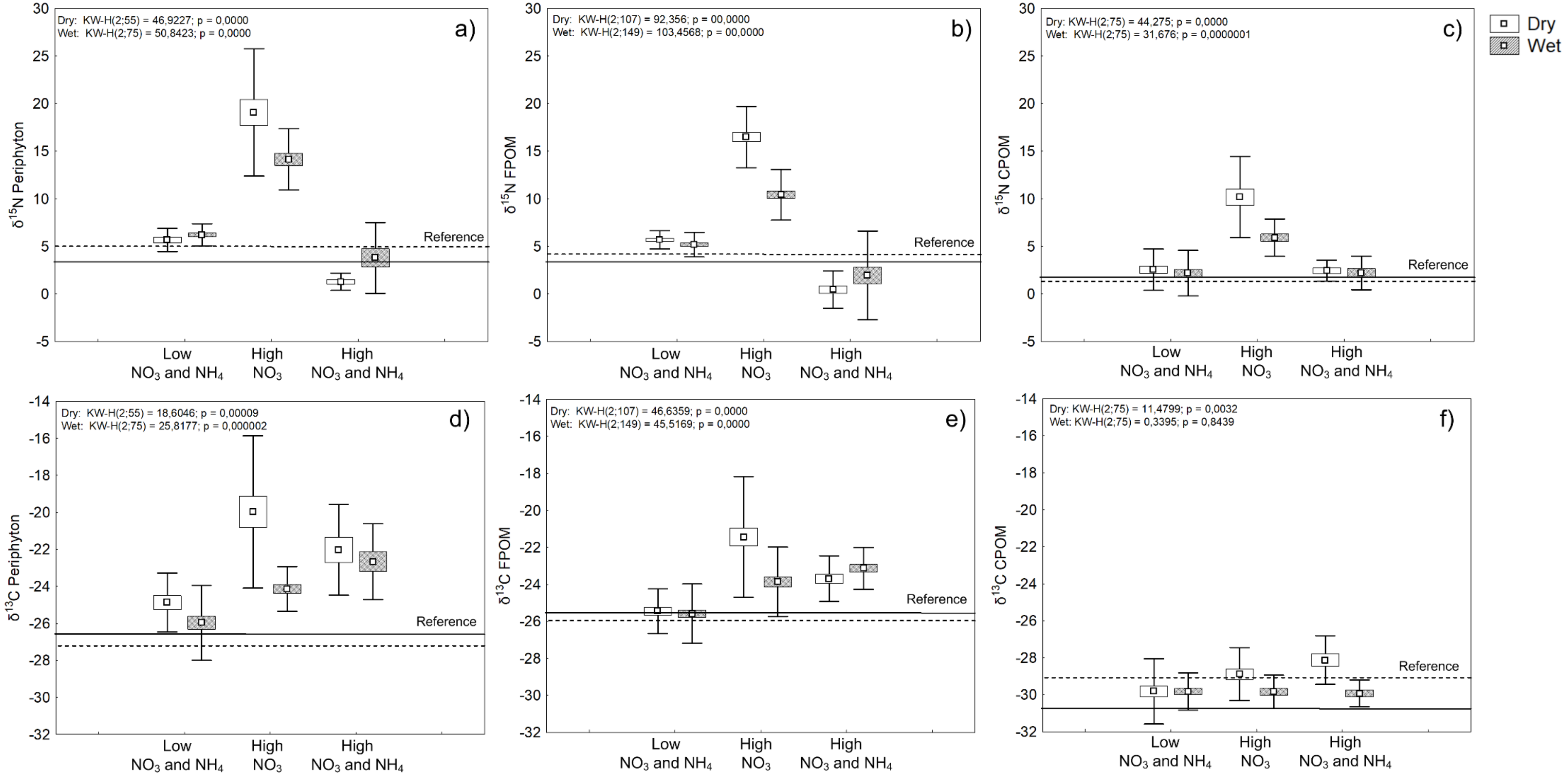


Figure 6. Mean and standard deviation of $\delta^{15}N$ (a, b and c) and $\delta^{13}C$ (d, e and f) of periphyton, CPOM and FPOM sampled in dry and wet seasons at sites with low NO_3^- and NH_4^+, high NO_3^-, and high NO_3^- and NH_4^+ concentrations in the Rio das Velhas basin. Mean values of $\delta^{15}N$ and $\delta^{13}C$ of the reference site (T6) are indicated with dashed (wet season) and solid (dry season) lines

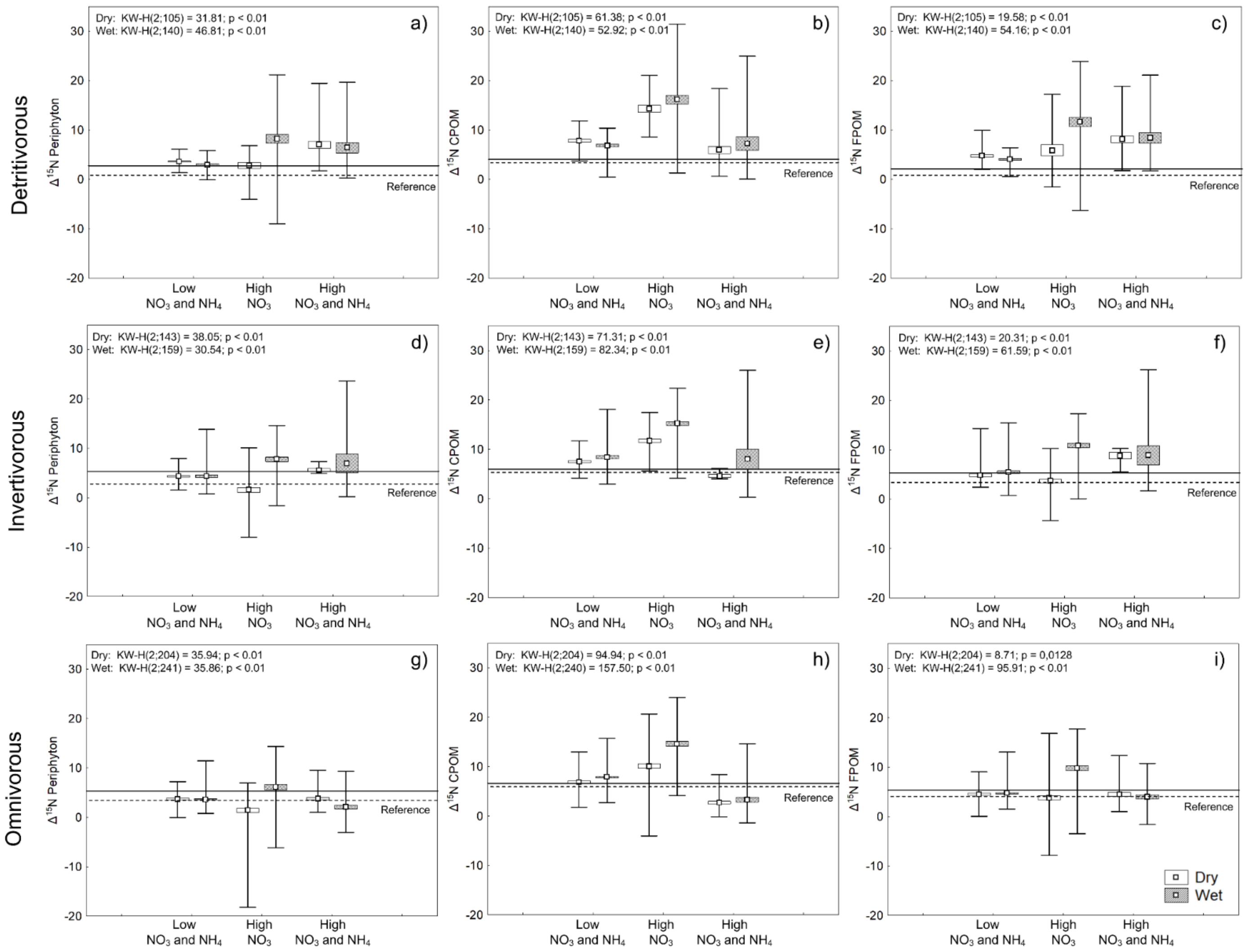


Figure 7. Delta ^{15}N (Δ^{15}N) calculated based on detritivorous (a, b and c), invertivorous (d, e and f), and omnivorous (g, h, and i) fishes and periphyton, CPOM and FPOM sampled in dry and wet seasons at sites with low NO_3^- and NH_4^+, sites with high NO_3^-, and sites with high NO_3^- and NH_4^+ concentrations in the Rio das Velhas basin. Box = Mean ± Standard Error (SE); Whisker = Min-Max

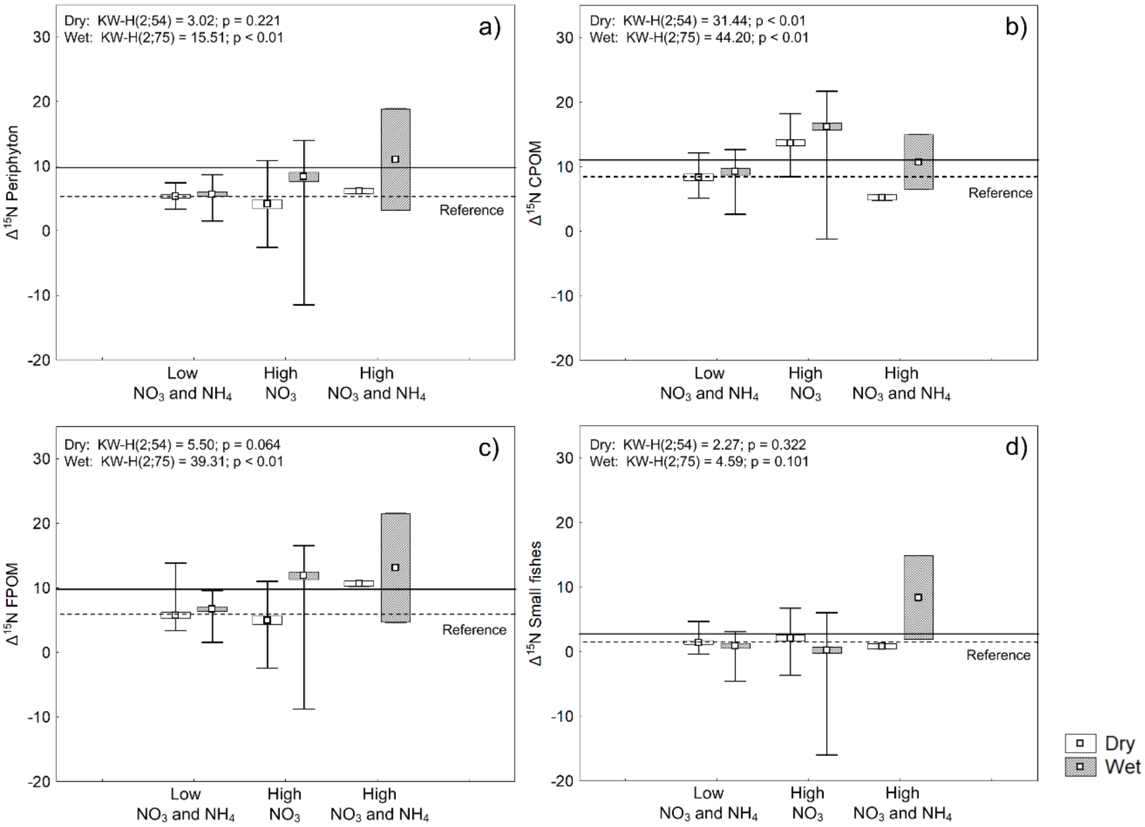


Figure 8. Delta ^{15}N (Δ^{15}N) calculated based on piscivorous fishes and periphyton (a), CPOM (b), FPOM (c) and small fishes (d) sampled in dry and wet seasons at sites with low NO_3^- and NH_4^+, sites with high NO_3^-, and sites with high NO_3^- and NH_4^+ concentrations in the Rio das Velhas basin. Box = Mean ± SE; Whisker = Min-Max

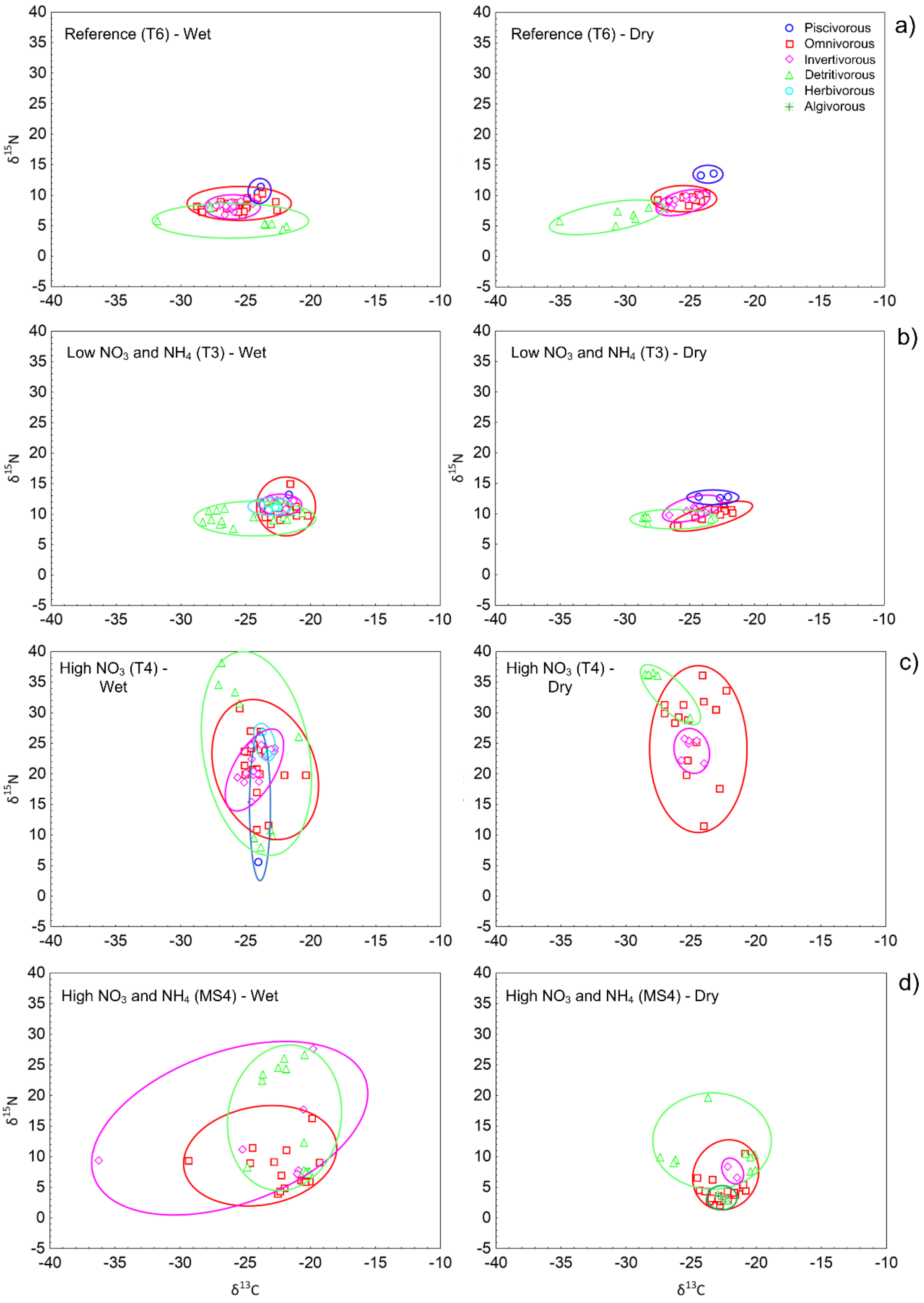


Figure 9. Food web structure of the (a) reference site - T6, (b) one site with low NO$_3$ and NH$_4^+$ concentrations - T3, (c) one site with high NO$_3$ – T4, and (d) one site with high NO$_3$ and NH$_4^+$ concentrations – MS4, in wet and dry season

Supplementary material

Table A1. Geographic location and description of each site sampled in the main stem and tributaries of Rio das Velhas basin

	Sites	Coordinates	Elevation (m)	Group	Description
Tributaries	T1	23K 605473 E 7823991 N	684	High NO_3 and NH_4	Polluted tributary. Receives a large amount of sewage, both from nearby cities and from several industries in the region (especially cement industries). Bad water quality and high toxic contaminants.
	T2	23K 632551 E 7832196 N	677	Low NO_3 and NH_4	Tributary in good conditions of conservation. Good water quality and low toxic contaminants.
	T3	23K 614931 E 7847433 N	651	Low NO_3 and NH_4	Tributary in good conditions of conservation. Good water quality and low toxic contaminants.
	T4	23K 599696 E 7871820 N	621	High NO_3	Polluted tributary. Receives untreated sewage from a large city (Sete Lagoas). Medium water quality and medium toxic contaminants.
	T5	23K 578454 E 7893841 N	632	Low NO_3 and NH_4	Tributary in intermediate conditions of conservation. Medium water quality and low toxic contaminants.
	T6	23K 641315 E 7861707 N	757	Reference	The most pristine tributary due to the location of its headwaters in the Serra do Cipó National Park. Presents good water quality and absence of toxic contaminants. Reference area in the basin.
	T7	23K 605789 E 7933699 N	567	Low NO_3 and NH_4	Tributary in intermediate conditions of conservation. Medium water quality and low toxic contaminants.
	T8	23K 586829 E 8010438 N	518	Low NO_3 and NH_4	Tributary in intermediate conditions of conservation. Medium water quality and low toxic contaminants.

Main stem	MS1	23K 649606 E 7753356 N	1010	Low NO_3 and NH_4	Site located in the headwater of rio das Velhas. Good water quality and absence of toxic contaminants.
	MS2	23K 622454 E 7785916 N	729	Low NO_3 and NH_4	Site with small influence of MRBH. Medium water quality and medium toxic contaminants.
	MS3	23K 618796 E 7806723 N	674	High NO_3 and NH_4	Site highly influenced by the MRBH with high sewage discharge. Bad water quality and medium toxic contaminants.
	MS4	23K 616174 E 7838041 N	658	High NO_3 and NH_4	Site highly influenced by the MRBH. High entrance of sewage. Bad water quality and medium toxic contaminants.
	MS5	23K 589298 E 7920498 N	567	High NO_3	Polluted site under influence of MRBH. Bad water quality and high toxic contaminants.
	MS6	23K 585926 E 7962502 N	552	High NO_3	Polluted site. Presents a partial improvement in the water quality as the distance from MRBH increases. Medium water quality and high toxic contaminants.
	MS7	23K 547752 E 8024649 N	495	High NO_3	Polluted site. Presents a partial improvement in the water quality as the distance from MRBH increases. Medium water quality and high toxic contaminants.
	MS8	23K 519793 E 8097515 N	464	High NO_3	Polluted site. Presents a partial improvement in the water quality as the distance from MRBH increases. Medium water quality and high toxic contaminants.

Table A2. Number of samples of each fish species sampled in Rio das Velhas Basin in dry (D) and wet (W) seasons.

Species	T1		T2		T3		T4		T5		T6		T7		T8		MS1		MS2		MS3		MS4		MS5		MS6		MS7		MS8		Total
	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	
Algivorous																																	
Apareiodon hasemani																			5								5		5		5		20
Apareiodon ibitiensis																							5		5			1		1		5	17
Detritivorous																																	
Bergiaria westermanni																											1						1
Curimatella lepidura																									1		5	5		5		5	21
Harttia longipinna					5						1																						6
Harttia torrenticola						5									5																		10
Hisonotus sp.2															4																		4
Hoplosternum littorale	3	4				1															5	5	5	6	1	2		1					33
Hypostomus auroguttatus				5		3																											8
Hypostomus commersoni	1	7																			5		4	3		1							21
Hypostomus francisci			5			5			4	3		1	1	2	3	1				3					1	3	3	5		3		5	48
Hypostomus lima	2						5	4	1	2									6	2									4				26
Hypostomus macrops																1					1			4	2	1							9
Hypostomus margaritifer													3																	2			5
Hypostomus sp.3															2																		2
Hypostomus sp.5												1				2																	3
Pareiorhaphis mutuca											3																						3
Steindachnerina elegans	3	4		5	1	7	1	5	5	3	2	5		3		5			1				1				5	2					58
Herbivorous																																	
Leporinus taeniatus													1	6		5									1								13
Myleus micans														3												1			5		1		10
Piabarchus stramineus				1		5		2					5	5		5										5			5		5		38

Schizodon knerii																											1			1	1		3
Invertivorous																																	
Astyanax rivularis																	5	5		1													11
Characidium fasciatum																	5	2															7
Characidium zebra											1																		1				2
Cichlasoma sanctifranciscense	5	1								1				4					4				1		5	5	1	1	1	1	1	5	36
Crenicichla lepidota																								1	1								2
Eigenmannia virescens				1	5	6			2		5	6		2	5	5									5		5	2			1	5	55
Gymnotus carapo																								2	1								3
Hasemania nana		5																															5
Hemigrammus marginatus							5												4			2						1	5	1			18
Lepidocharax burnsi			5	5		5		5	5	5	5	5			4	5			5	5													59
Leporellus vittatus																													3	1		2	6
Leporinus amblyrhynchus													1																				1
Leporinus marcgravii										3		5																					8
Neoplecostomus franciscoensis																	5	5															10
Orthospinus franciscensis																												1			1	2	4
Pachyurus francisci																												1				2	3
Phenacorhamdia tenebrosa					5																												5
Pimelodus fur				2									2	4													2		3				13
Pimelodus pohli							1	5																1	1		5	4		1		4	22
Roeboides xenodon																											2	1				1	4
Tetragonopterus chalceus																											1	1		1	1		4
Trachelyopterus galeatus								5																1	2	1				2			11
Triportheus guentheri																									3	1	5	5	5	5		5	29
Omnivorous																																	
Astyanax eigenmanniorum					5		4																										9

Astyanax fasciatus				3		1		3				1																				1	9
Astyanax intermedius																	5																5
Astyanax lacustris	2			1		5		2											4	2	1	3	1		5	5	3	1	5	3	5	3	51
Astyanax taeniatus				5		5	5	5	2	5		5		4	3	3		5	7	6	2	1	1	2	6		5	1	6		5		89
Australoheros facetus								1		1		3																					5
Callichthys callichthys																					1	5		5									11
Coptodon rendalli	3	5		5																					5								18
Knodus moenkhausii																			5				6		5								16
Moenkhausia costae																							1	1	1		5	1		1	1		11
Oreochromis niloticus		1																			5	5	8	5		5	2	1		1	5	3	41
Phalloceros uai	5	5		5		5	4	5				5							2	1						5	5			5		4	56
Piabina argentea			5	5	4	5			5	5	5	5	5		5	5				3									5	5		5	72
Poecilia reticulata		6		5			2	4		5		5							1		5	5	5	4	1			1			5		54
Rhamdia quelen								2		2								2	2	6													14
Serrapinnus heterodon																			2		3	1					1	5		1			13
Serrapinnus piaba		5									5											1									5		16
Piscivorous and parasites																																	
Acestrorhynchus lacustris														5													2	1	1				9
Anchoviella vaillanti																												5	1	5	1		12
Homodiaetus sp.											2																						2
Hoplias intermedius				6	4	1		2	4	3		2	1	2	3	5			2	1					3	3	4	4	1			1	52
Hoplias malabaricus	2																		7	1		1											11
Lophiosilurus alexandri																															1		1
Oligosarcus argenteus																	3	4															7
Pygocentrus piraya																											2	1	2	4		1	10
Serrasalmus brandtii		1																							2	5	5	5	3	4		3	28
Synbranchus marmoratus																														1			1

Total	26	44	15	54	29	59	27	50	28	38	29	49	19	40	34	42	23	23	57	31	28	29	38	35	57	43	75	57	61	54	44	62	1300
Species	9	11	3	14	7	14	8	14	8	12	9	13	8	11	9	11	5	6	15	11	9	10	11	12	21	14	23	25	18	22	16	19	72

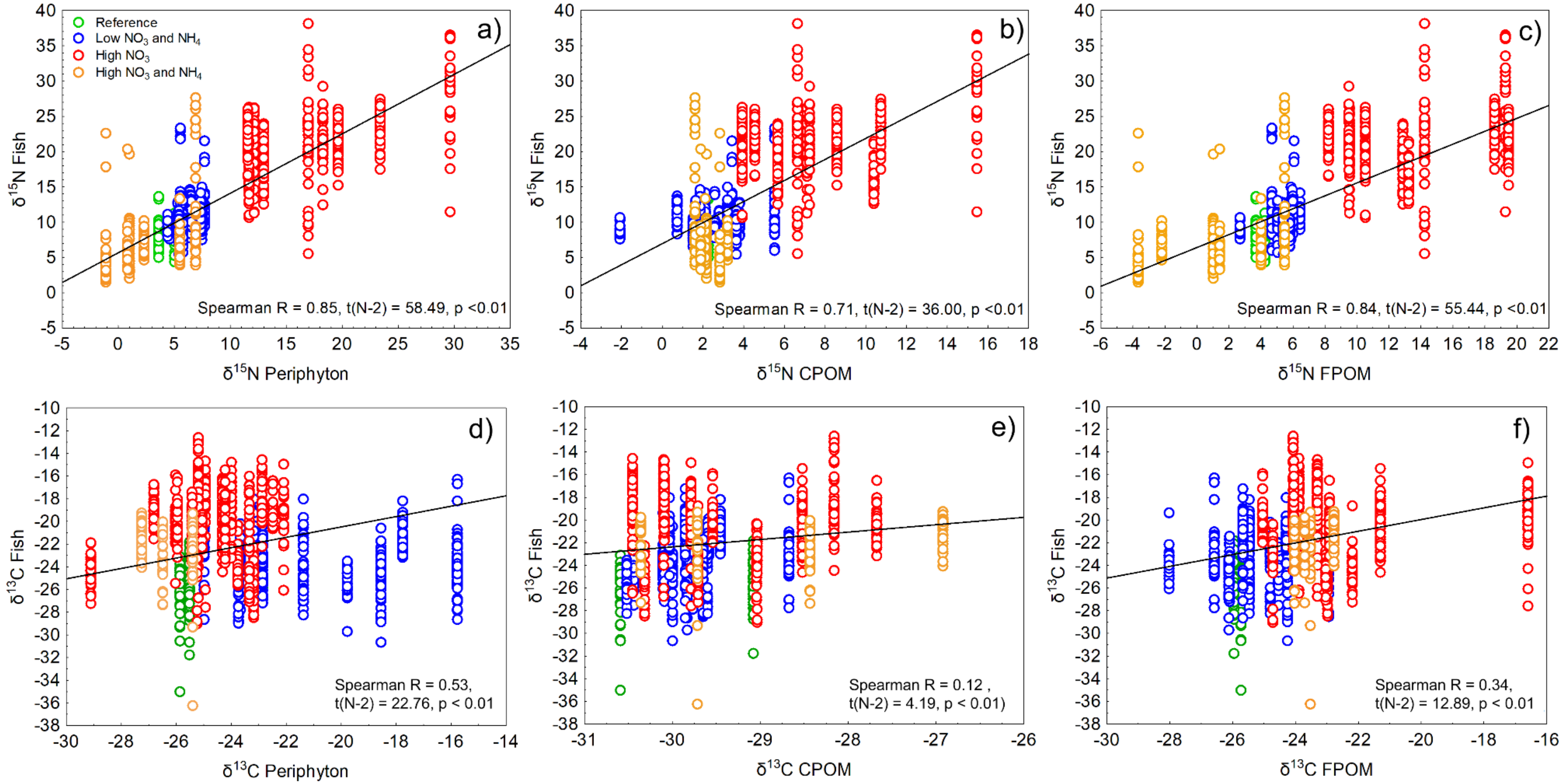


Figure A1. Relationship between δ^{15}N (a, b and c) and δ^{13}C (d, e and f) of basal resources (periphyton, CPOM and FPOM) and δ^{15}N of fishes sampled at Rio das Velhas basin

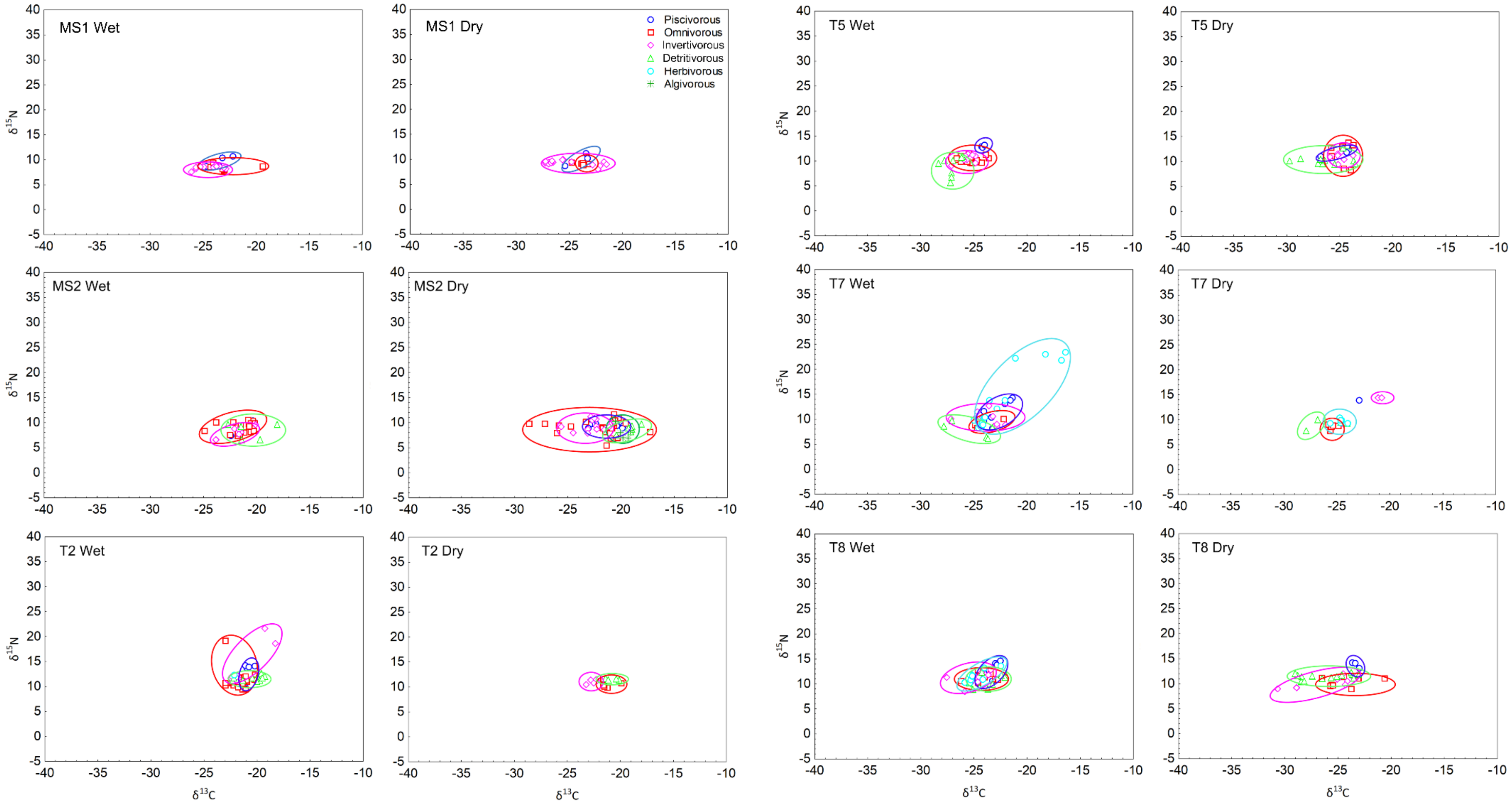


Figure A2. Food web structure of the remaining sites (not represented in the Figure 9) with low NO_3 and NH_4^+ concentrations in wet and dry seasons

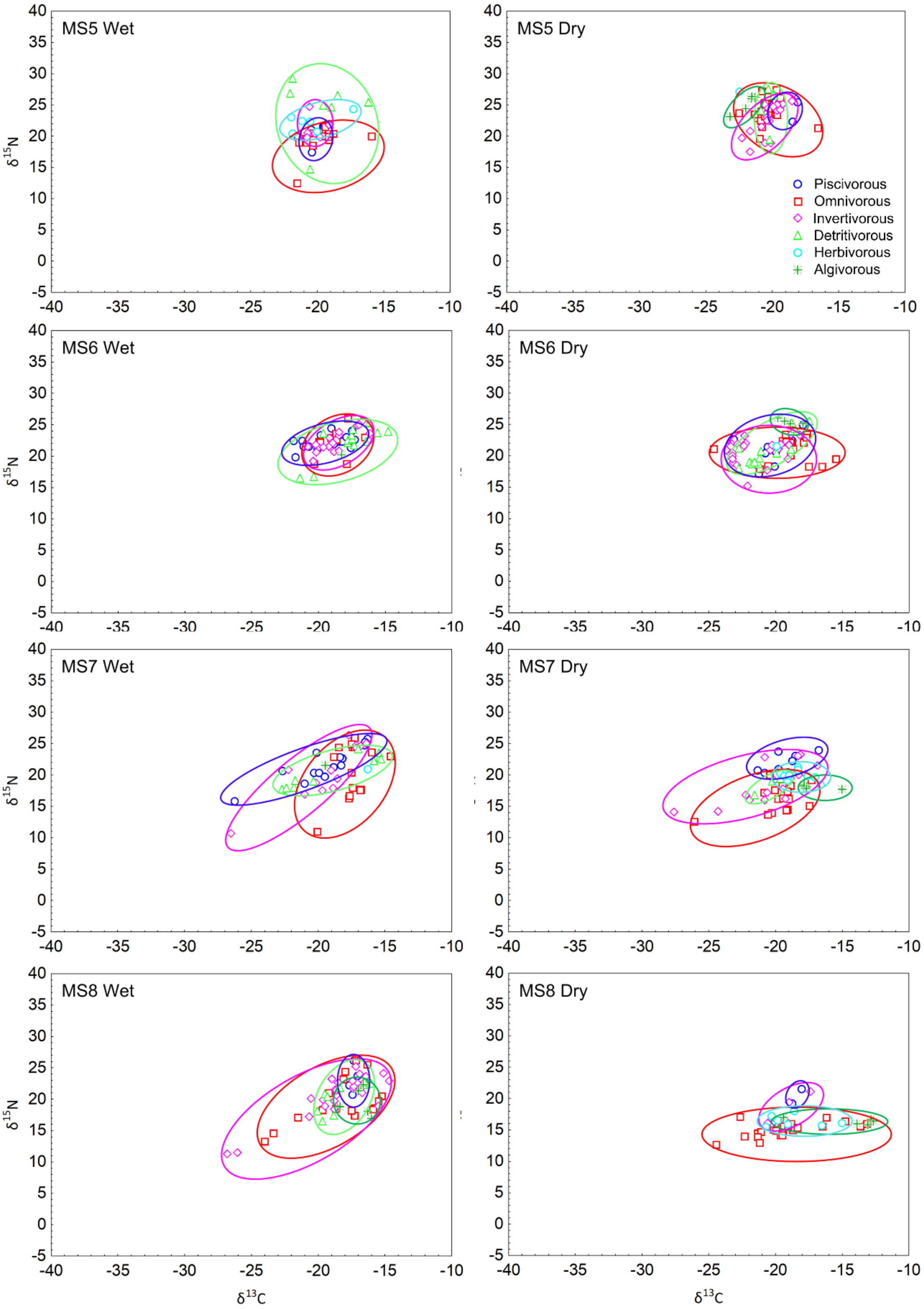


Figure A3. Food web structure of the remaining sites (not represented in the Figure 9) with high NO_3^- concentrations in wet and dry seasons

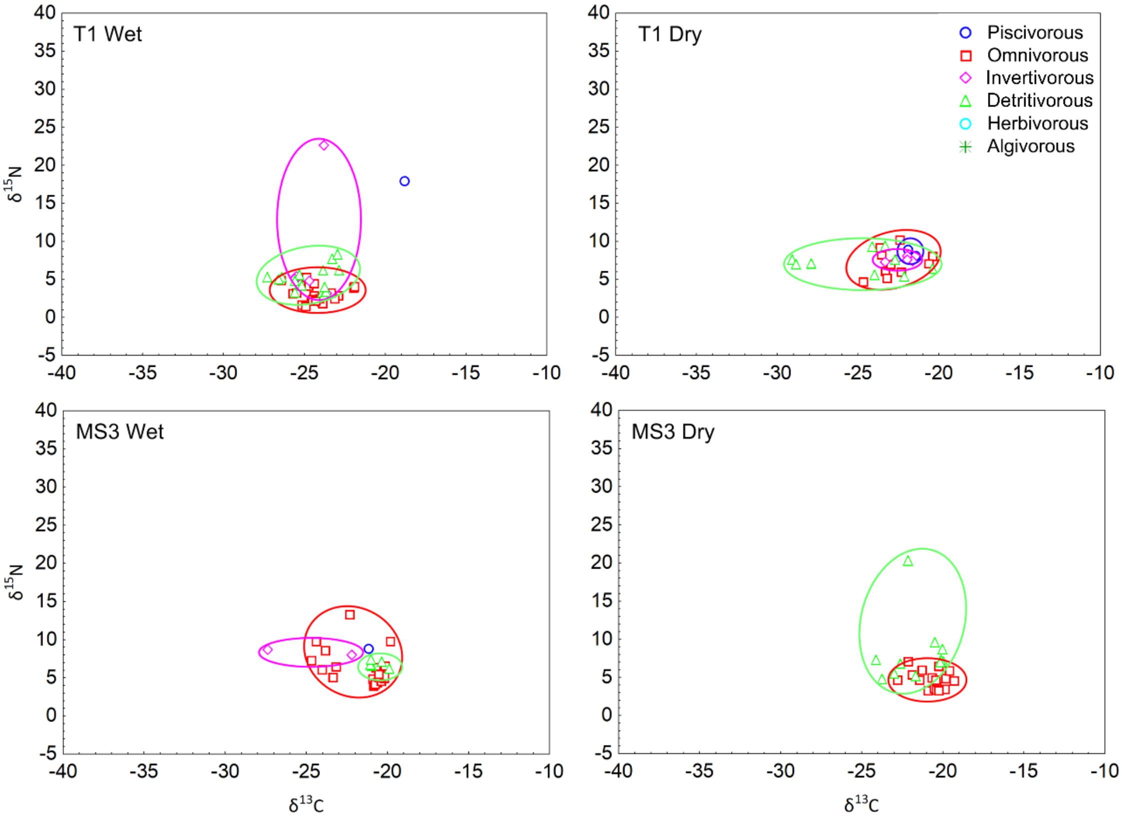


Figure A4. Food web structure of the remaining sites (not represented in the Figure 9) with high NO_3^- and NH_4^+ concentrations in the wet and the dry seasons

ARTIGO IV - ***Trophic diversity and carbon sources supporting fish communities along a longitudinal gradient in a polluted Brazilian river***

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Manuscrito redigido na língua e formato indicado para revista *Freshwater Biology*

(número de linhas foi retirado para melhor adequação ao formato da tese)

Abstract

In natural environments it is expected an increase on the number of species and trophic diversity along a longitudinal gradient of a river, from the headwaters to the river mouth. However, anthropogenic activities can display a great influence in the water quality and in the availability of habitats and resources, which can be reflected in aquatic organisms and change the expected patters. The objective of this study was to evaluate if the trophic diversity and the main carbon sources sustaining fish communities along a river gradient are influenced by the presence of pollution. The study was developed in eight sites distributed along a Brazilian river highly impacted by anthropogenic activities, especially discharge of domestic and industrial sewage, from a metropolitan region with more than five million human inhabitants. We calculated six metrics of trophic diversity and the main carbon sources sustaining fish communities through the data of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope rations of all fish species and its main food sources. Pollution promoted remarkably changes on trophic guilds, carbon sources and trophic diversity along the river gradient. Autotrophic resources were the main carbon sources to all trophic guilds in all sites, but the sewage-derived organic matter was an important carbon source to the fish community in the most polluted site. Trophic diversity increases along the river gradient; however, the presence of pollution promotes remarkably changes in all metrics independent of the number of species. Fish communities from the most polluted sites exhibited great carbon range and nitrogen range, large total area, high trophic diversity, small trophic redundancy and less even distribution of trophic niches. We conclude that under conditions of high pollution, other factors not necessarily reflecting trophic behaviour appear to influence the trophic diversity, as the existence of multiple isotopically distinct sources that have not been sampled creating a challenging isotopic baseline.

Keywords: Stables isotopes; Nitrogen; Sewage, Rio das Velhas; Aquatic pollution.

1 Introduction

One of the key stones in aquatic ecosystems studies is the understanding on the origin and flow of energy and matter along food webs. At least three theories, “River Continuum Concept” (RCC), “Flood Pulse Concept” (FPC), and “Riverine Productivity Model” (RPM), try to explain how the energy flow changes along the longitudinal and altitudinal gradient in natural lotic environments. According to the RCC (Vannote *et al.*, 1980), the input of allochthonous material in headwaters far exceeds the local primary production due to the presence of higher percentages of vegetal cover which contributes with large amounts of allochthonous detritus and reduces autotrophic production by shading. As stream width increases, the lower importance of terrestrial organic input coincides with enhanced significance of autochthonous primary production and organic transport from upstream (FPOM - fine particulate organic matter from processing of dead leaves and woody debris). Large rivers still receive quantities of FPOM from upstream, but the autotrophic production may often be limited by depth and turbidity (Vannote *et al.*, 1980). The importance of the connectivity and carbon sources flow from adjacent flooded areas was only considered in the FPC (Junk, Bayley & Sparks, 1989), which proposed that long and predictable pulses in discharge are “the major force controlling biota in river-floodplains". The major tenet of the RPM (Thorp & Delong, 1994), in turn, is that a substantial portion of the organic carbon actually "assimilated" by animals in certain types of large rivers is derived from a combination of local autochthonous production and direct inputs from the riparian zone during periods not limited to flood pulses. The authors also assume that in floodplain rivers instream primary productivity (especially from phytoplankton) is a significant contributor to secondary productivity.

Despite of the lack of consensus in determine the energy flow from upstream to downstream, it is expected that the changes in the river dimensions, and in the availability of habitats and resources, will be reflected in aquatic organisms along a longitudinal gradient of a river. The focus of the majority of studies are the changes in richness and composition (Ferreira & Petrere, 2009; de Carvalho *et al.*, 2015; Lazzarini Wolff & Segatti Hahn, 2017), which are closely related to functional, morphological, and trophic diversity (Petchey & Gaston, 2002; Baiser, Ardeshiri & Ellison, 2011; Junqueira *et al.*, 2012). However, anthropogenic activities can display a great influence in some aspects above mentioned, and promote remarkably changes in the expected patterns along a river.

The nutrient load (coming chiefly from human and animal wastes, detergents, and fertilizers) is one of the impacts of pollution on the quality of water in freshwater ecosystems (Kumaraswamy *et al.*, 2020). Great amounts of nutrients (as ammonium) may be lethal to aquatic organisms (Randall & Tsui, 2002), while smaller quantities of nutrients (as nitrate and phosphorus) do not cause an immediate impact, but accelerate eutrophication (Conley *et al.*, 2009). During eutrophication, excess nutrients act as fertilizer for autotrophic organisms (as algae and aquatic plants) causing plant overgrowth and harmful algal blooms, and, consequently, oxygen depletion from the water. In addition, the ingestion of harmful algae can promote the accumulation of toxins in aquatic organisms' body, which can be passed along the food chain (Mafra Junior, Fernandes & Proença, 2006). For acting on the availability of quality food, suitable habitat and adequate oxygen to survive, the aquatic pollution can profoundly change the structure of fish communities. The increase in autotrophic sources, for example, may favour algivorous, detritivorous and herbivorous guilds. Changes on macroinvertebrates biota (as the increase in chironomids in polluted sites) can be also reflected on fish diet and prey selection (de Carvalho *et al.*, 2019a b). In addition, the hypoxic condition can be lethal to some aquatic organisms, and may act by selecting organisms resistant to this condition (as exotic species).

By changing the richness and composition, the resources availability and the abundance of trophic guilds, aquatic pollution can consequently promote alterations on the trophic diversity along a river gradient. Therefore, the objective of this study was to evaluate if the trophic diversity and the carbon sources sustaining fish communities change along a longitudinal gradient in a river impacted by pollution. We used the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to test the hypothesis that at the sites close to the headwater (and before the sewage discharge), the allochthonous sources are the main carbon sources supporting the fish communities. Moreover, at these sites it is expected a high abundance of invertivorous and herbivorous fishes due the abundance of vegetation and insects from the riparian vegetation. At the sites under influence of big cities and influenced by discharge of domestic sewage, the hypothesis is that the autotrophic sources will be the main carbon source. It is also expected that the organic matter from sewage can be an important carbon source to the fish communities, as observed by de Carvalho et al. (2019b) for a poecilid species. At these sites, the algivorous, detritivorous and omnivorous fishes should be more abundant than the other trophic guilds due the abundance of autotrophic sources as algae and macrophytes. It is expected that at sites downstream the large cities and close to the river mouth, the autotrophic sources remain an important carbon source, as well as allochthonous resources carried from riparian vegetation during flood pulses.

Considering that it is expected natural changes along a gradient in a river (due the increase in the number of species and changes in the habitat structure from upstream to downstream), we calculated the metrics proposed by Layman et al. (2007) to test if the trophic structure of each site is related to the number of species, or if it is influenced by the presence of cities and discharge of sewage. We tested the hypothesis that the trophic structure and trophic ecology in each site are more related with the presence of pollution than with the number of species. It is expected that fish communities from polluted sites should exhibit smaller carbon range (species feeds from the same basal sources), smaller nitrogen range (species feeds in similar trophic levels), smaller total area, lower trophic diversity, more trophic redundancy and more even distribution of trophic niches in the fish communities.

2 Material and Methods

Study area

This study was conducted in the main stem of the Rio das Velhas basin, the largest tributary of the São Francisco River basin (Alves & Pompeu, 2010), located entirely in the territory of Minas Gerais state of Brazil, and covering 51 municipalities along its 801Km of extension (Figure 1). The basin, with a drainage area of 29.17 km^2, has estimated average annual flow rate of 300 m^3/s and average width of 38.3 m. The Metropolitan Region of Belo Horizonte (MRBH), the third largest urban conurbation in Brazil with almost 6 million inhabitants (IBGE, 2018) is located at the upper course of the Rio das Velhas. The Rio das Velhas is of significant economic and social importance because is responsible for most of the MRBH water supply.

The main pollution sources are located in the upper course, which receives the majority of domestic and industrial sewage from MRBH (Alves & Pompeu, 2010), with the estimated discharge of 700 liters of sewage per second in the river. In the Arrudas and Onça sub-basins (located at the upper section), there are at least 3125 industries (food, metallurgical, textile, chemical and pharmaceutical industries), of which about 50% have no appropriate treatment for their effluents and solid residues. In addition, much of the river is located in the Iron Quadrangle, a critical area because of numerous mining projects (Machado, Alves & Callisto, 2008). The middle and lower section of Rio das Velhas basin are also impacted by anthropogenic activities, including agriculture and industry. Nevertheless, discharge of domestic sewage from small cities is among the main pollution sources in the middle and lower sections.

Sampling design

We sampled a total of eight sites distributed along the main stem (site 1 to 8) of the Rio das Velhas basin (Figure 1). Sampling was performed in July of 2015 and 2016, totalizing two samplings in the dry season. With exception of the site 1 and 2, all sites along the main stem are influenced by the anthropogenic activities. The site 1 in located in the headwater of Rio das Velhas, and presents good water quality and absence of toxic contaminants. The site 2 is located before the MRBH, presenting medium water quality and medium toxic contaminants levels. The sites 3 and 4 are the sites highly influenced by the MRBH, by receiving a significant load of sewage, and consequently, presenting bad water quality and toxic contaminants. The site 5 is also a high polluted site, under the influence of MRBH and Ribeirão Jequitibá, a tributary that receives untreated sewage from a large city (Sete Lagoas). Therefore, exhibits bad water quality and high toxic contaminants levels. The sites 6, 7 and 8 are also polluted sites that presents a partial improvement in the water quality as the distance from MRBH increases. Both present medium water quality and high toxic contaminants.

Two Sewage Treatment Plants (STP’s) located in the Ribeirão Arrudas and Ribeirão da Onça, important tributaries that drains the city of Belo Horizonte and reach the Rio das Velhas before the site 3, were also sampled to obtain the isotopic composition of the sewage-derived particulate organic matter before and after the treatment.

Sampling

At each site we collected five samples for stable isotope analyses of: (a) each fish species (wherever possible); (b) all basal resources available (as periphyton/biofilm; filamentous algae; macrophytes; coarse particulate organic matter - CPOM; fine particulate organic matter – FPOM;

suspended matter; riparian vegetation and grasses). Storage and processing of resource samples occurred according to specific methodology detailed below. For isotopic analysis, we collected at least 2-5 mg of dry material from animal tissue and 5-10 mg of other organic samples. Upon completion of the preparation of the material, samples stored in *Eppendorf* tubes were forwarded to the Center for Nuclear Energy in Agriculture (CENA) at the University of São Paulo (USP) for isotopic analysis.

Fish

Fish were collected with gillnets (20 m long, with 3–16 cm stretch measure mesh), seines (5 m long, 1 mm mesh), cast nets (3 cm stretch measure mesh), and mosquito nets (80 cm in diameter, 1 mm mesh). Gill nets were fixed in the water column for 14 h overnight. Seines were used in shallow areas or littoral zones, mosquito nets were employed in near-shore aquatic macrophytes (both shorelines), undercut banks and in riffles, and cast nets were used in habitats too deep to wade. The three latter methods were employed for 1–3 h. In the field, after a preliminary identification, some specimens had a portion of muscle removed for isotopic analysis. The samples destined to isotopic analysis were kept frozen until laboratory processing to avoid deterioration of the material. In the laboratory, specimen identifications were confirmed, and the fish samples were lyophilized for 24 hours, and then ground to a fine homogeneous powder using a mortar and pestle. The remain specimens were fixed in formaldehyde 10% in field and preserved in 70% ethanol in laboratory.

All fish species were classified into trophic guilds (detritivorous, algivorous, herbivorous, omnivorous, invertivorous/insectivorous, and piscivorous/parasites) according the available literature (see supplementary material, Table A1).

Basal Resources

Periphyton (or biofilm) was collected by scraping stones using a small brush and was stored in distilled water. Samples of CPOM, filamentous algae and macrophytes were collected haphazardly on the riverbed of all sites. FPOM samples from sediment was obtained by resuspending the sediment and passing the suspended material through a phytoplankton net (45 µm mesh). Suspended matter (ie., seston) was obtained by fixing a phytoplankton net (45 µm mesh) in the water column for a period of three minutes. Riparian vegetation and grasses were collected haphazardly on the river banks. After collection, all samples were stored in plastic sample jars and held on ice for one to five hours prior to storage at a freezer. In the laboratory, periphyton, FPOM, and suspended matter samples were filtered through quartz fiber filters (Whatman® QMA quartz filters) using a filtration apparatus attached to a vacuum pump. All basal resource samples were oven dried at 60°C for a minimum of 48 hours. Afterwards, they were ground to a fine homogeneous powder using a mortar and pestle.

Data analyses

We analyzed a total of 1096 samples for C and N stable isotopes, including 578 individuals of 61 fish species (Table A1), 80 periphyton samples, 58 algae samples, 45 macrophytes samples, 80 CPOM samples, 79 FPOM samples, 76 suspended matter samples, 40 riparian vegetation samples, 40 grasses samples and 20 sewage samples from STP'S. To determine the isotopic ratio, a mass spectrometer system in the Continuous-flow (CF-IRMS) mode was used with a Carlo Erba elemental analyzer (CHN 1110) coupled to a Delta Plus mass spectrometer (Thermo Scientific). Results were expressed as the relative difference of international reference standards, in the delta notation (δ ‰), and calculated using the following formula:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^{3}$$

where X is ^{13}C or ^{15}N and R represents the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie & Prosser, 1996).

Statistical analysis

To evaluate the food web structure of fish communities in each site along the gradient of pollution, individuals of all fish species were plotted in *bi-plot* space according to the isotopic values of carbon (x-axis) and nitrogen (y-axis). Source contributions to fish diets were estimated for the eight sites based on stable isotope data analysed with Bayesian stable isotope mixing models (Moore & Semmens, 2008; Parnell *et al.*, 2010), specifically using the MixSIAR package in R (Stock and Semmens, 2016). It was conducted a Markov chain Monte Carlo sampling based on the following parameters: number of chains = 3; chain length = 100,000; burn in = 50,000; thin = 50 and model 4 (Resid*Process) error structure (Stock and Semmens, 2016). Diagnostic tests (Gelmin–Rubin, Heidelberger–Welch and

Geweke) and trace plots were examined for model convergence. The fractionation values used for consumers were 0.4 ± 1.3‰ for carbon and 2.54 ± 1.27‰ for nitrogen per trophic level (Post, 2002; Vanderklift & Ponsard, 2003). Only basal resources considered potential food sources to fish communities (evaluated through *bi-plot* figures) were used in the mixing models. For invertivorous fishes, due the absence of samples of terrestrial and aquatic insects, the fractionation values were multiplied by two. To omnivorous fishes, that consumes both insects and basal resources, it was considered the fractionation values multiplied by two to resources that are assimilated indirectly (trough insects) and by one to resources that are assimilated directly (as algae and macrophytes). For piscivorous and parasites fishes we considered the other fishes as food sources.

Six metrics originally proposed by Layman et al. (2007) and reformulated in a Bayesian framework by Jackson et al. (2011) were calculated, enabling a comparison of the structure and trophic ecology of each site through the use of the Stable Isotope Bayesian Ellipses package in R (SIBER, Jackson et al., 2011). The carbon range (CR) represents the variation in the x-axis and indicates the variety of resources used by the community. The nitrogen range (NR) provides a vertical representation of the structure of a food web (y-axis), and indicates the distance between the most and least enriched values of δ^{15}N consumers. The mean distance to centroid (CD) provides a measure of the average degree of trophic diversity within a trophic web and is calculated from the Euclidean distance of each species to the centroid. The mean nearest neighbour distance (MNND) represents the mean of the Euclidean distances to the nearest neighbour for each species in the *bi-plot* space and it is a measure of the total density of the community. Small MNND indicates greater trophic redundancy, i.e. food webs with a large proportion of species with similar trophic ecologies. The standard deviation of the nearest neighbour distance (SDNND) provides a measure of evenness of spatial density and packing of species in the bi-plot space that is less influenced than the MNND by the sample size. Low SDNND values suggest a more uniform distribution of trophic niches.

3 Results

Abundance and richness of trophic guilds

We sampled a total of 13634 fish specimens contemplating 71 species (supplementary material, Table A1). Mostly of the species were classified as invertivorous (24 species), followed by omnivorous (19 species), detritivorous (12 species), piscivorous (9 species), herbivorous (4 species), algivorous (2 species) and parasite (1 species). It was observed changes in the proportion of each guild along the gradient of pollution (Figure 2). At the headwater (site 1 – without pollution), more than 50% of the species were invertivorous. At the site immediately before the MRBH (site 2), the detritivorous fishes represented 60% of the community. At the sites located in the MRBH (sites 3 and 4), where occurs the higher discharge of sewage, most of fish species were detritivorous and omnivorous. At the subsequent sites, there was an increase in the number of invertivorous, herbivorous and piscivorous fishes. Piscivorous fishes were not sampled at the two most polluted sites (sites 3 and 4) and the highest number of piscivorous species were observed at the sites 1 and 7. Parasite species were only observed at the site 7.

Trophic structure and proportion of resources assimilated

The distribution of fish species and trophic guilds in the *bi-plot* space varied along the gradient upstream – downstream in the Rio das Velhas basin (Figure 3). Some basal resources do not seem to be consumed by fishes, such as grasses and riparian vegetation (therefore, these resources were not considered in the MixSIAR analysis). The FPOM exhibited similar isotopic compositions to the suspended material in almost all sites, and were pooled in the MixSIAR analysis (and classified only as FPOM). The isotopic composition of the sewage before the treatment (raw sewage - RS) was also similar with the sewage after the treatment (treated sewage -TS), and were pooled in the MixSIAR analysis and classified only as sewage (SW).

At the least disturbed site (site 1) the δ^{15}N of consumers did not exceed 12‰ and fishes from the same guilds exhibited similar diets with small variation in the carbon axis (Figure 3). Periphyton and FPOM were the main carbon sources for invertivorous and omnivorous fishes (Figure 4, Table A1). At the site 2 (still without influence of domestic sewage), the δ^{15}N of consumers did not exceed 13‰ and the fishes presented a wider distribution in the bi-plot space when compared with the site 1. Algae was the most consumed resource for detritivorous, algivorous, and omnivorous fishes, and the invertivorous fishes exhibited the most varied diet (Figure 4, Table A1). At the polluted site 3, the δ^{15}N of most of fishes did not exceed 13‰, with exception of one individual of a detritivorous species that showed δ^{15}N higher than 20‰. Detritivorous species, that consumed FPOM (37%), periphyton (28%) and sewage (27%) in similar proportions, were more δ^{15}N enriched than omnivorous fishes that consumed mainly periphyton (59%) (Figure 4, Table A1). Similar pattern was observed in the site 4. Again, detritivorous fishes were more δ^{15}N enriched than the other guilds, with one individual showing δ^{15}N around 20‰. However, the main food sources for fishes of all trophic guilds were derived from the sewage, representing 76% of the diet of the detritivorous, 53% of algivorous, 50% of omnivorous and 46% of invertivorous (Figure 4, Table A1). The proportion of sewage derived sources assimilation decreased considerably in the subsequent sites (sites 5, 6 ,7 and 8), while the autotrophic sources (algae, macrophytes and periphyton) and FPOM were the main carbon sources sustaining the fishes (Figure 4, Table A1). At the site 5, the δ^{15}N of fishes varied among 15‰ and 35‰, and gradually decreased at the sites 6, 7 and 8. Migratory species (as the species 10 and 52 in the Figure 3) exhibited δ^{15}N values significantly smaller than resident species.

Piscivorous fishes, were less enriched than fishes from other guilds in the sites 5 and 6 (Figure 3). Invertivorous fishes were potential preys of piscivorous fishes in all sites, while detritivorous fishes were those less predated (Figure 5f, Table A1). Parasites, sampled only at the site 7, exhibited preference by omnivorous and algivorous fishes (Figure 4g, Table A1).

Trophic diversity metrics

The richness and the Layman metrics varied along the river gradient (Figure 5). The carbon range changed according the changes in the number of fish species from upstream to downstream (Figure 5a). The nitrogen range also increased with the number of species, with exception of the site 3, which presented the highest NR, despite the decrease in the number of species (Figure 5b). The total area occupied by species in the isotopic niche showed a substantial increase along the river, and was not always correlated with the number of species in each site (Figure 5c). The trophic diversity (measured by the centroid distance) also increased along the gradient, with the highest values observed in the MRBH (site 3) (Figure 5d). At the sites immediately downstream the MRBH (site 3 and 4) it was also observed the smaller trophic redundancy (high mean nearest neighbor distance - MNND, Figure 5e) and less even distribution of trophic niches in the fish communities (high standard deviation nearest neighbor distance – SDNND, Figure 5f).

4 Discussion

Changes in trophic guilds abundance, carbon sources and trophic diversity were observed along the river gradient, with pollution promoting remarkably changes. The urban pollution from MRBH seems to favor the detritivores and omnivorous species, and negatively affect the piscivorous species. The carbon sources sustaining the fish communities also oscillated along the gradient. The hypothesis that the allochthonous sources could be the main carbon sources supporting fish communities in sites before the MRBH was refuted, since CPOM (and riparian vegetation and grasses) was a carbon source little assimilated by fishes. At the sites under influence of the discharge of domestic sewage, the hypothesis that the autotrophic sources and the organic matter from sewage could be an important carbon source to the fish communities, was confirmed, as well as the hypothesis that at the sites in the lower section of Rio das Velhas the autotrophic sources remain an important carbon source. The hypothesis that the trophic diversity in each site is more related with the presence of pollution than with the number of species was also confirmed. However, all of our prediction (that fish communities from disturbed sites should exhibit small carbon range and nitrogen range, small total area, less trophic diversity, more trophic redundancy and more even distribution of trophic niches) were the opposite of the expected.

Abundance and richness of trophic guilds

Alterations in water quality and habitat conditions commonly result in shifting availabilities of many food resources, and consequently, results in changes in fish communities (Karr, 1981). Therefore, the structure of a community’s trophic guilds may be considered as a good indicator of trophic integrity and is widely adopted in indices assessing the ecological integrity of aquatic environments (Karr, 1981; Henriques *et al.*, 2008; de Freitas Terra, Hughes & Araújo, 2013; de Carvalho *et al.*, 2017b) . Our results indicated that omnivorous and detritivorous fishes are favored by pollution, while piscivorous fishes were absent in highly polluted sites. According to Karr (1981), as a site declines in quality, the proportion of individuals with omnivorous habits increases, which likely is a result of degradation in the food base, especially invertebrates. The generalistic/opportunistic foraging ecology makes the omnivorous fishes successful relative to more specialized foragers (Karr, 1981). Detritivorous fishes may also be favored in degraded sites due the increase in detrital food mainly from the discharge of organic matter and sewage (Terra *et al.*, 2013). The importance of sewage sludge is confirmed to the exotic species, *Poecilia reticulata* (de Carvalho *et al.*, 2019b), that was found in high abundance in the sites influenced by MRBH (see Table A1).

In opposite, the presence of piscivorous fishes (top predators) is a good indicator of biotic integrity, since these populations tends to decline or disappear as the ecological integrity declines (Karr, 1981). Therefore, sites 1, 2, 5, 6, 7 and 8 that presented viable and healthy populations of piscivorous fishes (as *A. lacustres*; *A. vaillanti*; *H. intermedius*; *H. malabaricus*; *L. alexandri*; *O. argenteus*; *P. piraya*; *S. franciscanus*; *S. brandtii*) indicate a relatively healthy and trophic diverse community. Some of these sites also presented a high percentage of invertivorous species (especially the site 1 and 7), which are also a good indicator of biotic integrity, since they are affected by decreased invertebrate prey resulting from riparian vegetation removal and/or sewage discharge (Terra *et al.*, 2013; de Carvalho *et al.*, 2017b). Therefore, the absence of top predators and the small number of invertivorous species in the sites 3 and 4 reflected the poor biotic integrity of sites under the influence of MRBH.

Trophic structure and proportion of resources assimilated

One of the pioneering studies using stable isotopes in Brazil (Araujo-Lima *et al.*, 1986) highlighted the importance of autotrophic sources to detritivorous fishes. In our study, with exception of site 4 (where sewage was the most important carbon source), the autotrophic resources (filamentous algae, periphyton and macrophytes) were the most important resources sustaining the fish from all trophic guilds. In anthropically altered environments, especially when there is a high input of nutrients, it is expected an increase in the abundance of autotrophic sources as algae and macrophytes (Conley *et al.*, 2009), which can result in an increase in the consumption of these resources (Carvalho et al., 2015; de Carvalho et al., 2017a). In highly polluted sites, in turn, the assimilation of sewage-derived suspended particulate matter is disproportionately high, suggesting preferential assimilation. The carbon contribution from sewage origin varied between 46% (invertivorous) and 76% (detritivorous) in fishes downstream the MRBH (site 4), which highlighted the importance of carbon from urban effluents to the adapted fish fauna of these environments. This result is of concern, since the assimilation of sewage-derived particulate organic matter exposes aquatic organisms to high levels of contamination and may represent an important source of contaminants to higher trophic levels (DeBruyn & Rasmussen, 2002), including humans.

As the distance of MRBH increases, the importance of sewage-derived organic matter in the fish diet decreases. However, the great nitrogen enrichment observed in fishes and resources sampled in sites downstream MRBH (sites 5, 6, 7 and 8) is a strong indicative that other activities are also influencing the isotopic compositions of the resident aquatic organisms. Primary producers, as algae, macrophytes, and periphyton are sensitive to changes in environmental conditions (Gaiser et al., 2006), and their $\delta^{15}N$ has been used as pollution tracers in the last few decades (Costanzo *et al.*, 2001; Savage & Elmgren, 2004; Cohen & Fong, 2004; Schubert *et al.*, 2013; Wang *et al.*, 2016). In the present study, the observed $\delta^{15}N$ values close to 35‰ (in macrophytes of site 5) draw attention to possible activities polluting the river close these sites. Despite discharge of domestic sewage from small cities is among the main pollution sources in the middle and lower sections of the Rio da Velhas, other human activities (especially cattle raising and agriculture) directly influence the biotic integrity along the river gradient. Animal manure and sewage are enriched in ^{15}N due to ammonia volatilization during storage (Nikolenko *et al.*, 2018). Consequently, enrichment of $\delta^{15}N$- NO_3^- in manure and sewage (ranging between +5‰ and +35‰) and $\delta^{15}N$- NH_4^+ (ranging between +5‰ and +11‰) is reflected in the primary producers and consumers (Nikolenko *et al.*, 2018).

Similar changes on $\delta^{15}N$ of aquatic organisms were observed in The Grand River in southern Ontario (Loomer *et al.*, 2015). Loomer et al., (2015) observed a $\delta^{15}N$ enrichment in both invertebrates and fish in sites downstream two Municipal Wastewater Treatment Plants (MWTP), while downstream the MWTP with the poorest water quality they observed a depletion in $\delta^{15}N$ values. As mentioned above, usually sewage-derived organic matter is $\delta^{15}N$ enriched (Kendall, 1998), but depleted $\delta^{15}N$ values as those observed in the in the two STP's sampled in this present study is not unusual, and may occur as a consequence of a limited nitrification occurring during sewage treatment (Loomer *et al.*, 2015). Still in the study conducted by Loomer et al., (2015), carbon isotopes tended to be enriched downstream of the treatment plants, as well as observed in our results. Despite $\delta^{13}C$ values are not always reported as a tracer of pollution, it is necessary to consider that waste water is likely to affect carbon cycling by adding a new source of either organic or inorganic carbon into aquatic environments.

Trophic diversity metrics

Trophic diversity is expected to increase with the number of species in environments (Baiser *et al.*, 2011). However, despite the gradual increase in the richness and trophic diversity along the river gradient, we observed an unexpected peak of trophic diversity in a highly polluted site (where only 10 fish species were sampled). One of the hypotheses to explain the high trophic diversity in fish communities from a polluted site is the consumption of resources with variable $\delta^{13}C$ and $\delta^{15}N$. At anthropogenically altered sites it is expected a further inflow of sediments (organic and inorganic) from different sources such as: run-off from rural, agricultural and forested land, channel banks, urban road sediment and construction, sewage treatment stations, and others (Taylor *et al.*, 2008). Wastes from dump sites, landfills, and land spreads are usually washed into water bodies during soil erosion or run-off (Adeyemo, 2003). Therefore, the existence of unsampled resources with different $\delta^{13}C$ and $\delta^{15}N$ can be an explanation to the higher variation in the isotopic composition of fishes in the site immediately downstream the MRBH. Which likely explain the wide area occupied in the *bi-plot* space, as well as the smaller trophic redundancy and less even distribution of trophic niches in the fish communities.

The differences in the fish composition among communities (i.e. high abundance of generalist species in polluted sites) can also explain the high trophic diversity in the most polluted site. Polluted ecosystems usually exhibits more generalist's species (Santoro *et al.*, 2014), which use a wide diversity of resources. A high abundance of omnivorous species, which presents a generalist habit consuming a broad variety of resources, was also observed in our study. There is a tendency for more generalized populations to be more ecologically variable and to exhibit higher niche variation (Bolnick *et al.*, 2007). However, in our study, even the specialist species (e.g., those from *Hypostomus* genus) exhibited broader niches in polluted sites. In addition, the most generalist species (e.g., those from *Astyanax* genus, *Poecilia reticulata*, *Phalloceros uai*) were quite distributed along the sampled sites (Table A1), and exhibited broad niches only in polluted sites (see Alonso et al. 2019, de Carvalho et al. 2019b). Therefore, the availability of unsampled resources with variable $\delta^{13}C$ and $\delta^{15}N$ remains the strongest hypothesis explaining the great trophic diversity in the most polluted site.

Another striking pattern observed in highly polluted sites, was the high nitrogen range (NR). A larger nitrogen range suggests more trophic levels and thus a greater degree of trophic diversity (Layman *et al.*, 2007). Similar large NR are documented to fish communities (de Carvalho *et al.*, 2017a) and aquatic macroinvertebrates (Castro *et al.*, 2016) from streams influenced by sugarcane. However, in opposite to observed in our results, in these studies they observed smaller trophic diversity, and higher trophic redundancy and uniformity in anthropogenic altered sites. In the Rio das Velhas, only the carbon range (CR) oscillated according to the changes in number of species in the river gradient, which suggests that the carbon sources consumed by fish communities are more related with the communities' structure than with the pollution load.

We conclude that changes in the number of fish species, trophic guilds abundance and trophic diversity occurs along Rio da Velhas in response to both the physical characteristics of each habitat and the types of organic matter present. It is important to mention that the presence of unsampled food sources with variable $\delta^{13}C$ and $\delta^{15}N$ can have influence in the trophic diversity metrics in highly polluted sites. The changes on $\delta^{15}N$ values along the river gradient can also provide information about the integrity along the river gradient. However, although the variance in $\delta^{15}N$ values of primary producers and fish can indicate high pollution, identifying sources may be complicated where multiple sources are present with distinct signatures and biogeochemical processes that alter isotopic composition (Kendall, 1998), as likely is the case of Rio das Velhas.

Our results emphasizes the need for further studies on the effects of urban pollution on the aquatic organisms, since species richness and trophic diversity underlie strong linkages between food web structure and dynamics (Baiser *et al.*, 2011). In addition, water pollution can promote damage in several economic (fisheries, livestock and forestry), social (water supply), and ecological functions (groundwater recharge, nutrient recycling, and biodiversity maintenance). Therefore, more ecological and conservation research into pollution is needed given the misalignment between the projected high impact and relatively low research effort (around 5.4% of published articles) (Mazor et al., 2018).

Acknowledgments

We thank the Agência Peixe Vivo and the Comitê de Bacia Hidrográfica do Rio das Velhas CBH - Rio das Velhas) for project financial support and the Projeto Manuelzão for the logistical support. This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance code 001 and D.R.C. sandwich doctorate scholarship granted (88881.190508/2018-01). Thanks to Aline Junqueira Grossi (Federal University of Lavras, UFLA) and Luiza Hoehne (Federal University of Minas Gerais, UFMG) for the support on processing of samples. Thanks to the Benthos Ecology Laboratory (UFMG) and the Laboratory of Fish Ecology (UFLA) who assisted in the processing of samples and infrastructure, and to the Centre for Nuclear Energy in Agriculture (CENA) for their support and partnership in the isotopic analysis. P.S.P. received a research grant and a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (303548/2017-7) and from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (PPM-00237/13).

References

Adeyemo O.K. (2003). Consequences of Pollution and Degradation of Nigerian Aquatic Environment on Fisheries Resources. *The Environmentalist* **23**, 297–306. https://doi.org/10.1023/B:ENVR.0000031357.89548.fb

Alonso M.B., de Carvalho D.R., Alves C.B.M., Moreira M.Z. & Pompeu P.S. (2019). Changes in trophic characteristics of two fish species of Astyanax (Teleostei: Characidae) in response to aquatic pollution. *Zoologia* **36**, 1–12. https://doi.org/10.3897/zoologia.36.e30445

Alves C.B.M. & Pompeu P. dos S. (2010). A fauna de peixes da bacia do rio das Velhas no final do século XX. In: *Peixes do rio das Velhas: passado e presente*. (Eds C.B.M. Alves & P.S. Pompeu), pp. 167–189. ARGVMENTVM, Belo Horizonte.

Araujo-Lima C.A.R.M., Forsberg B.R., Victoria R. & Martinelli L. (1986). Energy sources for detritivorous fish in the Amazon. *Science* **234**, 1256–1258. https://doi.org/10.1126/science.234.4781.1256

Baiser B., Ardeshiri R.S. & Ellison A.M. (2011). Species Richness and Trophic Diversity Increase Decomposition in a Co-Evolved Food Web. *PLoS ONE* **6**, e20672. https://doi.org/10.1371/journal.pone.0020672

Barrie A. & Prosser S.J. (1996). Mass Spectrometry of Soils. In: *Mass Spectrometry of Soils*. p. 536. Marcel Dekker, Inc.

Bolnick D.I., Svanback R., Araujo M.S. & Persson L. (2007). Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proceedings of the National Academy of Sciences* **104**, 10075–10079. https://doi.org/10.1073/pnas.0703743104

Carvalho D.R., Castro D., 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**, 559–578. https://doi.org/10.1111/jfb.12734

de Carvalho D.R., Casarim R., Pereira R.B. & Pompeu P.S. (2015). Habitat structure determining the spatial distribution of ichthyofauna in a brazilian stream | Estrutura do habitat determinando a distribui??o espacial da ictiofauna de um c?rrego brasileiro. *Acta Scientiarum - Biological Sciences* **37**. https://doi.org/10.4025/actascibiolsci.v37i4.28063

de Carvalho D.R., de Castro D.M.P., Callisto M., Chaves A.J. de M., Moreira M.Z. & Pompeu P.S. (2019a). Stable isotopes and stomach content analyses indicate omnivorous habits and opportunistic feeding behavior of an invasive fish. *Aquatic Ecology* **3**. https://doi.org/10.1007/s10452-019-09695-3

de Carvalho D.R., de Castro D.M.P., Callisto M., Moreira M.Z. & Pompeu P.S. (2017a). 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/s10750-017-3130-6

de Carvalho D.R., Flecker A.S., Alves C.B.M., Sparks J.P. & Pompeu P.S. (2019b). Trophic responses to aquatic pollution of native and exotic livebearer fishes. *Science of The Total Environment* **681**, 503–515. https://doi.org/10.1016/j.scitotenv.2019.05.092

de Carvalho D.R., Leal C.G., Junqueira N.T., de Castro M.A., Fagundes D.C., Alves C.B.M., *et al.* (2017b). A fish-based multimetric index for Brazilian savanna streams. *Ecological Indicators* **77**, 386–396. https://doi.org/10.1016/j.ecolind.2017.02.032

Castro D.M.P., De Carvalho D.R., Pompeu P.D.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*. https://doi.org/10.1371/journal.pone.0150527

Cohen R.A. & Fong P. (2005). Experimental evidence supports the use of δ15N content of the opportunistic green macroalga Enteromorpha intestinalis (Chlorophyta) to determine nitrogen sources to estuaries. *Journal of Phycology* **41**, 287–293. https://doi.org/10.1111/j.1529-8817.2005.04022.x

Cohen R.A. & Fong P. (2004). Nitrogen uptake and assimilation in Enteromorpha intestinalis (L.) Link (Chlorophyta): using 15N to determine preference during simultaneous pulses of nitrate and ammonium. *Journal of Experimental Marine Biology and Ecology* **309**, 67–77. https://doi.org/10.1016/j.jembe.2004.03.009

Conley D.J., Paerl H.W., Howarth R.W., Boesch D.F., Seitzinger S.P., Havens K.E., *et al.* (2009). Controlling Eutrophication: Nitrogen and Phosphorus. *Science* **323**, 1014–1015. https://doi.org/10.1126/science.1167755

Costanzo S.D., O'Donohue M.J., Dennison W.C., Loneragan N.R. & Thomas M. (2001). A New Approach for Detecting and Mapping Sewage Impacts. *Marine Pollution Bulletin* **42**, 149–156. https://doi.org/10.1016/S0025-326X(00)00125-9

DeBruyn A.M.H. & Rasmussen J.B. (2002). Quantifying assimilation of sewage-derived organic matter by riverine benthos. *Ecological Applications* **12**, 511–520. https://doi.org/https://doi.org/10.1890/1051-0761(2002)012[0511:QAOSDO]2.0.CO;2

Derse E., Knee K.L., Wankel S.D., Kendall C., Berg, C.J. & Paytan A. (2007). Identifying Sources of Nitrogen to Hanalei Bay, Kauai, Utilizing the Nitrogen Isotope Signature of Macroalgae. *Environmental Science & Technology* **41**, 5217–5223. https://doi.org/10.1021/es0700449

Ferreira F.C. & Petrere M. (2009). The fish zonation of the Itanhaém river basin in the Atlantic Forest of southeast Brazil. *Hydrobiologia* **636**, 11–34. https://doi.org/10.1007/s10750-009-9932-4

de Freitas Terra B., Hughes R.M. & Araújo F.G. (2013). Sampling Sufficiency for Fish Assemblage Surveys of Tropical Atlantic Forest Streams, Southeastern Brazil. *Fisheries* **38**, 150–158. https://doi.org/10.1080/03632415.2013.775572

Gaiser E.E., Childers D.L., Jones R.D., Richards J.H., Scinto L.J. & Trexler J.C. (2006). Periphyton responses to eutrophication in the Florida Everglades: Cross-system patterns of structural and compositional change. *Limnology and Oceanography* **51**, 617–630. https://doi.org/10.4319/lo.2006.51.1_part_2.0617

Henriques S., Pais M.P., Costa M.J. & Cabral H. (2008). Efficacy of adapted estuarine fish-based multimetric indices as tools for evaluating ecological status of the marine environment. *Marine Pollution Bulletin* **56**, 1696–1713. https://doi.org/10.1016/j.marpolbul.2008.07.008

IBGE (2018). Estimativas de população. *Instituto Brasileiro de Geografia e Estatística*

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**, 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Junk W.J., Bayley P.B. & Sparks R.E. (1989). The flood pulse concept in river-floodplain systems. In: *Proceedings of the International Large River Symposium (LARS)*. (Ed. D.P. Dodge), pp. 110–127. Canadian Special Publication of Fisheries and Aquatic Sciences.

Junqueira N.T., Leal C.G., Alves C.B.M. & Pompeu P.S. (2012). Morphological diversity of fish along the rio das Velhas, Minas Gerais, Brazil. *Neotropical Ichthyology* **10**, 417–424. https://doi.org/10.1590/S1679-62252012005000004

Karr J.R. (1981). Assessment of Biotic Integrity Using Fish Communities. *Fisheries* **6**, 21–27. https://doi.org/10.1577/1548-8446(1981)006<0021:AOBIUF>2.0.CO;2

Kendall C. (1998). *Tracing Nitrogen Sources and Cycling in Catchments*. (Eds C. Kendall & J.J. McDonnell), Elsevier B.V.

Kumaraswamy T.R., Javeed S., Javaid M. & Naika K. (2020). Impact of Pollution on Quality of Freshwater Ecosystems. In: *Fresh Water Pollution Dynamics and Remediation*. pp. 69–81. Springer Singapore, Singapore.

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**, 42–48. https://doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2

Lazzarini Wolff L. & Segatti Hahn N. (2017). Fish habitat associations along a longitudinal gradient in a preserved coastal Atlantic stream, Brazil. *Zoologia* **34**, 1–13. https://doi.org/10.3897/zoologia.34.e12975

Loomer H.A., Oakes K.D., Schiff S.L., Taylor W.D. & Servos M.R. (2015). Use of Stable Isotopes to Trace Municipal Wastewater Effluents into Food Webs within a Highly Developed River System. *River Research and Applications* **31**, 1093–1100. https://doi.org/10.1002/rra.2826

Machado A.T.M., Alves C.B.M. & Callisto M. (2008). Projeto Manuelzão: metodologia e resultados.

In: *Projeto Manuelzão. A história da mobilização que começou em torno de um rio*. (Eds A.H. Lisboa, E.M.A. Goulart; & L.F.M. Diniz), pp. 39–54. Instituto Guaicuy - SOS Rio das Velhas, Belo Horizonte.

Mafra Junior L.L., Fernandes L.F. & Proença L.A.O. (2006). Harmful algae and toxis in paranaguá bay , Brazil: bases for monitoring. *Brazilian Journal of Oceanography* **54**, 107–121. https://doi.org/10.1590/S1679-87592006000200002

Mazor T., Doropoulos C., Schwarzmueller F., Gladish D.W., Kumaran N., Merkel K., *et al.* (2018). Global mismatch of policy and research on drivers of biodiversity loss. *Nature Ecology & Evolution* **2**, 1071–1074. https://doi.org/10.1038/s41559-018-0563-x

Moore J.W. & Semmens B.X. (2008). Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters* **11**, 470–480. https://doi.org/10.1111/j.1461-0248.2008.01163.x

Nikolenko O., Jurado A., Borges A. V, 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.2017.10.086

Ochoa-Izaguirre M.J. & Soto-Jiménez M.F. (2013). Evaluación de las fuentes de nitrógeno en el sistema lagunar de Urías, golfo de California, mediante el uso de isótopos estables en macroalgas. *Ciencias Marinas* **39**, 413–430. https://doi.org/http://dx.doi.org/10.7773/cm.v39i4.2285

Ochoa-Izaguirre M.J. & Soto-Jiménez M.F. (2015). Variability in nitrogen stable isotope ratios of macroalgae: consequences for the identification of nitrogen sources. *Journal of Phycology* **51**, 46–65. https://doi.org/10.1111/jpy.12250

Parnell A.C., Inger R., Bearhop S. & Jackson A.L. (2010). Source partitioning using stable isotopes: coping with too much variation. *PloS one* **5**, e9672. https://doi.org/10.1371/journal.pone.0009672

Petchey O.L. & Gaston K.J. (2002). Functional diversity (FD), species richness and community composition. *Ecology Letters* **5**, 402–411. https://doi.org/10.1046/j.1461-0248.2002.00339.x

Post D.M. (2002). Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. *Ecology* **83**, 703. https://doi.org/10.2307/3071875

Randall D.. & Tsui T.K.. (2002). Ammonia toxicity in fish. *Marine Pollution Bulletin* **45**, 17–23.

https://doi.org/10.1016/S0025-326X(02)00227-8

Santoro R., Bentivoglio F., Carlino P., Calizza E., Costantini M.L. & Rossi L. (2014). Sensitivity of food webs to nitrogen pollution: A study of three transitional water ecosystems embedded in agricultural landscapes. *Transitional Waters Bulletin* **8**, 84–97. https://doi.org/10.1285/i1825229Xv8n1p84

Savage C. & Elmgren R. (2004). Macroalgal (Fucus vesiculosus) δ N Values Trace Decrease in Sewage Influence. *Ecological Applications* **14**, 517–526

Schubert P.R., Karez R., Reusch T.B.H. & Dierking J. (2013). Isotopic signatures of eelgrass (Zostera marina L.) as bioindicator of anthropogenic nutrient input in the western Baltic Sea. *Marine Pollution Bulletin* **72**, 64–70. https://doi.org/10.1016/j.marpolbul.2013.04.029

Stock B. & Semmens B. (2016a). MixSIAR GUI user manual. *Version 3.1*, 1–59. https://doi.org/10.5281/zenodo.47719

Stock B.C. & Semmens B.X. (2016b). Unifying error strucctures in commonly used biotracer mixing models. *Ecology* **97**, 576–582. https://doi.org/10.1002/ecy.1517

Taylor K.G., Owens P.N., Batalla R.J. & Garcia C. (2008). Sediment and contaminant sources and transfers in river basins. In: *Sustainable Management of Sediment Resources*. (Ed. P.N. Owens), pp. 83–135.

Terra B.D.F., Hughes R.M., Francelino M.R. & Araújo F.G. (2013). Assessment of biotic condition of Atlantic Rain Forest streams: A fish-based multimetric approach. *Ecological Indicators* **34**, 136–148. https://doi.org/10.1016/j.ecolind.2013.05.001

Thornber C.S., DiMilla P., Nixon S.W. & McKinney R.A. (2008). Natural and anthropogenic nitrogen uptake by bloom-forming macroalgae. *Marine Pollution Bulletin* **56**, 261–269. https://doi.org/10.1016/j.marpolbul.2007.10.031

Thorp J.H. & Delong M.D. (1994). The Riverine Productivity Model: An Heuristic View of Carbon Sources and Organic Processing in Large River Ecosystems. *Oikos* **70**, 305. https://doi.org/10.2307/3545642

Vanderklift M. a & Ponsard S. (2003). Sources of variation in consumer-diet 15N enrichment: a meta-analysis. *Oecologia* **136**, 169–182. https://doi.org/10.1007/s00442-003-1270-z

Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E. (1980). The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**, 130–137.

https://doi.org/10.1139/f80-017

Viana I.G. & Bode A. (2013). Stable nitrogen isotopes in coastal macroalgae: Geographic and anthropogenic variability. *Science of The Total Environment* **443**, 887–895. https://doi.org/10.1016/j.scitotenv.2012.11.065

Viana I.G. & Bode A. (2015). Variability in δ 15 N of intertidal brown algae along a salinity gradient: Differential impact of nitrogen sources. *Science of The Total Environment* **512–513**, 167–176. https://doi.org/10.1016/j.scitotenv.2015.01.019

Wang Y., Liu D., Richard P. & Di B. (2016). Selection of effective macroalgal species and tracing nitrogen sources on the different part of Yantai coast, China indicated by macroalgal δ15N values. *Science of The Total Environment* **542**, 306–314. https://doi.org/10.1016/j.scitotenv.2015.10.059

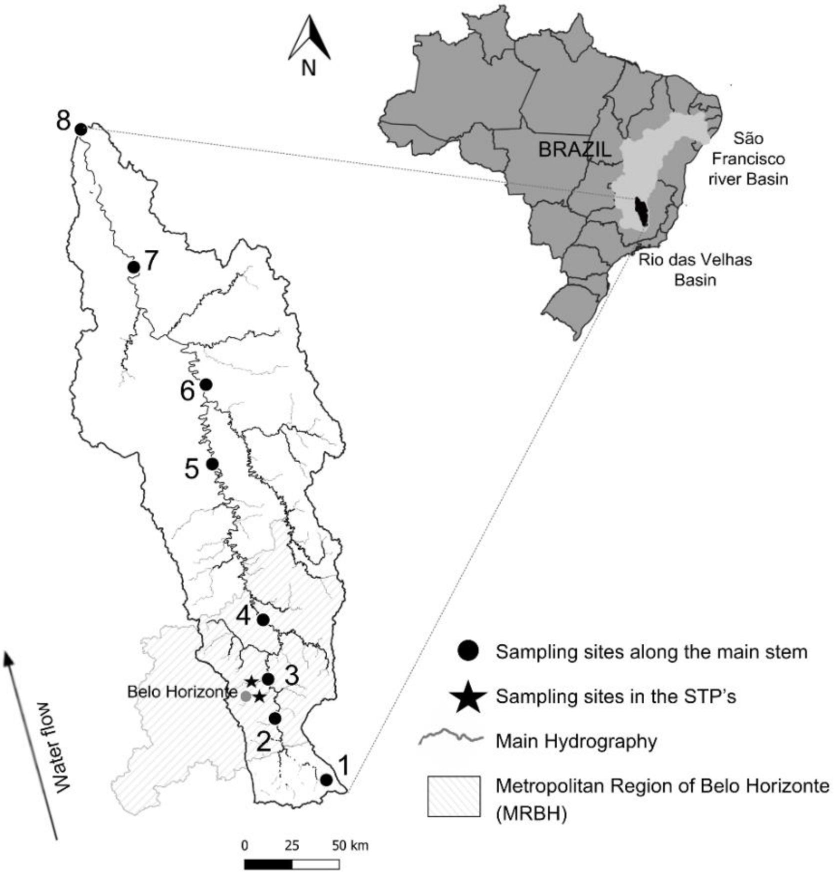


Figure 1 Sampling area covering eight sampling sites at the main stem of the Rio das Velhas basin and two sampling sites in the Sewage Treatment Plants (STP) located at the Metropolitan Region of Belo Horizonte (MRBH)

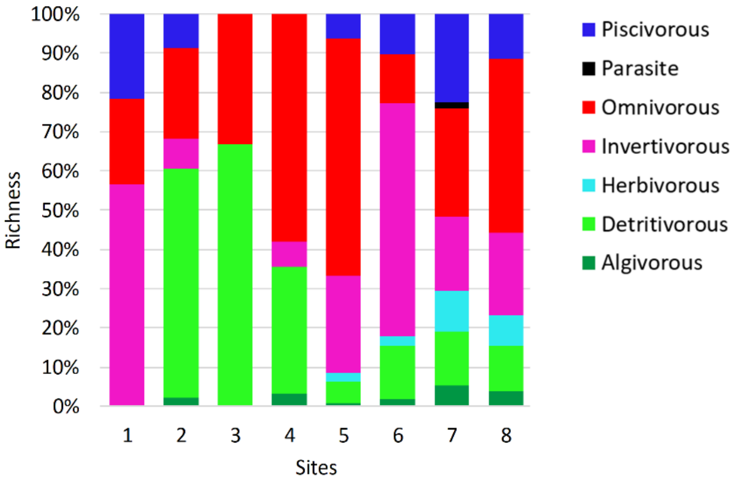


Figure 2 Relative number of species (richness) per guild sampled at eight sites distributed along the Rio das Velhas

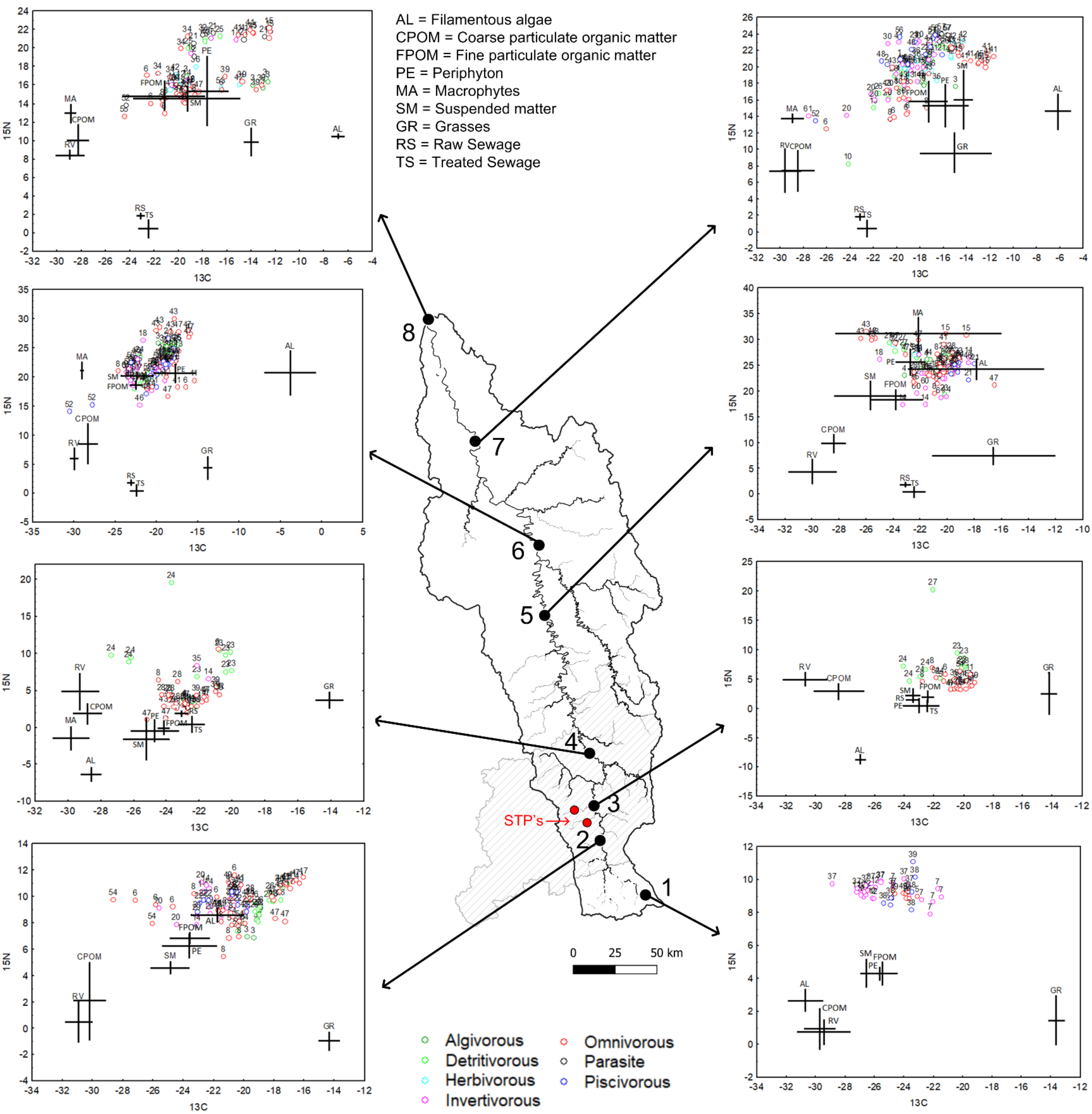


Figure 3 *Bi-plot* space representing the trophic structure of fish communities at the eight sites sampled along the Rio das Velhas. STP = Sewage treatment plants. *Acestrorhynchus lacustris* (1); *Anchoviella vaillanti* (2); *Apareiodon hasemani* (3); *Apareiodon ibitiensis* (4); *Astyanax intermedius* (5); *Astyanax lacustris* (6); *Astyanax rivularis* (7); *Astyanax taeniatus* (8); *Bergiaria westermanni* (9); *Brycon orthotaenia** (10); *Callichthys callichthys* (11); *Characidium fasciatum* (12); *Characidium zebra* (13); *Cichlasoma sanctifranciscense* (14); *Coptodon rendalli* (15); *Crenicichla lepidota* (16); *Curimatella lepidura* (17); *Eigenmannia virescens* (18); *Gymnotus carapo* (19); *Hemigrammus marginatus* (20); *Hoplias intermedius* (21); *Hoplias malabaricus* (22); *Hoplosternum littorale* (23); *Hypostomus commersoni* (24); *Hypostomus francisci* (25); *Hypostomus lima* (26); *Hypostomus macrops* (27); *Knodus moenkhausii* (28); *Lepidocharax burnsi* (29); *Leporellus vittatus* (30); *Leporinus taeniatus* (31);

Lophiosilurus alexandri (32); *Megaleporinus obtusidens* *(33); *Megaleporinus reinhardti** (34); *Moenkhausia costae* (35); *Myleus micans* (36); *Neoplecostomus franciscoensis* (37); *Oligosarcus argenteus* (38); *Oreochromis niloticus* (39); *Orthospinus franciscensis* (40); *Phalloceros uai* (41); *Piabarchus stramineus* (42); *Piabina argentea* (43); *Pimelodus fur* (44); *Pimelodus maculatus** (45); *Pimelodus pohli* (46); *Poecilia reticulata* (47); *Pygocentrus piraya* (48); *Rhamdia quelen* (49); *Rhinelepis aspera** (50); *Roeboides xenodon* (51); *Salminus franciscanus** (52); *Schizodon knerii* (53); *Serrapinnus heterodon* (54); *Serrapinnus piaba* (55); *Serrasalmus brandtii* (56); *Stegophilus insidiosus* (57); *Steindachnerina elegans* (58); *Tetragonopterus chalceus* (59); *Trachelyopterus galeatus* (60); *Triportheus guentheri* (61). *Migratory species

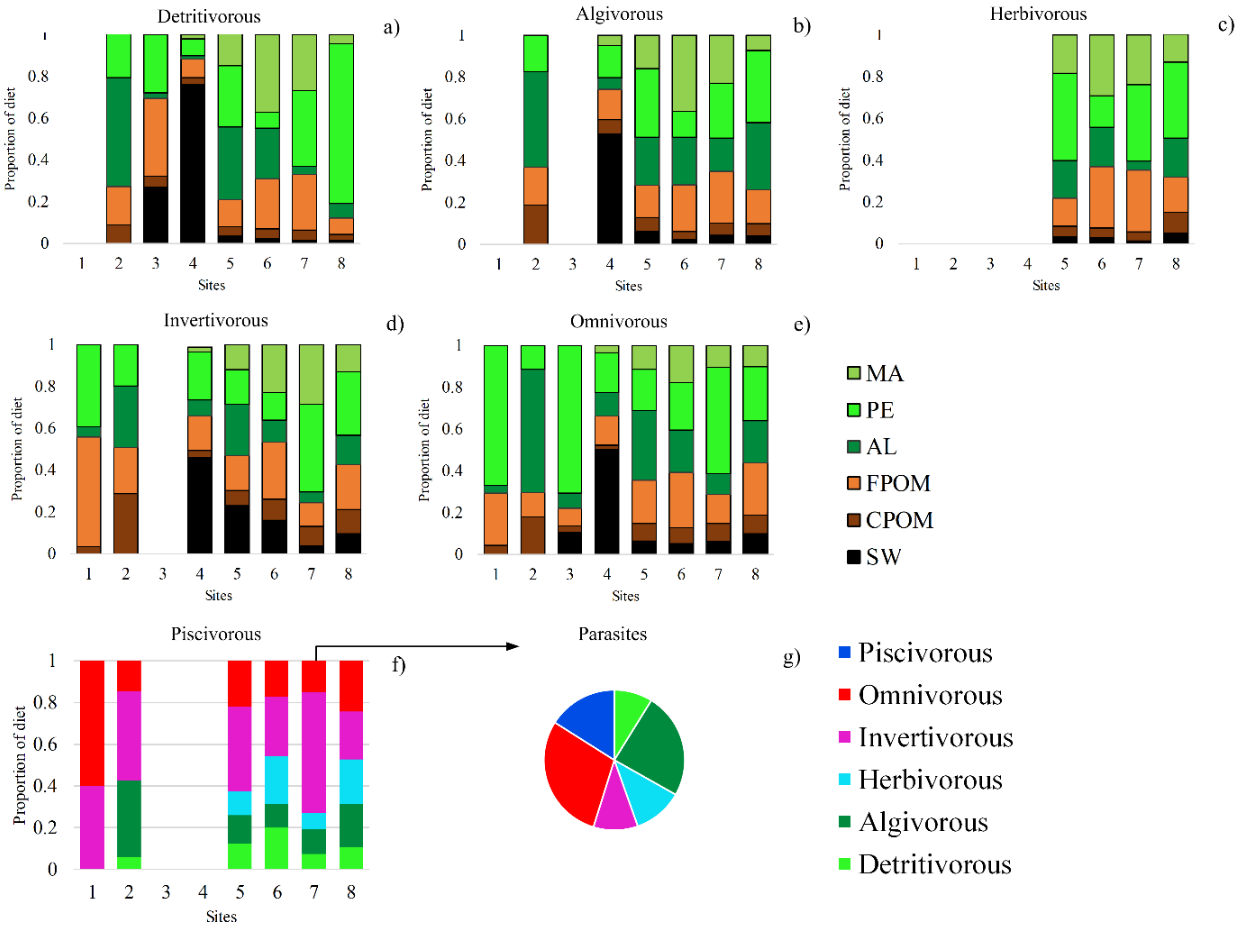


Figure 4 Proportion of basal resources in the diet of fish trophic guilds sampled at the eight sites sampled along the gradient in the Rio das Velhas. AL= Filamentous algae; CPOM= Coarse particulate organic matter; FPOM= fine particulate organic matter (grouped with suspended matter samples); PE= Periphyton; SW=Sewage (raw and treated sewage samples); and MA= macrophytes

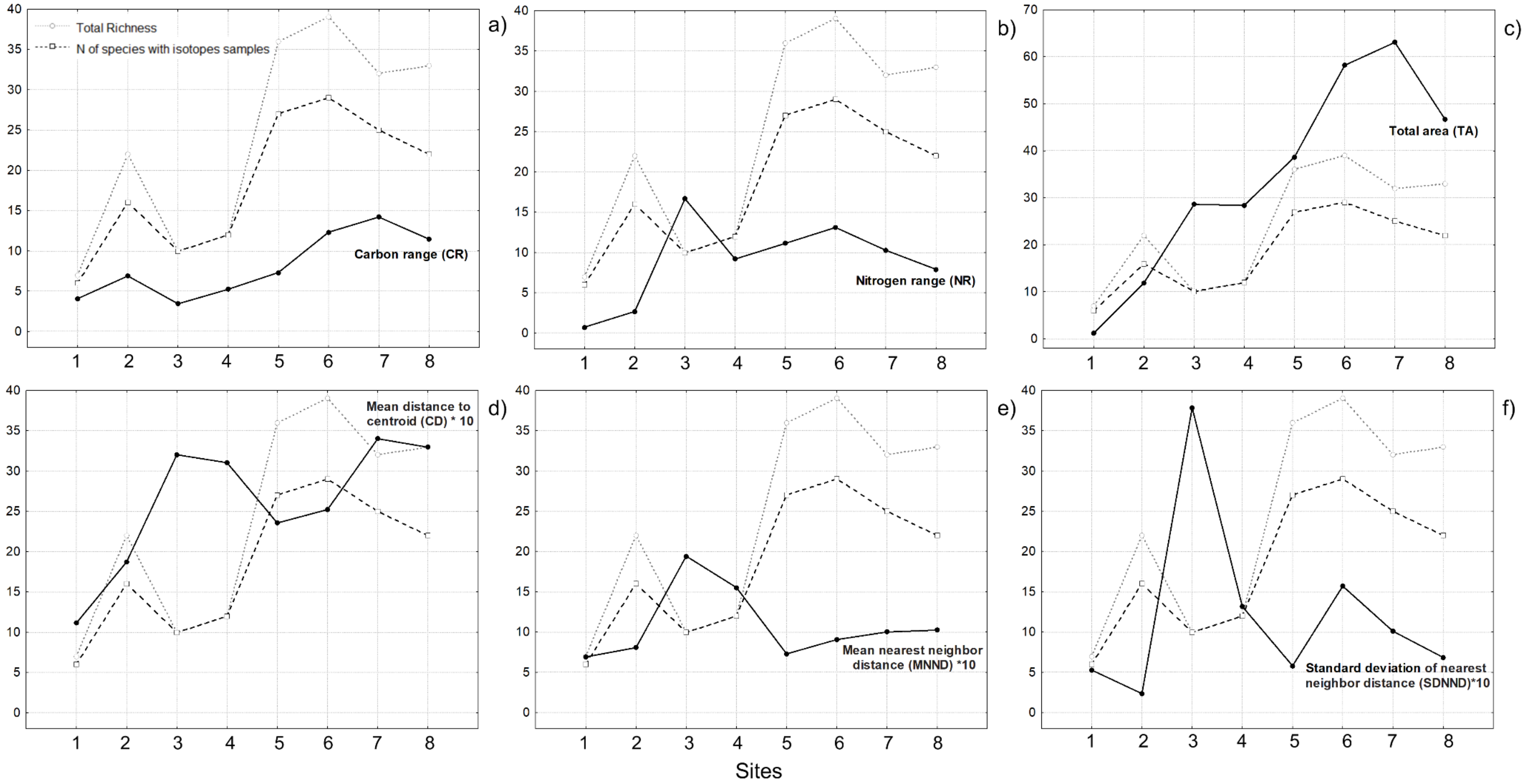


Figure 5 Total richness (dotted line - light gray), number of species sampled to stable isotopes analysis (dotted line - black) and Layman metrics (solid line - black): CR (a), NR (b), TA (c), CD (d), MNND (e) and SDNND (f) for each site distributed along the Rio das Velhas (sites 1 to 8). *CD, MNND and SDNND values were multiplied 10 times to facilitate the results interpretation

Supplementary material

Table A1 Number of individuals of each fish species sampled at the eight sites along a gradient o pollution in the Rio das Velhas. Number of stable isotopes samples are indicated between parenthesis

Species	**Sites**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
Algivorous									
Apareiodon hasemani		12 (5)				84 (5)	584 (5)	155 (5)	**835 (20)**
Apareiodon ibitiensis		1		12 (5)	470 (5)	11	46		**540 (10)**
Detritivorous									
Bergiaria westermanni						2 (1)			**2 (1)**
Brycon orthotaenia						1	4 (4)		**5 (4)**
Curimatella lepidura					1 (1)	68 (5)		4	**73 (6)**
Hoplosternum littorale			6 (5)	6 (6)	1 (1)				**13 (12)**
Hypostomus commersoni			8 (5)	4 (4)	1 (1)	1 (1)			**14 (11)**
Hypostomus francisci					1 (1)	8 (8)	11 (3)	41 (4)	**61 (16)**
Hypostomus lima		51 (13)					20 (7)		**71 (20)**
Hypostomus macrops			1 (1)		6 (6)	1			**8 (7)**
Hypostomus sp.4		1							**1**
Prochilodus costatus		1			1			1	**3**
Rhinelepis áspera						3 (1)			**3 (1)**
Steindachnerina elegans		3 (2)		1 (1)	1 (1)	33 (5)			**38 (9)**
Herbivorous									
Leporinus taeniatus					3 (3)				**3 (3)**
Myleus micans						4	17 (5)	4 (1)	**25 (6)**
Piabarchus stramineus					2098	42	2183 (10)	368 (5)	**4691 (15)**
Schizodon knerii						1 (1)	2	1 (1)	**4 (2)**
Invertivorous									
Astyanax rivularis	263 (6)	174							**437 (6)**
Characidium fasciatum	31 (10)								**31 (10)**

Characidium zebra					1		1 (1)		**2 (1)**
Cichlasoma sanctifranciscense		5 (4)		1 (1)	15 (8)	1 (1)	3 (2)	2 (2)	**27 (18)**
Crenicichla lepidota					1 (1)				**1 (1)**
Eigenmannia virescens					36 (7)	55 (7)		1 (1)	**92 (15)**
Gymnotus carapo					1 (1)				**1 (1)**
Hasemania nana					20	1	1		**22**
Hemigrammus gracilis		3							**3**
Hemigrammus marginatus		5 (4)					12 (5)		**17 (9)**
Hyphessobrycon santae	86								**86**
Lepidocharax burnsi		275 (5)			1		1		**277 (5)**
Leporellus vittatus							4 (3)	3	**7 (3)**
Megaleporinus elongatus						1			**1**
Moenkhausia costae				2 (1)	1 (1)	26 (5)		2 (1)	**31 (8)**
Neoplecostomus franciscoensis	66 (10)								**66 (10)**
Orthospinus franciscensis						2		2 (1)	**4 (1)**
Pimelodus fur						15 (2)	8 (5)		**23 (7)**
Pimelodus maculatus						2 (1)			**2 (1)**
Pimelodus pohli					2 (1)	19 (7)	3 (2)	1	**25 (10)**
Roeboides xenodon						11 (2)		1	**12 (2)**
Tetragonopterus chalceus						4		2 (1)	**6 (1)**
Trachelyopterus galeatus					2 (2)	1 (1)		2	**5 (3)**
Triportheus guentheri					6 (3)	74 (5)	23 (5)	2	**105 (13)**
Omnivorous									
Astyanax intermedius	32 (5)								**32 (5)**
Astyanax lacustris		25 (5)	2 (1)	2 (1)	82 (5)	10 (3)	17 (5)	17 (5)	**155 (25)**
Astyanax sp.					1				**1**
Astyanax taeniatus		48 (7)	2 (2)	1 (1)	45 (6)	14 (5)	35 (6)	12 (5)	**157 (32)**
Callichthys callichthys			1 (1)						**1 (1)**
Coptodon rendalli			2 (1)		205 (10)		12 (5)	9 (4)	**228 (20)**
Franciscodoras marmoratus						1			**1**

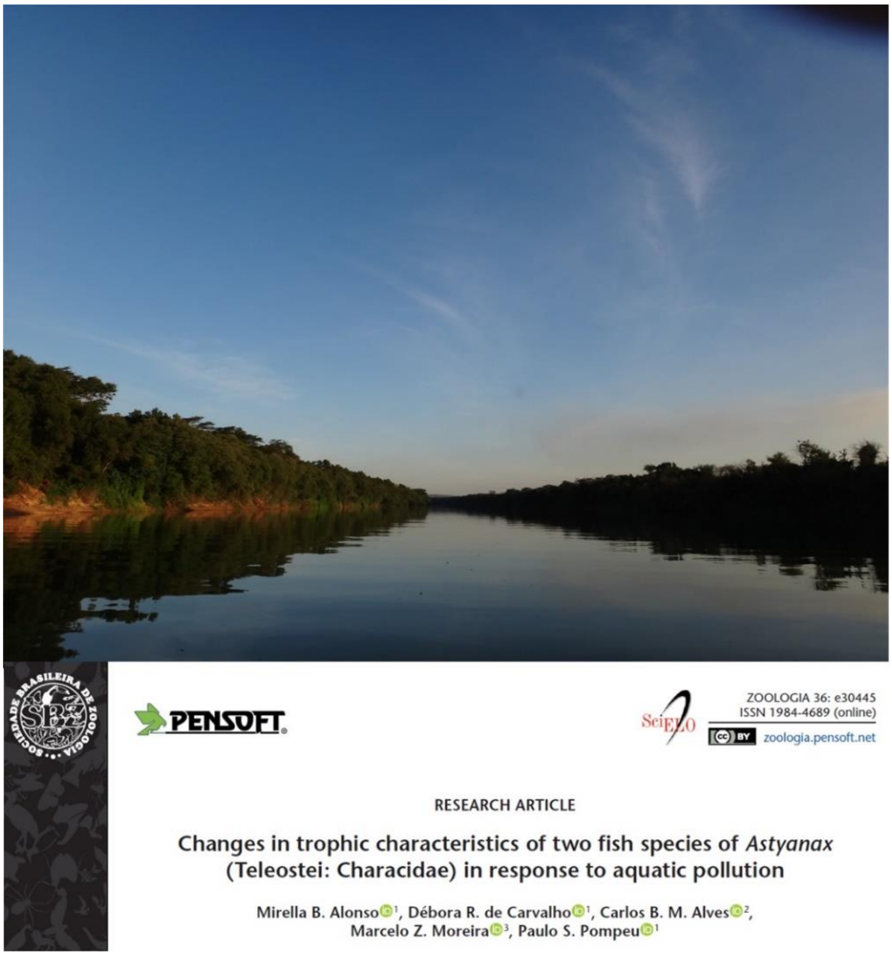
Geophagus brasiliensis		1							**1**
Knodus moenkhausii		174 (5)		11 (6)	147 (5)	31	7	11	**381 (16)**
Megaleporinus obtusidens					2 (2)			1	**3 (2)**
Megaleporinus reinhardti					1 (1)		2	11 (5)	**14 (6)**
Oreochromis niloticus			16 (5)	126 (8)		3 (2)		6 (5)	**151 (20)**
Phalloceros uai		28 (7)			2 (2)	21 (5)	23 (5)	47 (4)	**121 (23)**
Piabina argentea		77 (5)		4 (4)	5 (5)	52 (5)	135 (10)		**273 (29)**
Poecilia reticulata		26 (6)	439 (10)	3031 (9)	13 (5)	18 (5)		47 (5)	**3574 (40)**
Psellogrammus kennedyi							1		**1**
Rhamdia quelen	6 (3)	5 (5)							**11 (8)**
Serrapinnus heterodon		699 (7)	3 (3)			3 (1)		1	**706 (11)**
Serrapinnus piaba					7			23 (5)	**30 (5)**
Parasite									
Stegophilus insidiosus							13 (5)		**13 (5)**
Piscivorous									
Acestrorhynchus lacustris					1	3 (2)	1 (1)		**5 (3)**
Anchoviella vaillanti							2 (1)	1 (1)	**3 (2)**
Hoplias intermedius		4 (2)			6 (5)	11 (6)	8 (5)	4 (4)	**33 (22)**
Hoplias malabaricus		9 (9)							**9 (9)**
Lophiosilurus alexandri								2 (1)	**2 (1)**
Oligosarcus argenteus	12 (7)								**12 (7)**
Pygocentrus piraya						2 (2)	2 (2)		**4 (4)**
Salminus franciscanus					1	3 (2)	1 (1)	1 (1)	**6 (4)**
Serrasalmus brandtii					8 (2)	17 (5)	7 (3)	1	**33 (10)**
Total Richness	**7 (6)**	**22 (16)**	**10 (10)**	**12 (12)**	**36 (27)**	**40 (29)**	**32 (25)**	**33 (22)**	**71 (61)**
Total Abundance	**496 (41)**	**1627 (91)**	**480 (34)**	**3201 (47)**	**3195 (91)**	**660 (101)**	**3189 (106)**	**786 (67)**	**13634 (578)**

Table A2 Bayesian stable isotope mixing model (MixSIAR) output results. Mean and standard deviation of the food source proportions in the diet of fish trophic guilds sampled along the Rio das Velhas (sites 1 to 8). AL= Filamentous algae; CPOM= Coarse particulate organic matter; FPOM= fine particulate organic matter (grouped with suspended matter samples); PE= Periphyton; SW=Sewage (raw and treated sewage samples); and MA= macrophytes. Bold= resources that contributes more than 20% to fish diet

Trophic guilds	Source	Sites															
		1		2		3		4		5		6		7		8	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Detritivorous	AL			**0.52**	**±0.14**	0.03	±0.03	0.02	±0.02	**0.35**	**±0.21**	**0.24**	**±0.06**	0.04	±0.04	0.07	±0.05
	CPOM			0.09	±0.06	0.05	±0.05	0.03	±0.05	0.05	±0.05	0.05	±0.05	0.05	±0.10	0.03	±0.04
	FPOM			0.18	±0.15	**0.37**	±0.27	0.09	±0.18	0.13	±0.12	**0.24**	**±0.18**	**0.27**	**±0.30**	0.08	±0.09
	PE			0.21	±0.17	**0.28**	**±0.24**	0.08	±0.17	**0.29**	**±0.22**	0.08	±0.07	**0.36**	**±0.25**	**0.76**	**±0.13**
	SW					**0.27**	**±0.24**	**0.76**	**±0.26**	0.04	±0.03	0.02	±0.03	0.01	±0.01	0.01	±0.02
	MA							0.02	±0.04	0.16	±0.10	**0.37**	**±0.13**	**0.27**	**±0.10**	0.05	±0.06
Algivorous	AL			**0.46**	**±0.12**			0.05	±0.05	**0.23**	**±0.12**	**0.23**	**±0.05**	0.16	±0.09	**0.32**	**±0.09**
	CPOM			0.19	±0.10			0.07	±0.06	0.07	±0.06	0.04	±0.04	0.06	±0.05	0.06	±0.05
	FPOM			0.18	±0.14			0.15	±0.12	0.15	±0.11	**0.22**	**±0.14**	**0.25**	**±0.18**	0.16	±0.13
	PE			0.18	±0.15			0.15	±0.12	**0.33**	**±0.17**	0.13	±0.11	**0.26**	**±0.15**	**0.35**	**±0.11**
	SW							**0.53**	**±0.15**	0.06	±0.05	0.02	±0.02	0.04	±0.04	0.04	±0.04
	MA							0.05	±0.05	0.16	±0.10	**0.36**	**±0.12**	**0.23**	**±0.09**	0.07	±0.06
Herbivorous	AL									0.18	±0.17	0.19	±0.10	0.04	±0.04	0.19	±0.08
	CPOM									0.05	±0.06	0.05	±0.06	0.04	±0.08	0.10	±0.10
	FPOM									0.13	±0.13	**0.29**	**±0.23**	**0.30**	**±0.30**	0.17	±0.16
	PE									**0.42**	**±0.25**	0.15	±0.16	**0.36**	**±0.25**	**0.36**	**±0.14**
	SW									0.03	±0.03	0.03	±0.04	0.01	±0.01	0.05	±0.06
	MA									0.18	±0.16	**0.29**	**±0.16**	0.24	±0.10	0.13	±0.12
Invertivorous	AL	0.05	±0.04	**0.29**	**±0.17**			0.08	±0.06	**0.25**	**±0.09**	0.10	±0.04	0.05	±0.04	0.14	±0.07
	CPOM	0.04	±0.03	**0.29**	**±0.11**			0.03	±0.04	0.07	±0.07	0.10	±0.07	0.09	±0.08	0.11	±0.09
	FPOM	**0.52**	**±0.25**	**0.22**	**±0.15**			0.17	±0.15	0.17	±0.10	**0.27**	±0.14	0.11	±0.10	**0.22**	**±0.16**
	PE	**0.39**	**±0.25**	**0.20**	**±0.14**			**0.23**	**±0.19**	0.17	±0.11	0.13	±0.09	**0.42**	**±0.11**	**0.30**	**±0.17**
	SW							**0.46**	**±0.20**	**0.23**	**±0.07**	0.16	±0.05	0.04	±0.03	0.10	±0.06

	MA							0.02	±0.04	0.12	±0.08	**0.23**	**±0.10**	**0.29**	**±0.07**	0.13	±0.09
Omnivorous	AL	0.04	±0.03	**0.59**	**±0.15**	0.07	0.04	0.11	±0.06	**0.33**	**0.12**	**0.20**	**0.06**	0.10	0.06	**0.20**	**±0.06**
	CPOM	0.04	±0.04	0.18	±0.10	0.03	0.03	0.02	±0.02	0.08	0.06	0.08	0.06	0.09	0.06	0.09	±0.07
	FPOM	**0.25**	**±0.16**	0.12	±0.10	0.09	0.08	0.14	±0.11	**0.21**	**0.13**	**0.26**	**0.16**	0.14	0.10	**0.25**	**±0.18**
	PE	**0.67**	**±0.16**	0.11	±0.10	**0.71**	**0.12**	0.19	±0.16	**0.20**	**0.12**	**0.23**	**0.13**	**0.51**	**0.12**	**0.26**	**±0.17**
	SW					0.11	0.09	**0.50**	**±0.17**	0.06	0.05	0.05	0.04	0.06	0.04	0.10	±0.06
	MA							0.03	±0.04	0.12	0.08	0.18	0.11	0.11	0.07	0.10	±0.07
Piscivorous	Detritivorous			0.06	±0.06							**0.20**	**±0.15**	0.07	±0.07	0.11	±0.08
	Algivorous			**0.36**	**±0.16**					0.12	±0.11	0.11	±0.10	0.12	±0.10	**0.21**	**±0.13**
	Herbivorous									0.11	±0.12	**0.23**	**±0.13**	0.08	±0.07	**0.21**	**±0.15**
	Invertivorous	**0.40**	**±0.22**	**0.43**	**±0.16**					**0.40**	**±0.22**	**0.28**	**±0.15**	**0.58**	**±0.19**	**0.23**	**±0.15**
	Omnivorous	**0.60**	**±0.22**	0.15	±0.13					**0.22**	**±0.17**	0.17	±0.13	0.15	±0.14	**0.25**	**±0.14**
Parasites	Detritivorous													0.09	±0.09		
	Algivorous													**0.24**	**±0.16**		
	Herbivorous													0.11	±0.11		
	Invertivorous													0.10	±0.09		
	Omnivorous													**0.29**	**±0.20**		
	Piscivorous													0.16	±0.14		

TERCEIRA PARTE



ZOOLOGIA 36: e30445
ISSN 1984-4689 (online)

zoologia.pensoft.net

RESEARCH ARTICLE

Changes in trophic characteristics of two fish species of *Astyanax* (Teleostei: Characidae) in response to aquatic pollution

Mirella B. Alonso[1], Débora R. de Carvalho[1], Carlos B. M. Alves[2],
Marcelo Z. Moreira[3], Paulo S. Pompeu[1]

1 CONCLUSÃO GERAL

Nos quatro capítulos desenvolvidos nessa tese, conseguimos avaliar os impactos da poluição sobre os peixes, abrangendo desde do nível específico até à nível de comunidade, respondendo quatro perguntas distintas:

1) Espécies nativas e espécies exóticas são afetadas pela poluição de maneira similar?

Observou-se que a poluição afeta a dieta e o nicho trófico das duas espécies de forma similar, entretanto a espécie exótica se alimenta diretamente do esgoto (além de ser encontrada em grande abundância em locais poluídos), o que pode ser uma característica que facilite a existência dessa espécie em locais muito poluídos.

2) Amostras de peixes e perifíton podem ser utilizadas para traçar a poluição?

Observamos que tanto a assinatura de nitrogênio do perifíton quanto a dos cascudos varia temporalmente e espacialmente em toda bacia, e que podem ser utilizadas como indicadores de poluição. Entretanto, o perifíton é mais sensível a mudanças nas concentrações de nitrato e amônia entre estações do que os peixes. A grande variação espacial nas assinaturas isotópicas (de valores negativos até valores extremamente altos) sugere que diferentes atividades antrópicas estão influenciando diferentes regiões da bacia do Rio das Velhas. Entretanto, devido à grande diversidade de fontes de nitrogênio na bacia do Rio das Velhas, o $\delta^{15}N$ do perifíton e dos cascudos serão melhor utilizados como indicadores da intensidade de poluição por nitrogênio, mas não como traçadores de fontes individuais de nitrogênio.

3) A poluição altera o nicho trófico das comunidades de peixes?

Vimos que em locais sem poluição, o nicho isotópico das comunidades de peixes é similar ao nicho do ponto mais preservado na bacia (Rio cipó, considerado como referência em conservação). Já nos pontos poluídos da bacia, observa-se que esse nicho é bem diferente do ponto de referência, indicando alteração na estrutura trófica desses locais. Em todos pontos poluídos, observou-se uma grande variação no eixo do $\delta^{15}N$, o que provavelmente ocorre devido a elevada concentração de nitrogênio (especialmente nitrato) proveniente de atividades antrópicas. Nestes pontos, também houve um maior enriquecimento de $\delta^{15}N$ nos peixes detritívoros, o que não é esperado para peixes que se alimentam na base da teia trófica.

4) A diversidade trófica e as fontes de carbono que sustentam as comunidades de peixes mudam ao longo de um gradiente de poluição?

De maneira geral, recursos autotróficos (algas, macrófitas e perifíton) são as principais fontes de carbono para os peixes do Rio das Velhas. Entretanto, vimos que nos pontos imediatamente após a Região Metropolitana de Belo Horizonte o esgoto é uma fonte alimentar importante para peixes de todas as guildas alimentares. Métricas indicadoras de diversidade trófica também foram afetadas pela poluição. Apesar de haver uma redução no número de espécies nos locais mais poluídos, houve um aumento na diversidade trófica, e uma menor redundância nos itens consumidos pelos peixes.

Através dos resultados destes quatro capítulos, concluímos que os isótopos estáveis constituem uma importante ferramenta na avaliação dos efeitos da poluição sobre as comunidades aquáticas. Além disso, os peixes, que alteram seu comportamento trófico em resposta a poluição, se mostraram bons indicadores de qualidade ambiental, podendo ser utilizados em programas de monitoramento ambiental não só do Rio das Velhas, mas em outras bacias hidrográficas.

Nossos resultados também chamam atenção para a necessidade de maiores esforços no tratamento de esgoto das cidades distribuídas ao longo da bacia do Rio das Velhas. Apesar de grande parte das cidades realizar o tratamento secundário do esgoto, percebe-se que ainda existe uma grande descarga de poluentes na bacia, inclusive de esgoto sem nenhum tratamento. A melhoria na qualidade na água se faz importante não só pela preservação dos organismos aquáticos e manutenção do funcionamento do ecossistema, mas também para o fornecimento de água para uma região com mais de cinco milhões de habitantes.

2 PRODUTOS SECUNDÁRIOS

Durante o período de doutorado também participei da coorientação de duas alunas de graduação, Mirella Bisso Alonso e Marina Rodrigues, que tiveram seus projetos de iniciação científica vinculados ao meu projeto de doutorado.

Durante a graduação, Mirella desenvolveu dois projetos distintos. O primeiro projeto avaliou se duas espécies do mesmo gênero, *Astyanax taeniatus* e *Astyanax lacustris*, alteram suas características tróficas de forma similar em resposta a poluição. Deste projeto se resultou o artigo “Changes in trophic characteristics of two fish species of *Astyanax* (Teleostei: Characidae) in response to aquatic pollution” que foi publicado na revista “Zoologia”. Neste artigo vimos que ambas espécies se alimentam preferencialmente de algas em locais poluídos e de perifíton em locais sem poluição. Além disso, observou-se que o nicho trófico das duas espécies apresenta maior sobreposição em locais mais poluídos.

No segundo projeto, Mirella caracterizou a estrutura trófica da comunidade de peixes de um riacho em condição de referência, o Rio Cipó, um dos afluentes mais preservados da bacia do Rio das Velhas por estar localizado dentro do Parque Nacional da Serra do Cipó. Deste projeto resultou o artigo “Trophic structure of a fish community in a reference condition river” que está em fase de formatação para ser submetido na revista “Environmental Biology of Fishes”. Ao caracterizar a estrutura trófica do Rio Cipó, observamos que os peixes da guilda dos detritívoros foram os que apresentaram maior variação nos recursos consumidos, o que resultou em uma grande amplitude de nicho. Os peixes dessa guilda, apesar de se alimentarem dos mesmos recursos (algas, perifíton e CPOM), variaram a proporção de assimilação de cada recurso. Peixes da guilda dos herbívoros também apresentaram uma grande amplitude de nicho, o que ocorreu devido à grande discrepância na assinatura isotópica da espécie *Myleus micans* (pacú). Essa espécie de hábito herbívoro apresentou composição isotópica de δ^{15}N e δ^{13}C muito mais enriquecida em comparação as demais espécies e recursos alimentares. Resultado que sugere que o pacú pode estar se alimentando em diferentes regiões da bacia do Rio das Velhas, onde os recursos alimentares são mais enriquecidos em consequência da poluição.

O projeto da Marina, por sua vez, objetivou avaliar se a presença de poluição promove alterações similares nos hábitos alimentares de espécies pertencentes a diferentes guildas tróficas, a espécie detritívora, *Hypostomus francisci*, e a espécie piscívora, *Hoplias intermedius*. Deste projeto resultou o artigo “Convergent responses of fish belonging to different feeding guilds to sewage pollution” que foi recentemente aceito para publicação na revista “Neotropical Ichthyology”. Neste estudo observamos que ambas espécies alteram sua dieta em resposta a poluição, entretanto a espécie detritívora apresenta maior plasticidade trófica. O nicho isotópico de ambas as espécies foi mais amplo em locais menos perturbados e mais enriquecido em $\delta^{15}N$ em regiões poluídas (sendo o $\delta^{15}N$ da espécie detritívora mais enriquecido que o da espécie piscívora). Em conclusão, ambas as espécies apresentaram respostas isotópicas semelhantes à poluição ambiental. No entanto, a espécie detritívora foi mais sensível a essas alterações e, portanto, é provavelmente uma melhor indicadora de condição ambiental do que a espécie piscívora.

Somados aos manuscritos frutos da tese, geramos sete manuscritos até o momento. Esperamos, a partir destes trabalhos, fornecer mais informações acerca da qualidade ambiental da bacia do Rio das Velhas, assim como esclarecer como a presença da poluição afeta o comportamento alimentar dos peixes da bacia.

ARTIGO V - ***Changes in trophic characteristics of two fish species of the genus Astyanax in response to aquatic pollution***

Mirella Bisso Alonso; Débora Reis de Carvalho, Carlos Bernardo Mascarenhas Alves;
Marcelo Zacharias Moreira; Paulo Santos Pompeu

Manuscrito publicado na revista *Zoologia*

(Manuscrito redigido na língua e formato indicado para revista)

144
ZOOLOGIA 36: e30445
ISSN 1984-4689 (online)

zoologia.pensoft.net

RESEARCH ARTICLE

Changes in trophic characteristics of two fish species of *Astyanax* (Teleostei: Characidae) in response to aquatic pollution

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http://zoobank.org/2139337D-1A2B-49F2-8D78-A5694B66A376

ABSTRACT. The trophic plasticity of most fish species of *Astyanax* Baird & Girard, 1854 in response to environmental changes and resource availability is high. This work evaluates the differences in the trophic characteristics of two congeneric species, *Astyanax taeniatus* (Jenyns, 1842) and *Astyanax lacustris* (Lütken, 1875), in Rio das Velhas Basin, which is highly impacted by the discharge of sewage from the Metropolitan Region of Belo Horizonte (MRBH). Eight sites were sampled and grouped into three regions: upper course (two sites upstream of the MRBH); middle course (three sites located in the middle portion of the Rio das Velhas, region with greater influence of the MRBH), and lower course (three sites downstream of the MRBH). Samples of fish and food resources were collected from all sites to obtain the isotopic composition of nitrogen (δ^{15}N) and carbon (δ^{13}C), and the stomach contents of the two species was analized. The most common items in the stomach of *A. lacustris* and *A. taeniatus*, respectively, were from plants and insects, followed by algae/periphyton (especially at the low course of Rio das Velhas). In contrast, stable isotope analyses indicated that algae (in polluted sites) and periphyton (in least-disturbed sites) were best assimilated both species. Both analyses indicated that the trophic niches of the two species overlap more in more polluted sites relative to less polluted sites. *Astyanax taeniatus* and *A. lacustris* only presented different isotopic composition of carbon and nitrogen in the upper course of the Rio das Velhas, probably in response to the greater diversity of food items consumed by each species. In the other regions, the species presented similar isotopic signatures, with δ^{15}N and δ^{13}C notably enriched in the most polluted regions (middle and low course). Our results suggest that pollution acts by increasing trophic niche overlap of these species, altering the type of resources most assimilated, and promoting a greater enrichment of δ^{15}N in fish and resources.

KEY WORDS. *Astyanax lacustris*, *Astyanax taeniatus*; carbon, nitrogen enrichment, stable isotopes, stomach contents.

INTRODUCTION

In many developing countries, a large proportion of untreated raw sewage is released into aquatic environments (Macedo and Sipaúba-Tavares 2010), increasing the load of organic matter and pollutants in rivers, which are considered as the main drivers of artificial eutrophication in these environments (Tundisi and Tundisi 2008). Nutrients from human activities, when released into the water, contribute to the rapid growth of algal blooms and aquatic plants, altering the physico-chemical and ecological conditions of aquatic systems (Pereira and Mercante 2005, Hicks et al. 2016, Baeta et al. 2017). Among the consequences of an increase in primary productivity is the rapid reduction in water oxygen levels, which has drastic effects on fish and invertebrate communities (Macedo and Sipaúba-Tavares 2010, Baeta et al. 2017). In addition, changes in primary productivity in response to pollutants affect directly the diets of these aquatic consumers (Cabana and Rasmussen 1996, Esteves and Aranha 1999), and may also be responsible for promoting the local extinction of specialist and less tolerant species.

By favoring primary productivity, environmental pollution of aquatic systems may homogenize the type of resources available to organisms in higher trophic levels. This process of homogenization in aquatic systems has been also described in several taxonomic groups such as diatoms, zooplankton and macroinvertebrates (Lougheed et al. 2008). Such changes in the balance of available resources may consequently affect the food web since changes in nutritional composition or abundances of basal food sources can induce shifts in primary consumers or even their exclusion (Hall 2004). However, the effects of this homogenization of producer communities on upper trophic levels remains unclear.

According to ecological theory, generalist species are less sensitive to environmental change than specialists as they have the capacity of varing their diet according to the availability of resources present in their respective habitats (Tundisi and Tundisi 2008). Therefore, trophic plasticity is an important strategy to allow species to tolerate changes in environment condition (Gerking 1994, Wootton 1999). Species of the American fish *Astyanax* Baird & Girard, 1854 are well known for their broad geographic distribution and their ability to inhabit environments with differing levels of preservation, including highly polluted environments (eg., Souza and Lima-Júnior 2013, Carvalho et al. 2015). *Astyanax* is composed of approximately 100 species that are distributed from the southern United States to northern Argentina (Eigenmann 1921, Géry 1997, Weitzman and Fink 1983). Most species have omnivorous feeding habits, with diets composed of animal and vegetable items, of both autochthonous and allochthonous origins (e.g., Esteves 1996, Vilela et al. 2002, Cassemiro et al. 2002, Bennemann et al. 2005). In addition, some species of this genus present generalist feeding habits and high trophic plasticity in response to environmental changes and resource availability (Lobón-Cerviá and Bennemann 2000, Carvalho et al. 2015), which increases their chance of survival in disturbed habitats (Menezes et al. 2007). However, congeneric species (species of the same genus) may respond differently to changes in the aquatic environment and the availability of resources.

One way to identify how distinct species respond to changes in the environment is by comparing their feeding habits in regions under differing levels of human disturbance (e.g, Carvalho et al. 2015). Accordingly, analyses of stomach contents and stable isotopes (carbon and nitrogen), can be used simultaneously for robust and reliable assessment of feeding habits (e.g., Carassou et al. 2017, Connan 2017). Stomach contents analyses provide useful taxonomic information on consumed prey items (Beaudoin et al. 1999). However, there are often uncertainties in the identification of such items, due to the different stages of digestion of food items, and that not all ingested items are in fact assimilated into biomass (Manetta and Benedito-Cecílio 2003). Stable isotopes analyses, on the other hand, provide information on the energy flow in food chains (Peterson and Fry 1987, Kling et al. 1992). The nitrogen ($\delta^{15}N$) isotope is consistently fractionated throughout the trophic web, allowing researchers to make inferences about the trophic relationships of consumers with their diet (Vander Zanden et al. 1997, Post 2002, Vanderklift and Ponsard 2003). The carbon ($\delta^{13}C$) isotope, in turn, allows to delineate the energy flow in environments that present several foods with different carbon values (Manetta and Benedito-Cecílio 2003).

Based on this information, we aimed to evaluate how trophic characteristics of two congeneric species, *Astyanax lacustris* (Lütken, 1875) and *Astyanax taeniatus* (Jenyns, 1842), change across an environmental pollution gradient. The diet and the trophic niches occupied by these two species were evaluated in different regions of a highly disturbed Neotropical river basin, the Rio das Velhas, south east Brazil. The main source of disturbance in this river basin is the discharge of untreated domestic and industrial sewage from a large nearby urban conurbation. We tested the following hypotheses: 1) Under natural (undisturbed) conditions, the congeneric species occupy different trophic niches, and consequently present little food overlap; 2) However, along a gradient of pollution, due to the simplification (homogenization) of the available resources, and due to their high trophic plasticity, both species will increase their food overlap and will present more similar isotopic signatures.

MATERIAL AND METHODS

The study was conducted in the Rio das Velhas Basin, southeast Brazil, with sampling sites located in the main channel of the Rio das Velhas. The Rio das Velhas is the largest tributary of the São Francisco river Basin (Alves and Pompeu 2001), and is located entirely in the territory of Minas Gerais state (CETEC 1983), covering 51 municipalities. The basin, with a drainage area of 29,173 km^2, has an average annual flow rate at its mouth of 300 m^3/s and average width of 38.3m (CETEC 1983). The Rio das Velhas is of significant economic and social importance. Its upper course is located at the Metropolitan Region of Belo Horizonte (MRBH), the third largest urban conurbation in Brazil, with almost six million inhabitants, and is the main water supply.

Eight sites were sampled along the Rio das Velhas channel (RV-01 to RV-08), which were divided into three regions (upper, middle and lower course). The upper course of the Rio das Velhas (Upper RV) corresponds to the region with the best water quality (RV-01 and RV-02). The middle course (Middle RV) is in the region with the greatest influence of the MRBH, characterized by the discharge of large amounts of domestic and industrial sewage (RV-03, RV-04, and RV-05). The lower course (Low RV), in turn, is the most distant region from the MRBH and is close to the river mouth (RV-06, RV-07, and RV-08). In this region the river partly recovers its quality, due to the presence of numerous well preserved tributaries (Alves and Pompeu 2001) (Table 1, Fig. 1).

Two sewage treatment plants (STP), Arrudas and Onça, were also sampled to obtain complementary samples of the suspended material to obtain the isotopic composition of the raw

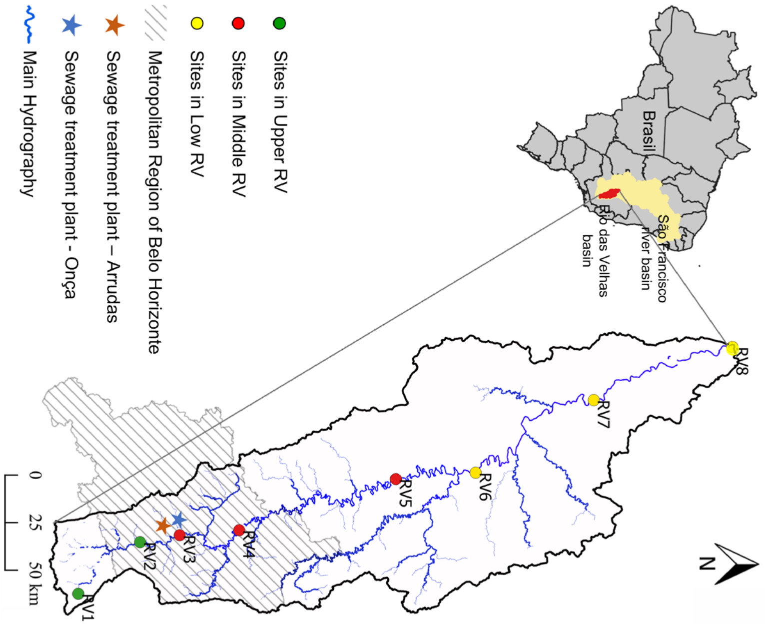


Figure 1. Sampling network in the Rio das Velhas Basin, Minas Gerais, Brasil.

sewage. All the sites were sampled between the years 2015 and 2016, in the dry (May to August) and wet (October to January) seasons (Table 1, Fig. 1).

The information about degradation level of sampling sites was obtained through data from literature (Feio et al. 2015). The sites RV08, RV11, RV10, RV12, RV13, RV14, RV15 and RV16 (Feio et al. 2015) were considered as the correspondents of RV-01, RV-02, RV-03, RV-04, RV-05, RV-06, RV-07 and RV-08, respectively (Table 1). Degradation levels range from I (preserved) to IV (degraded).

Data about water quality, hypereutrophic condition, toxic contamination and pressure factors in the study sites were accessed through IGAM's website (http://portalinfohidro.igam.mg.gov.br), which monitors water quality quarterly at several points across the Rio das Velhas Basin. The IGAM monitoring sites: BV001, BV139, BV105; BV137; BV141, BV150, BV151 and BV149, were considered as sampling points: RV-01, RV-02, RV-03, RV-04, RV-05, RV-06, RV-07 and RV-08, respectively (Table 1). Mean values of conductivity, dissolved oxygen, total ammoniacal nitrogen and total phosphorous were obtained from IGAM measurements carried out in the years 2015 and 2016. The hypereutrophic condition, toxic contamination and pressure factors acting in the study sites were extracted from the quarterly report of the year 2017.

Table 1. Geographic location (in degrees/minutes/seconds and UTM, date, altitude and municipality) and water quality of the sampling sites sampled in the main channel of Rio das Velhas. Cond.: Condutivity (µS/cm), D.O.: Dissolved oxygen (mg/l), Am. nitr.: Ammoniacal nitrogen (mg/l), Phosp.: Total phosphorus (mg/l), Tox. contam.: Toxic contamination, Deg. level: degradation level ranging from I to IV (Feio et al. 2015). *Sites with hypereutrophic condition according IGAM.

Characteristics of sampling sites						Water Quality						
Regions	Sampling points	Date of sampling	Coordinates	Altitude (m)	Municipality	Cond.	D.O.	Am. nitr.	Phosp.	Tox. contam.	Deg. level	Pressure factors
Upper RV	RV-01	20/08/2015 21/01/2016 10/06/2016	20°18'42.8"S 43°34'01.5"W 23K 649606 E 7753356 W	1010	Ouro Preto	26.09	8.10	0.11	0.06		I	
Upper RV	RV-02	19/08/2015 20/01/2016 9/06/2016	20°01'10.7"S 43°49'45.4"W 23K 622454 E 7785916 W	729	Nova Lima	73.21	7.54	0.12	0.08		II	
Middle RV	RV-03	17/08/2015 19/01/2016 7/06/2016	19°49'54.8"S 43°51'56.2"W 23K 618796 E 7806723 W	674	Santa Luzia	345.15	2.95	5.40	0.69	Total ammoniacal nitrogen	III	Discharge of domestic sewage
Middle RV	RV-04	18/08/2015 18/01/2016 8/06/2016	19°32'56.7"S 43°53'33.3"W 23K 616174 E 7838041 W	658	Lagoa Santa	330.92	4.29	4.96	0.49	Total ammoniacal nitrogen	III	Discharge of domestic sewage
Middle RV	RV-05	10/08/2015 11/01/2016 31/05/2016	18°48'19.2"S 44°09'09.2"W 23K 589298 E 7920498 W	567	Curvelo	287.20	7.25	0.92	0.41	Arsenic and total ammoniacal nitrogen	III*	Gold metallurgy and discharge of domestic sewage
Low RV	RV-06	11/08/2015 12/01/2016 1/06/2016	18°25'33.2"S 44°11'10.9"W 23K 585926 E 7962502 W	552	Corinto	203.23	7.30	0.23	0.22	Arsenic	II	Agriculture
Low RV	RV-07	13/08/2015 13/01/2016 3/06/2016	17°51'55.4"S 44°32'57.4"W 23 K 547752 E 8024649 W	495	Lassance	162.21	7.63	0.21	0.17	Arsenic	II	Discharge of domestic sewage and agriculture (sugar cane)
Low RV	RV-08	12/08/2015 14/01/2016 2/06/2016	17°12'25.9"S 44°48'49.8"W 23 K 519793 E 8097515 W	464	Várzea da Palma	153.00	8.35	0.14	0.11	Arsenic	II	Discharge of domestic sewage
Sewage MRBH	STP Arrudas	20/07/2016, 25/01/2017			Sabará							
Sewage MRBH	STP Onça	20/07/2016, 18/01/2017			Belo Horizonte							

Captures of specimens of *A. lacustris* and *A. taeniatus* were carried out with gillnets with mesh sizes of 2.4, 3.0 and 4.0 cm between opposing nodes and with cast nets, seines and sieves. A total of 137 individuals of *A. lacustris* and 103 individuals of *A. taeniatus* was sampled in the three regions. The captures with gillnets represented 63% of sampling. For the stable isotope analyses, we collected at least five samples of each species at each sampling site (whenever possible). In the field, dorsal muscle was removed for large specimens and for small the whole fish was used removing the digestive tract. All samples were kept frozen until laboratory processing to avoid decomposition and deterioration of the material. In the laboratory, the fish samples were lyophilized for 24 hours, ground to fine and homogeneous powder using mortar and pestle and stored in eppendorf tubes.

The individuals that were not selected to stable isotopes analyses were fixed in formalin (10%) in the field, washed in water after fixation and transferred to alcohol (70%) in laboratory. Individuals predated or in high stage of decomposition were discarded. The remain individuals were used to stomach contents analyses in laboratory, where they had their stomach contents carefully removed. The same individuals were not used for both isotopic and stomach contents analyses because the stomach contents were analyzed following the results of stable isotope analyses, when we detect the need for complementary information.

Whenever possible, we collected five samples of all basal food resources available at each sampling site: periphyton, filamentous algae, suspended matter, fine particulate organic matter (FPOM) from sediments, vegetation (grasses and riparian vegetation), coarse particulate organic matter (CPOM), and aquatic macrophytes. Complementary samples of the suspended material were made at the sewage treatment plants to obtain the isotope signature of the raw sewage.

Samples of algae, aquatic macrophytes, vegetation and CPOM were collected at all sites where they were present, stored in plastic bottles and kept frozen until laboratory processing. Filamentous algae and aquatic macrophytes were collected manually in each site where they were present. Leaves from pasture (grasses) and from the natural riparian vegetation were manually collected along river banks in each site, with the most common species being prioritized at the site. The CPOM was randomly collected from leaf litter deposits in the streams.

Liquid samples, like periphyton, suspended matter (including sewage samples) and sediment, were collected at each site and kept frozen until processing in laboratory, where they were filtered using a filtration device attached to a vacuum pump using calcined quartz fiber filters (Whatman® QMA quartz filters). The periphyton was collected by scraping rocks with a brush and placing the material in a plastic bottle with distilled water. FPOM samples were collected from sediment deposits revolving in each sampling site and stored in plastic bottles. The suspended matter presented in the sampling sites and at STPs were collected with a phytoplankton net (0.45 mm mesh) deployed for a period of three minutes at each sampling site.

In the laboratory, all basal resource samples were dried in an oven at 60 °C for 48 hours and then ground with a mortar and pestle and stored in Eppendorf tubes.

The contents of 44 stomachs of *A. lacustris*, and 31 stomachs of *A. taeniatus* were analyzed in total. Food items were weighed (0.001 g accuracy/ wet weight) and identified under stereomicroscope to the lowest taxonomic category possible. The frequency of occurrence (Fi = number of times item i occurred, divided by the total number of stomachs) and the relative weight (Pi = sum of the weight of item i divided by the sum of the weight of all items) of each item were obtained. The food index (IA), proposed by Kawakami and Vazzoler (1980), was then calculated for each species and region, according to the formula: $IAi = (Fi.Pi)/\Sigma Fi.Pi$, where, IAi = food index of item i; Fi = frequency of occurrence of item i, and Pi = weight of item i.

The degree of overlap in food items between species was calculated using the simplified Morisita index (Morisita-Horn index) (Krebs 2014), according to the formula below: $C_H = 2\Sigma Pij.Pik/\Sigma P^2ij + \Sigma P^2ik$, where, C_H = Simplified Morisita Index of overlap (Horn 1966) between species j and species k; Pij = Proportion resource i is of the total resources used by species j; Pik = Proportion resource i is of the total resources used by species k, and n = Total number of resource states (I = 1, 2, 3, ... n).

For the food items characterization, "detritus" was considered dead particulate organic material, "sediment" included inorganic particles, and "plant remnants" were related to fragment of terrestrial vegetation.

A total of 42 samples of *A. lacustris*, 47 samples of *A. taeniatus* and 703 basal resources samples were sent to the Center for Nuclear Energy in Agriculture (CENA) at University of São Paulo (USP) for isotopic analysis. About 2–5 mg of dry animal tissue material and approximately 5–10 mg of basal resources samples were selected for analysis.

To determine the isotopic ratio, a mass spectrometer system in the Continuous-flow (CF-IRMS) mode was used with a Carlo Erba elemental analyzer (CHN 1110) coupled to a Delta Plus mass spectrometer (Thermo Scientific). Results were expressed as relative difference of international reference standards, in the delta notation (δ ‰), and calculated using the following formula: $\delta X = [(R_{sample}/R_{standard})^{-1}] \times 10^3$, where X is ^{13}C or ^{15}N and R represents the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie and Prosser 1996).

Differences in isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ of consumers and resources between the three regions were tested using one-way analysis of variance (ANOVAs) when the normality and homoscedasticity assumptions were met. The nonparametric Kruskal-Wallis test was used for data with non-normal distribution. When significant differences ($p < 0.05$) were observed, means were compared using the post-hoc Tukey's test. We also tested if isotopic signatures of *A. lacustris* and *A. taeniatus* presented variation between the dry and wet season, using t-tests (normal distribution) and Mann-Whitney tests (non-parametric). These analyses were performed in the software Statistica 6.0 (Statsoft 2004).

To evaluate the trophic structure of *A. lacustris* and *A. taeniatus* populations, individuals of the two species were plotted in the bi-plot space according to the isotopic values of carbon (x-axis) and nitrogen (y-axis) in each region (Fig. S1). Source contributions to the *A. lacustris* and *A. taeniatus* diet were estimated for the three regions based on stable isotope data analyzed through Bayesian stable isotope mixed models (Moore and Semmens 2008, Parnell et al. 2010), specifically using the MixSIAR package in R (Stock and Semmens 2016a). For both analyses, only the autochtonous sources, algae and periphyton, and the allochtounous sources, leaves of riparian vegetation and grasses were taken into account. The samples of sewage were also considered to sites "Middle RV" and "Low RV" since they are located in the area under influence of pollution. We used Markov chain Monte Carlo sampling based on the following parameters: number of chains = 3; chain length = 100,000; burn in = 50,000; thin = 50 and model 4 (Resid*Process) error structure (Stock and Semmens 2016b). Diagnostic tests (Gelmin-Rubin, Heidelberger-Welch and Geweke) and trace plots were examined for model convergence. The fractionation values used for consumers were 0.4 ± 1.3 ‰ for Carbon and 2.54 ± 1.27‰ for Nitrogen (Vanderklift and Ponsard 2003, Post 2002). Both the graphical representation and the partition analysis were done using the MixSIAR package in the R programming environment (Stock and Semmens 2016a).

The isotopic niches of *A. lacustris* and *A. taeniatus* in both regions (Upper RV, Middle RV and Low RV) were quantified based on standard ellipse areas (SEA – expressed in ‰^{2}) through use of the Stable Isotope Bayesian Ellipses package in R (SIBER, Jackson et al. 2011). The standard ellipse area (SEA) represents the core isotopic niche space and it is a proxy of the richness and evenness of resources consumed by the population (Bearhop et al. 2004). All measures were "bootstrapped" (n = 10,000, indicated by the letter "b") to compare groups with different sample sizes. A small sample size correction (indicated by the subscript letter "c") was applied to SEA to increase the accuracy of the comparisons, enabling the comparison of niches of populations with different sample sizes (Jackson et al. 2011). The SEAc allows to calculate the degree of niche overlap (in percentage, where 100% indicates total overlap) and can be used as a quantitative measure of diet similarity among different species (Hill et al. 2015).

RESULTS

Stomach contents

Only two stomachs were found empty, both of *A. taeniatus* sampled in Upper RV. Plant and insect remnants were the predominant items in the stomachs of *A. lacustris* and *A. taeniatus*, respectively (Table 2). However, both species presented variations in the type and proportion of ingested food items in each study region. In Upper RV, *A. lacustris* feed more on plant remnants, aquatic insects and detritus, while *A. taeniatus* feed on sediments and insect remnants. In the middle RV, *A. lacustris* maintained its diet based on plant remnants, however there was an increase of insect remnants. In this region *A. taeniatus* feed on insect remnants and aquatic insects. In the low RV, the most consumed item by *A. lacustris* was algae/periphyton, a pattern also observed for *A. taeniatus*, albeit to a lesser extent (Table 2).

Variation in resources used by *A. lacustris* and *A. taeniatus* was reflected in the food overlap of the two species in each region. The lowest food overlap was observed in the Upper RV (0%), followed by middle RV (34%) and low RV(83%).

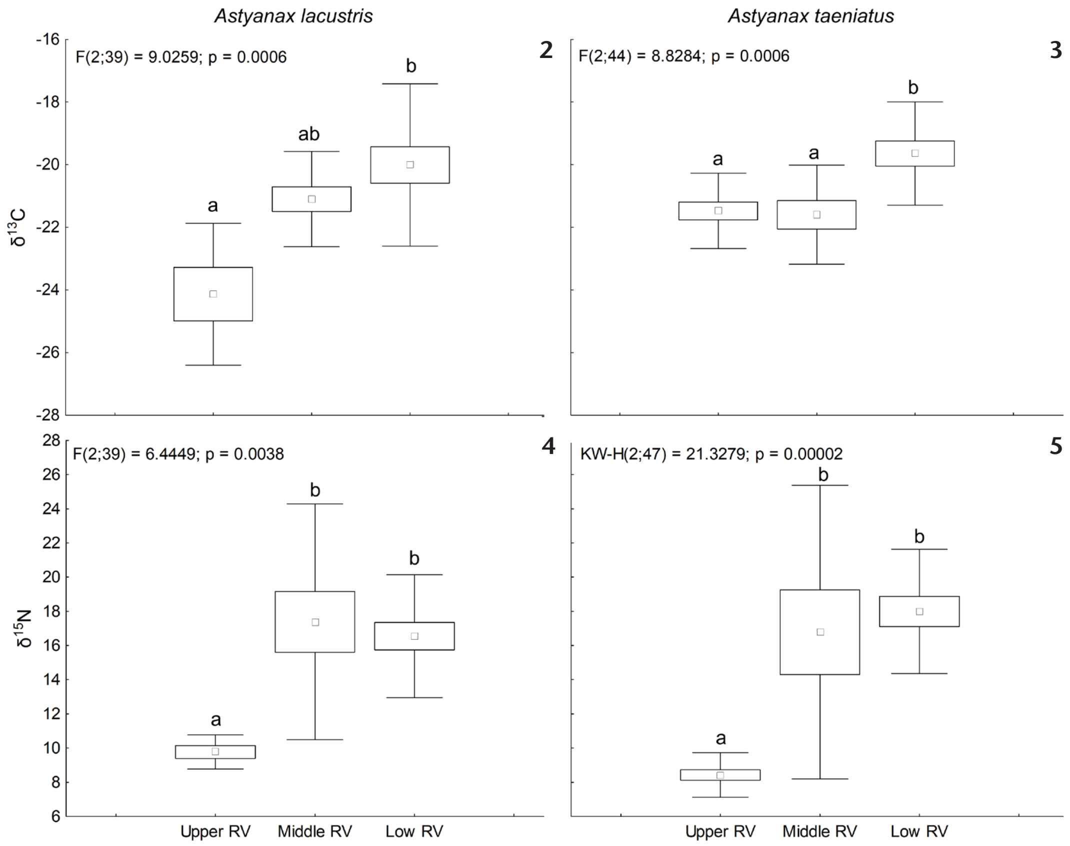
Table 2. Food index (AI), frequency of occurrence (Freq.) and weight of each food item found in the stomachs of the species *A. lacustris* and *A. taeniatus* in each sampled region of the Rio das Velhas Basin.

	Item	Upper RV			Middle RV			Low RV		
		IA	Freq.	Weight	IA	Freq.	Weight	IA	Freq.	Weight
Astyanax lacustris	Algae/ Periphyton	0.00	0.00	0.00	0.00	0.08	0.00	0.80	0.32	0.85
	Aq. Macrophytes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.01
	Sediment	0.00	0.00	0.00	0.00	0.15	0.00	0.02	0.24	0.03
	Detritus	0.20	0.17	0.33	0.00	0.00	0.00	0.00	0.00	0.00
	Plant remnants	0.40	0.33	0.33	0.53	0.54	0.44	0.00	0.16	0.00
	Aquatic insects	0.40	0.67	0.33	0.00	0.23	0.01	0.00	0.16	0.00
	Terrestrial insects	0.00	0.00	0.00	0.02	0.08	0.12	0.00	0.04	0.00
	Insects remnants	0.00	0.67	0.00	0.44	0.46	0.43	0.17	0.56	0.10
Astyanax taeniatus	Algae/ Periphyton	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.40	0.27
	Aq. Macrophytes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Sediment	0.43	0.50	0.47	0.00	0.00	0.00	0.00	0.13	0.00
	Detritus	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.07	0.06
	Plant remnants	0.00	0.10	0.00	0.12	0.17	0.14	0.15	0.20	0.18
	Aquatic insects	0.00	0.20	0.00	0.64	0.17	0.77	0.00	0.00	0.00
	Terrestrial insects	0.00	0.00	0.00	0.02	0.17	0.03	0.10	0.07	0.34
	Insects remnants	0.57	0.60	0.52	0.22	0.67	0.07	0.28	0.40	0.16

Stable isotopes

The $\delta^{13}C$ and $\delta^{15}N$ of *A. lacustris* and *A. taeniatus* is different among the three regions (Figs 2–5). The $\delta^{13}C$ of *A. lacustris* is significantly different between Upper and Low RV (Fig. 2), while the $\delta^{13}C$ of *A. taeniatus* in Low RV is different of all other regions (Fig. 3). The $\delta^{15}N$ values of both species in Upper RV are different to the $\delta^{15}N$ values in Middle and Low RV (Figs 4, 5). When the comparison was made between the species, it was possible to observe that the isotopic composition of *A. lacustris* and *A. taeniatus* were different only in the upper RV for both carbon ($p < 0.01$) and nitrogen ($p = 0.02$). In other regions – middle RV ($\delta^{13}C$: $p = 0.41$ and $\delta^{15}N$: $p = 0.83$) and low RV ($\delta^{13}C$: $p = 0.61$ and $\delta^{15}N$: $p = 0.23$) – there was no variation between species. In addition, only the *A. taeniatus* presented variation in the $\delta^{15}N$ values between seasons ($p = 0.02$), with more enriched values being observed in the dry season.

Basal resources presented extensive variation in their isotopic composition, except for riparian vegetation and grasses, that did not vary in $\delta^{13}C$ between the three sampled regions (Table 3). Strikingly, autotrophic resources (algae, periphyton and aquatic



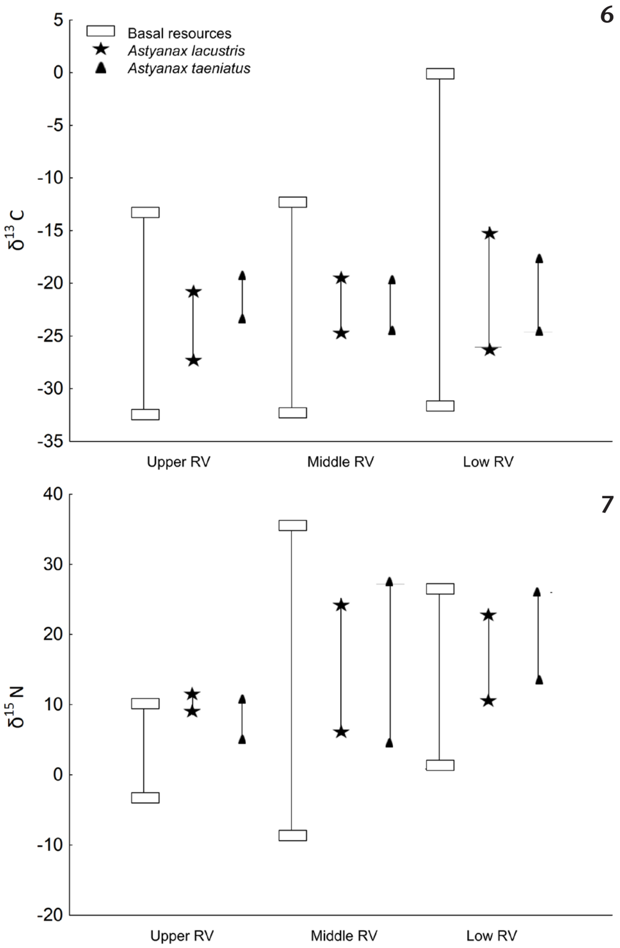
Figures 2–5. Variation in the isotopic composition of carbon (2–3) and nitrogen (4–5) in the species *A. lacustris* (2, 4) and *A. taeniatus* (3, 5) among the studied regions. Mean (small box), standard error (bars) and standard deviation (large box). Letters (a and b) indicate significant differences according to post-hoc Tukey's test.

Table 3. Variation in the carbon and nitrogen isotopic composition of the resources sampled in the three regions of the Rio das Velhas Basin. Letters (a, b and c) indicate significant differences according to post-hoc Tukey's test. AL: filamentous algae, CPOM: coarse particulate organic matter, FPOM: fine particulate organic matter, GR: grasses, MA: macrophytes, PE: periphyton, RV: riparian vegetation, SM: suspended matter and SW = raw sewage.

	$\delta^{13}C$ (mean and SD)				$\delta^{15}N$ (mean and SD)			
	Upper RV	Middle RV	Low RV	p	Upper RV	Middle RV	Low RV	p
AL	-27.74±4,66a	-22.84±6,35a	-5.52±2,47b	<0.01	4.67±2,99a	8.38±16,38ab	15.85±4,81b	<0.01
CPOM	-30.01±1,17a	-29.00±1,46b	-28.98±1,53b	<0.01	0.94±2,72a	4.48±3,86b	7.53±3,52c	<0.01
FPOM	-25.08±1,75a	-23.55±1,33b	-21.33±3,07c	<0.01	5.03±1,70a	6.66±7,10a	14.26±3,75b	<0.01
GR	-13.98±0,87a	-17.21±6,45a	-16.27±5,67a	0.56	0.28±1,81a	4.56±3,66b	8.00±3,89c	<0.01
MA	-30.54±1,08a	-25.94±5,91ac	-29.00±0,75c	<0.01	8.46±1,93a	19.55±14,18b	15.47±3,37b	<0.01
PE	-25.07±2,20a	-23.62±1,61a	-19.19±3,69b	<0.01	5.26±1,20a	9.15±10,72a	15.36±4,08b	<0.01
RV	-30.20±1,78a	-29.99±1,86a	-29.51±1,30a	0.54	0.64±1,72a	4.78±2,43b	7.28±2,39c	<0.01
SM	-25.88±1,67a	-24.56±1,75b	-20.45±4,62c	<0.01	4.47±1,39a	5.59±7,24a	14.15±4,96b	<0.01

macrophytes) showed highly enriched nitrogen isotopic values in the most polluted regions (middle and low RV) (Figs 6, 7). The range of carbon values of basal resources and fish species was higher in the low RV region and similar in other regions (Fig. 6). The range of nitrogen isotopic values, in turn, was higher in middle and low RV regions (Fig. 7).

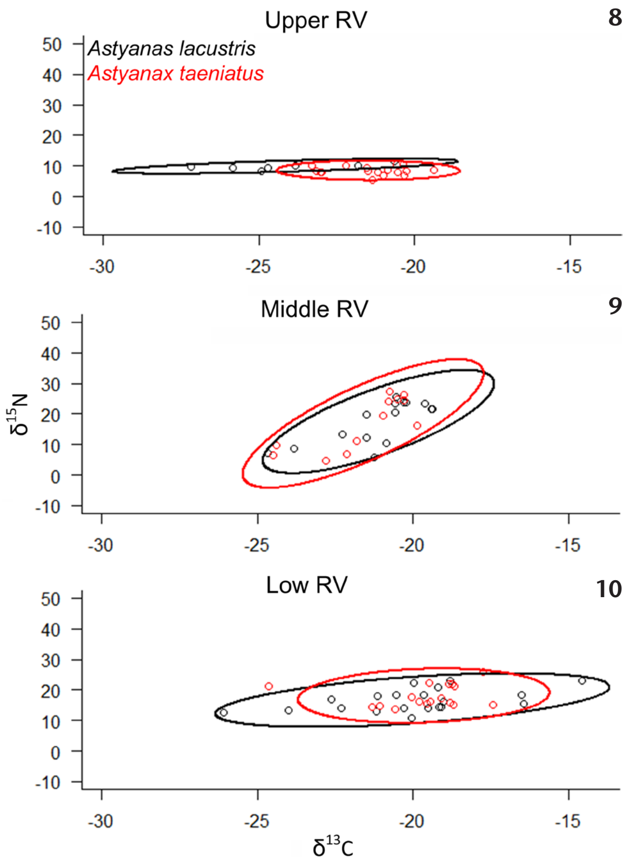
According to the partition analysis, in the upper RV, periphyton was the most assimilated basal resource, followed



Figures 6–7. Isotopic values range for $\delta^{13}C$ (6) and $\delta^{15}N$ (7) of basal resources, *A. lacustris* and *A. taeniatus* sampled in three regions of Rio das Velhas basin.

by filamentous algae and grasses (mainly for *A. taeniatus*). In the middle course, both species assimilated more carbon from filamentous algae and the other resources had similar contributions. In the lower course, the periphyton was again the most assimilated resource by *A. lacustris* and *A. taeniatus*. However, riparian vegetation had a greater contribution in this site than in other sites, being the second most consumed resource by both species (Table 4).

Results of isotopic niche overlap were similar to those observed in the stomach contents analyses. We again observed a slight overlap of trophic niches in the upper RV (23%) (Fig. 8, see also Fig. S1). In this region, with no influence of the sewage from the MRBH, the two species presented little overlap in assimilated carbon sources and appeared to occupy the same trophic level (Fig. 8). In the middle course of the Rio das Velhas, where the discharge of sewage is high, the carbon and nitrogen values of the two species were very similar, presenting high overlap (71%). In addition, a large variation in nitrogen signatures for both species was observed in this region, with an amplitude of 4.68 to 27.22 ‰ (Figs 7, 9). In the low RV it was also observed a high niche overlap (62%) especially in the carbon source (Fig. 10). However, there was a decrease in variation of nitrogen isotopic composition (10.97 to 25.96 ‰) (Fig. 7).



Figures 8–10. Trophic niche of *A. lacustris* and *A. taeniatus* (evaluated by the ellipse area with 95% confidence interval) in the Upper (8), Middle (9) and Low (10) regions of the Rio das Velhas Basin.

Table 4. Mean proportion of each basal resource assimilated by *Astyanax lacustris* and *A. taeniatus* at each sampling site. AL: filamentous algae, SW: raw sewage, GR: grasses, RV: riparian vegetation, PE: periphyton.

	Rio das Velhas regions	Basal resources				
		AL	SW	GR	RV	PE
A. lacustris	Upper	0.31		0.06	0.04	0.59
	Middle	0.58	0.09	0.08	0.08	0.18
	Low	0.04	0.04	0.05	0.14	0.74
A. taeniatus	Upper	0.19		0.22	0.02	0.57
	Middle	0.62	0.07	0.05	0.08	0.19
	Low	0.03	0.02	0.03	0.11	0.81

DISCUSSION

Food overlap between the two congeneric species was low in the least-disturbed region (upper Rio das Velhas), confirming our first hypothesis, that closely-related sympatric species diverge in their trophic niche to allow coexistence. In this study, the species *A. lacustris* and *A. taeniatus* presented high trophic plasticity in response to pollutants, increasing their food overlap and presenting similar isotopic signatures in the heavily polluted areas. Such aspect confirm our second hypothesis, that human disturbance promotes homogenization of fish species' diets. Despite plant and insect remnants were the predominant items in the stomachs of *A. lacustris* and *A. taeniatus*, algae and periphyton were also important food items (especially in lower sites). The importance of autochthonous resources as food items was highlighted in the partition analysis, which indicated that algae (in polluted regions) and periphyton (in least-disturbed region) were the most assimilated resources for both species.

The variation in $\delta^{13}C$ and $\delta^{15}N$ compositions and in stomach contents of *A. lacustris* and *A. taeniatus* along the Rio das Velhas highlight their generalistic habits and high trophic plasticity (Manna et al. 2012, Carvalho et al. 2015) probably as a result of resource availability found in the aquatic environment (Lobón-Cerviá and Bennemann 2000). The *A. taeniatus* also changes its $\delta^{15}N$ compositions between seasons (with enriched values in the dry season) which can be due changes on trophic levels (Vander Zanden et al. 1997), but also can occur in response to a higher enrichment in $\delta^{15}N$ values of resources in dry seasons.

The predominance of insects and plant remains in their stomach contents as well as the consumption of algae/periphyton are in agreement with the literature (e.g. Andrian et al. 2001, Casatti et al. 2003, Bennemann et al. 2005, Souza and Lima-Júnior 2013). It is likely that periphyton (in least-disturbed sites) and algae (in more degraded sites) are also being consumed indirectly through the consumption of insects. Castro et al. (2016) also observed a trend of changes in macroinvertebrates assimilation of algae and periphyton between degraded and preserved environments, which reinforces our statement. These changes in the type and proportion of autochthonous resources that sustain the two species are probably due to changes in environmental conditions as a result of pollution. Periphyton (or biofilm) is defined as an integral and independent micro-ecosystem in aquatic ecosystems, harboring biotic components (like algae, fungi, bacteria, protozoans, metazoans) and abiotic components (like substrata, extracellular polymeric substance and detritus) (Wu 2016). These organisms occur on the surface of rocks and submerged vegetation (Tundisi and Tundisi 2008), in environments with good water quality and greater presence of rocks and wood that favor the proliferation of periphyton. On the other hand, an increase of nutrients in the aquatic environment triggers a marked increase in algae (Tundisi and Tundisi 2008). Therefore, the expected greater abundance of algae in areas under the influence of pollution and of periphyton in sites with better environmental conditions, explain the shifts on basal resources assimilated by fish species.

In this study, stomach contents and stable isotopes analyses showed that there is a tendency to niche overlapping in *A. lacustris* and *A. taeniatus* in the presence of pollutants. The percentage of niche overlap observed by stomach contents and stable isotopes analyses were not the same, which is expected since not all items found in fish stomachs are assimilated (Manetta and Benedito-Cecílio 2003). In addition, the items consumed only occasionally or accidentally by individuals are observed on stomach contents, but will not be reflected on isotopic composition of fish. The greater overlap observed in the middle and lower course of Rio das Velhas could be due to the lower heterogeneity and resource availability in impacted sites (Gutiérrez-Cánovas et al. 2015). Fish tend to exhibit greater selectivity and specialization in the resources consumed in heterogeneous aquatic ecosystems, while in environments with few resources (or predominance of a single resource), fish tend to share the same food items (Knoppel 1970, Hurlbert 1978). Although we did not measure algae abundance, it is expected that in polluted sites populations reach high densities (e.g. Lata Dora et al. 2010, Macedo and Sipaúba-Tavares 2010), becoming an important food source consumed either directly or indirectly by generalist species, which can explain the higher niche overlap between *A. lacustris* and *A. taeniatus* in the middle and lower regions.

Trophic niche amplitude differed between regions. In the undisturbed region (Upper RV), both species had a broader trophic niche on the horizontal axis (niche with great carbon range). This trend is expected in food webs in which there are multiple basal resources with varying $\delta^{13}C$ values, enabling niche diversification at the base of a food web (Layman et al. 2007), which indicates that *A. lacustris* and *A. taeniatus* feed on a greater range of resources under natural conditions. On the other hand, in the most polluted region (Middle RV), the two species presented a narrow carbon range and a large nitrogen range (more vertical trophic niche). The narrow carbon range may be occurring in response to the restriction and homogenization of available food resources (the opposite of what has been observed in preserved sites). A larger range in $\delta^{15}N$ sometimes suggests more trophic levels and thus a greater degree of trophic diversity (Layman et al. 2007), however, probably this is not the explanation to the niche verticalization observed in this study, but the greater enrichment observed in the basal resources. This verticalization of the trophic niche has been found in fish (De Carvalho et al. 2017) and macroinvertebrates (Castro et al. 2016) in environments impacted by other anthropogenic activities (sugarcane).

The enriched nitrogen values of fish and resources especially in the middle section are probably related to the influence of sewage effluents, since $\delta^{15}N$ values of domestic wastes ranges between 7‰ to 38‰ (Dailer et al. 2010). Domestic wastes are nitrogen enriched especially because of isotopic fractionation during nitrification and volatilization in the case of ammonium, or denitrification in the case of nitrate (Nikolenko et al. 2018).

Therefore, the uptake of enriched δ^{15}N by primary producers are reflected in the entire food web (McClelland et al. 1997). Changes in δ^{15}N values along the pollution gradient, with a particularly large increase in regions affected by sewage effluent, were similar to those reported in studies of macroinvertebrates (e.g. Morrissey et al. 2013, Pastor et al. 2014, Baumgartner and Robinson 2016), and primary producers (e.g. MCclelland and Valiela 1998, Cole et al. 2004, Wang et al. 2016). Together, results of these studies, including ours, support the finding that high δ^{15}N values are good indicators of anthropogenic stress in aquatic systems.

Therefore, stomach contents and stable isotope analyses were very useful to evaluate the effects of the presence of pollutants in the trophic ecology of two congeneric species. It was possible to observe that even where species originally present different feeding habits (verified through the analysis of the stomach contents), food webs were mainly based on autochthonous items, such as algae and periphyton (verified through the isotopic analysis), assimilated directly and indirectly through aquatic insects. The presence of pollution, besides triggering increased food overlap between *A. lacustris* and *A. taeniatus*, also promoted an enrichment in δ^{15}N values of fish and resources. The δ^{15}N values of fish seems to be an effective means to detect anthropogenic impacts in aquatic ecosystems. In addition to providing important information on species biology, our work contributes to elucidate one of the 100 key ecological issues (Sutherland et al. 2013): How do resource pulses affect resource use and interactions between organisms?

ACKNOWLEDGMENTS

We thank the Agência Peixe Vivo and the Comitê de Bacia Hidrográfica do Rio das Velhas (CBH, Rio das Velhas) for the project financial support and the Projeto Manuelzão for the logistical support. Thanks to Aline J. Grossi (Universidade Federal de Lavras, UFLA) and Luiza Hoehne (Universidade Federal de Minas Gerais, UFMG) for the support on processing of samples. Thanks also to the Benthos Ecology Laboratory (UFMG) and the Laboratory of Fish Ecology (UFLA) that allowed processing of samples and infrastructure, and to the Centro de Energia Nuclear na Agricultura (CENA) for their support and partnership in the isotopic analysis. PSP received a research grant and a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 303548/2017-7) and from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG PPM-00608/15).This study was financed in part by the Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES) – Finance code 32004010017P3. The manuscript underwent grammar revision by Alistair Campbell.

LITERATURE CITED

Alves CBM, Pompeu PS (2001) A Fauna de Peixes da Bacia do Rio das Velhas no Final do Século XX. In: Alves CBM, Pompeu PS (Eds) Peixes do rio das Velhas: passado e presente. Belo Horizonte, SEGRAC, 165–187.

Andrian IF, Silva HB, Peretti D (2001) Dieta de *Astyanax bimaculatus* (Linnaeus, 1758) (Characiformes, Characidae), da área de influência do reservatório de Corumbá. Estado de Goiás, Brasil. Acta Scientiarum 23(2): 435–440. http://dx.doi.org/10.4025/actascibiolsci.v23i0.2735

Baeta A, Vieira LR, Lírio AV, Canhoto C, Marques JC, Guilhermino L (2017) Use of stable isotope ratios of fish larvae as indicators to assess diets and patterns of anthropogenic nitrogen pollution in estuarine ecosystems. Ecological Indicators 83: 112–121. https://doi.org/10.1016/j.ecolind.2017.07.062

Barrie A, Prosser SJ (1996) Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. In: Boutton TW, Yamasaki S (Eds) Mass spectrometry of soils. New York, Marcel Dekker, 1–46.

Baumgartner SD, Robinson CT (2016) Changes in macroinvertebrate trophic structure along a land-use gradient within a lowland stream network. Aquatic Sciences 79(2): 407–418. https://doi.org/10.1007/s00027-016-0506-z

Bearhop SC, Adams E, Waldron S, Fuller AA, MacLeod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology 73: 1007–1012. https://doi.org/10.1111/j.0021-8790.2004.00861.x

Beaudoin CP, Tonn WML, Prepas EEL, Wassenaar LI (1999) Individual specialization and trophic adaptability of norther pike (*Esox Lucius*): an isotope and dietary analysis. Oecologia 120(3): 386–396. https://doi.org/10.1007/s004420050871

Bennemann ST, Gealh AM, Orsi ML, Souza LM (2005) Ocorrência e ecologia trófica de quatro espécies de *Astyanax* (Characidae) em diferentes rios da bacia do rio Tibagi, Paraná, Brasil. Iheringia, Série Zoologia 95(3): 247–254. https://doi.org/10.1590/S0073-47212005000300004

Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. Ecology 93(20): 10844–10847. https://doi.org/10.1073/pnas.93.20.10844

Carassou L, Whitfield AK, Moyo S, Richoux NB (2017) Dietary tracers and stomach contents reveal pronounced alimentary flexibility in the freshwater mullet (*Myxus capensis*, Mugilidae) concomitant with ontogenetic shifts in habitat use and seasonal food availability. Hydrobiologia 799(1): 327–348. https://doi.org/10.1007/s10750-017-3230-3.

Carvalho DR, Castro D, Callisto M, Moreira MZ, Pompeu PS (2015) Isotopic variation in five species of stream fish under the influence of different land uses. Journal of Fish Biology 87(3): 559–578. https://doi.org/10.1111/jfb.12734

Casatti L, Mendes HF, Ferreira KM (2003) Aquatic macrophytes as feeding site for small fishes in the Rosana reservoir, Paranapanema river, Southeastern Brazil. Brazilian Journal of Biology 63(2): 213–222. https://doi.org/10.1590/S1519-69842003000200006

Cassemiro FAS, Hahn NS, Fugi R (2002) Avaliação da dieta de *Astyanax altiparanae* Garutti and Britski, 2000 (Osteichthyes, Tetragonopterinae) antes e após a formação do reservatório de salto Caxias, Estado do Paraná, Brasil. Acta Scientiarum 24(2): 419–425. https://doi.org/10.4025/actascibiolsci.v24i0.2314

Castro DMP, De Carvalho DR, Pompeu PS, Moreira MZ, Nardoto GB, Callisto M (2016) Land Use Influences Niche Size and the Assimilation of Resources by Benthic Macroinvertebrates in Tropical Headwater Streams. PLos ONE 11: e0150527. https://doi.org/10.1371/journal.pone.0150527

CETEC (1983) Diagnóstico Ambiental do Estado de Minas Gerais. Belo Horizonte, Fundação Centro Tecnológico de Minas Gerais/CETEC, Série de Publicações Técnicas/SPT-010, 158 pp.

Cole ML, Valiela I, Kroeger KD, Tomasky GL, Cebrian J, Wigand C, McKinney RA, Grady SP, da Silva MHC (2004) Assessment of a $\delta^{15}N$ Isotopic Method to Indicate Anthropogenic Eutrophication in Aquatic Ecosystems. Journal of Environmental Quality 33(1): 124–132. https://doi.org/10.2134/jeq2004.1240

Connan M, Bonnevie BT, Hagen C, van der Lingen CD, McQuaid C (2017) Diet specialization in a colonial seabird studied using three complementary dietary techniques: effects of intrinsic and extrinsic factors, Marine Biology 164: 171. https://doi.org/10.1007/s00227-017-3201-2

Dailer ML, Knox RS, Smith JE, Napier M, Smith CM (2010) Using $\delta^{15}N$ values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai'i, USA. Marine Pollution Bulletin 60(5): 655–671. https://doi.org/10.1016/j.marpolbul.2009.12.021

De Carvalho DR, Castro DMP, Callisto M, Moreira MZ, Pompeu PS (2017) The trophic structure of fish communities from streams in the Brazilian Cerrado under different land uses: an approach using stable isotopes. Hydrobiologia 795(1): 199–217. https://doi.org/10.1007/s10750-017-3130-6

Eigenmann CH (1921) The American Characidae. Memoirs of the Museum of Comparative Zoology 43(3): 209–310.

Esteves KE (1996) Feeding Ecology of three *Astyanax* species (Characidae, Tetragonopterinae) from a foodplain lake of Mogi-Guaçú River, Paraná River Basin, Brazil. Environmental Biology of Fishes 46(1): 83–101. https://doi.org/10.1007/BF00001701

Esteves KE, Aranha JMR (1999) Ecologia trófica de peixes de riachos. Oecologia Brasiliensis 6: 157–182.

Feio MJ, Ferreira WR, Macedo DR, Eller AP, Alves CBM, França JS, Callisto M. (2015) Defining and testing targets for the recovery of tropical streams based on macroinvertebrate communities and abiotic conditions. River Research and Applications 31(1): 70–84. https://doi.org/10.1002/rra.2716

Gerking SD (1994) Feeding ecology of fish. Califórnia, Academic Press, 416 pp.

Géry J (1977) Characoids of the world. Neptune City, T.F.H. Publications, 672 pp.

Gutiérrez-Cánovas C, Sánchez-Fernández D, Velasco J, Millán A, Bonada N (2015) Similarity in the difference: changes in community functional features along natural and anthropogenic stress gradients. Ecology 96(9): 2458–2466. https://www.jstor.org/stable/24702350

Hall SR (2004) Stoichiometrically Explicit Competition between Grazers: Species Replacement, Coexistence, and Priority Effects along Resource Supply Gradients. American Society of Naturalists 164(2): 157–172. https://doi.org/10.1086/422201

Hicks KA, Loomer HA, Fuzzen MLM, Kleywegt S, Tetreault GR, McMaster ME, Servos MR (2016) $\delta^{15}N$ tracks changes in the assimilation of sewage-derived nutrients into a riverine food web before and after major process alterations at two municipal wastewater treatment plants. Ecological Indicators 72: 747–758. https://doi.org/10.1016/j.ecolind.2016.09.011

Hill JM, Jones RW, Hill MP, Weyl OLF (2015) Comparisons of isotopic niche widths of some invasive and indigenous fauna in a South African river. Freshwater Biology 60(5): 893–902. https://doi.org/10.1111/fwb.12542

Horn HS (1966) Measurement of "overlap" in comparative ecological studies. The American Naturalist 100(914): 419-424.

Hurlbert SH (1978) The measurement of niche overlap and some relatives. Ecology 59(1): 67–77. https://doi.org/10.2307/1936632

Jackson AL, Inger R, Parnell AC, 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

Kling GW, Fry B, O'Brien W (1992) Stable isotopes and planktonic trophic structure in arctic lakes. Ecology 73(2): 561–566. https://doi.org/10.2307/1940762

Knoppel HA (1970) Food of Central Amazonian fishes. Amazoniana 2: 257–352.

Kawakami E, Vazzoler G (1980) Método gráfico e estimativa de índice alimentar aplicado no estudo de alimentação de peixes. Boletim do Instituto Oceangráfico 29(2): 205–207. https://doi.org/10.1590/S0373-55241980000200043

Krebs CJ (2014) Niche measures and resource preferences. In: Krebs CJ (Ed.) Ecological methodology. New York, Harper & Row, 3rd ed., 597–653.

Lata Dora S, Maiti SK, Tiwary RK, Anshumali (2010) Algae as an indicator of river water pollution – A Review. The Bioscan 2: 413–422.

Layman CA, Arrington DA, Montana CG, Post DM (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

Lobón-Cerviá J, Bennemann S (2000) Temporal trophic shifts and feeding diversity in two sympatric, neotropical, omnivorous fishes: *Astyanax bimaculatus* and *Pimelodus maculatus* in Rio Tibagi (Paraná, Southern Brazil). Archiv fuer Hydrobiologie 149(2): 285–306. https://doi.org/10.1127/archiv-hydrobiol/149/2000/285

Lougheed VL, Mcintosh MD, Parker CA, Stevenson RJ (2008) Wetland degradation leads to homogenization of the biota local and landscape scales. Freshwater Biology 53(12): 2402–2413. https://doi.org/10.1111/j.1365-2427.2008.02064.x

Macedo CF, Sipaúba-Tavares LH (2010) Eutrofização e qualidade da água na piscicultura: consequências e recomendações. Boletim do Instituto de Pesca 36(2): 149–163.

Manetta GI, Benedito-Cecílio E (2003) Aplicação da técnica de isótopos estáveis na estimativa da taxa de turnover em estudos ecológicos: uma síntese. Acta Scientiarum, Biological Sciences 25(1): 121–129. https://doi.org/10.4025/actascibiolsci.v25i1.2090

Manna LR, Rezende CF, Mazzoni R (2012) Plasticity in the diet of Astyanax taeniatus in a coastal stream from south-east Brazil. Brazilian Journal of Biology 72(4): 919–928. https://doi.org/10.1590/S1519-69842012000500020

McClelland JW, Valiela I, Michener RH (1997) Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. American Society of Limnology and Oceanography 42(5): 930–937. https://doi.org/10.4319/lo.1997.42.5.0930

McClelland JW, Valiela I (1998) Liking nitrogen in estuarine producers to land-derived sources. American Society of Limnology and Oceanography 43(4): 577–585. https://doi.org/10.4319/lo.1998.43.4.0577

Menezes NA, Weitzman S, Oyakawa OT, Lima F, Castro R, Weitzman M (2007) Peixes de água doce da Mata Atlântica. São Paulo, Museu de Zoologia/USP, Conservação Internacional, FAPESP, CNPq, 407 pp.

Morrissey CA, Boldt A, Mapstone A, Newton J, Ormerod SJ (2013) Stable isotopes as indicators of wastewater effects on the macroinvertebrates of urban rivers. Hydrobiologia 700(1): 231–244. https://doi.org/10.1007/s10750-012-1233-7

Moore JW, Semmens BX (2008) Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11(5): 470–480. https://doi.org/10.1111/j.1461-0248.2008.01163.x

Nikolenko O, Jurado A, Borges AV, 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.2017.10.086

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS One 5: e09672. https://doi.org/10.1371/journal.pone.0009672

Pastor A, Peipoch M, Cañas L, Chappuuis E, Ribot M, Gacia E, Riera JL, Martí E, Sabater F (2014) Nitrogen stable isotopes in primary uptake compartments across streams differing in nutrient availability. Envirанmental Science & Technology 47(18): 10155–10162. https://doi.org/10.1007/s00027-013-0330-7

Pereira LPF, Mercante CTJ (2005) A amônia no sistema de criação de peixes e seus efeitos sobre a qualidade de água. Uma revisão. Boletim do Instituto de Pesca 31(1): 81–88.

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18: 293–320. https://doi.org/10.1146/annurev.es.18.110187.001453

Post DM (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

Souza RG, Lima-Junior SE (2013) Influence of environmental quality on the diet of *Astyanax* in a microbasin of central western Brazil. Acta Scientiarum, Biological Sciences 35(2): 177–184. https://doi.org/10.4025/actascibiolsci.v35i2.15570

StatSoft (2004). STATISTICA: data analysis software system. Tulsa, StatSoft Inc, v. 7. Available online at: http://www.statsoft.com

Stock BC, Semmens BX (2016a) MixSIAR GUI. User Manual, v. 3.1. Available online at: https://github.com/brianstock/MixSIAR

Stock BC, Semmens BX (2016b) Unifying error structures in commonly used biotracer mixing models. Ecology 97(10): 2562–2569. https://doi.org/10.1002/ecy.1517

Sutherland WJ, Freckleton RP, Godfray HCJ, Beissinger SR, Benton T, Cameron DD, Hails RS (2013) Identification of 100 fundamental ecological questions. Journal of Ecology 101(1): 58–67. https://doi.org/10.1111/1365-2745.12025

Tundisi JG, Tundisi TM (2008) Limnologia. São Paulo, Oficina de Texto, 631 pp.

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δ^{15}N enrichment: a meta-analysis. Oecologia 136(2): 169–182. https://doi.org/10.1007/s00442-003-1270-z

Vander Zanden M, Cabana G, Rasmussen JB (1997) Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ^{15}N) and literature dietary data. Canadian Journal of Fisheries and Aquatic Sciences 54(5): 1142–1158. https://doi.org/10.1139/f97-016

Vilela FS, Becker FG, Hartz SM (2002) Diet of *Astyanax* species (Teleostei, Characidae) in an Atlantic forest river in Southern Brazil. Brazilian Archives of Biology and Technology 43(2): 223–232. https://doi.org/10.1590/S1516-89132002000200015

Wang Y, Liu D, Richard P, Di B (2016) Selection of effective macroalgal species and tracing nitrogen sources on the different part of Yantai coast, China indicated by macroalgal δ^{15}N values. Science of The Total Environment 542(Part A): 306–314. https://doi.org/10.1016/j.scitotenv.2015.10.059

Weitzman SH, Fink W (1983) Relationships of the Neon Tetras, a group of South American freshwater fishes (Teleostei, Characidae) with comments on the phylogeny of new world Characiforms. Bulletin of the Museum of Comparative Zoology 150(6): 339–395.

Wootton RJ (1999) Ecology of teleost fishes. The Netherlands, Kluwer Academic Publishers, 2nd ed., 386 pp.

Wu Y (2016) Periphyton: functions and application in environmental remediation. Amsterdam, Elsevier, 402pp.

Supplementary material 1

Figure S1. Distribution of *A. lacustris* (red points) and *A. taeniatus* (blue points) species in the bi-plot space by study regions.

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Data type: species data

Link: https://doi.org/10.3897/zoologia.36.e30445.suppl1

Submitted: October 9, 2018
Accepted: January 25, 2019
Available online: July 5, 2019
Editorial responsibility: Vinicius Abilhoa

Author Contributions: PSP, DRC and CBMA designed the experiments; DRC, PSP, and MBA conducted the experiments; MZM, PSP, MBA and DRC analyzed the data; MBA, DRC, CBMA, MZM and PSP wrote the paper.

Competing Interests: The authors have declared that no competing interests exist.

ARTIGO VI - ***Trophic structure of a fish community in a reference condition river***

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Manuscrito redigido na língua e formato indicado para revista *Environmental Biology of Fishes*

(número de linhas foi retirado para melhor adequação ao formato da tese)

Abstract

Natural environments described as reference conditions are areas where the impacts of human activities are minimal or non-existent. The objective of this work was to characterize the trophic structure of a riverine fish community in reference conditions, using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes. Samples were taken at two points along the Cipó River, a major tributary within the basin of the das Velhas River, in the state of Minas Gerais, Brazil. To obtain the isotopic composition of nitrogen and carbon, data were collected on fish and available food resources. The species pacú, *Myleus micans* (an herbivorous species) was found to differ substantially within the trophic web by presenting the most enriched values of $\delta^{15}N$ and $\delta^{13}C$. This suggests that the species has a large spatial range and may be feeding in places with resources enriched in $\delta^{15}N$ and $\delta^{13}C$, outside of the Cipó River. Variation in $\delta^{15}N$ and $\delta^{13}C$ was observed both between guilds and within guilds, with detritivorous fish presenting the greatest variation in $\delta^{13}C$. Although all detritivorous species appear to consume the same basal resources (algae, periphyton and CPOM), the proportion of assimilation of each resource varied among them. The catfish species, *Hypostomus* sp., *Pareiorhaphis mutuca* and *Harttia longipinna*, were found to have a diet predominantly based on CPOM. *Hypostomus francisci* and *Hypostomus margaritifer* consumed mainly periphyton, while *Steindachnerina elegans* consumed predominantly algae and CPOM. The isotopic niche of herbivorous fish was also broad, particularly due to the large variation in $\delta^{13}C$ and $\delta^{15}N$ for the species *M. micans*. The range and overlap of isotope niches of the other guilds (parasitic, piscivores, omnivores, invertivores and algivores) corresponded with what would be expected in a typical trophic web. Through the results of this work we highlight the need for more refined and accurate classification of fish in trophic guilds. We stipulate that improved knowledge on trophic structure of riverine fish under reference conditions can provide relevant information for proposed recovery goals for degraded environments, particularly through the establishment of isotopic reference levels.

Keywords: Trophic web. Ichthyofauna. Stable isotopes. Carbon. Nitrogen.

Introduction

Anthropogenic activities such as industrialization, land use change and urbanization pose major threats to aquatic environments (Martins et al. 2018). Such activities may alter the structure and functioning of aquatic ecosystems (Feld and Hering 2007; Ligeiro et al. 2013), as well as promote changes in biota (Vander Laan et al. 2013; Baumgartner and Robinson 2017). Fish are among the aquatic organisms impacted by environmental changes, from small changes in their communities (e.g. in response to changes in the availability of food resources), to critical episodes that result in mortality. At the trophic level, fish communities impacted by human activities may see a reduction in trophic guilds and an increase in generalist species, stemming from limiting conditions of physical and chemical parameters (dissolved oxygen, for example) (Pompeu et al. 2005), changes in water quality (such as changes in phosphorus and nitrogen concentrations) and availability of food resources (Karr

1981; Casatti et al. 2009; Alexandre et al. 2010; Eichbaum Esteves and Alexandre 2011; Bonato et al. 2012).

The description of trophic relationships is often fundamental when conducting ecological studies (Vander Zanden et al. 1997). A food web is generally used to represent the trophic structure and dietary relationships in a given community (Vander Zanden, M. Jake Rasmussen 1999). A crosswise description of trophic interactions in a food web can reveal the flow of matter and energy through ecosystems, detecting trophic links within a group of consumers (Santos 2009), and also establish a trophic structure comparable to other systems (Jepsen and Winemiller 2002; Santos 2009).). In many studies on the trophic ecology of fish, species are classified into guilds, grouping species by their use of food resources (Adams 1985; Simberloff and Dayan 1991; Luczkovich et al. 2002). A commonly observed trophic structure in fish communities includes detritivorous, algivorous and herbivorous species near the base of the food chain, omnivorous and invertivorous species at intermediate levels, and piscivorous species occupying the top (Cohen et al. 1985; Jepsen and Winemiller 2002; Anderson et al. 2007). Parasitic species, when present in a community, can reach the highest trophic level (Arias-González and Morand 2006).

Stable isotope analysis is an important tool to help define and understand the structure and functioning of food webs and energy flow in aquatic ecosystems (Peterson and Fry 1987; Vander Zanden and Rasmussen 2001; Rybczynski et al. 2008; Cucherousset et al. 2012; Davis et al. 2012). Isotopic analysis provides information on long-term trophic relationships, as well as assimilated diet (Hesslein et al. 1993; Rybczynski et al. 2008; Cucherousset et al. 2012; Davis et al. 2012). The most commonly used stable isotopes in ecological studies of aquatic ecosystems are carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (Manetta and Benedito-Cecilio 2003; Cucherousset et al. 2012); both of which are widely used to evaluate the impacts of anthropogenic activities on trophic structure (Castro et al. 2016; López-Rasgado et al. 2016; de Carvalho et al. 2017; Baeta et al. 2017). In studies on the trophic ecology of fish, the isotopic composition of $\delta^{13}C$ in fish tissue is compared with the isotopic composition of the resources used in their diet, since the components of a food web will reflect the isotopic carbon composition of their food sources (Fry and Sherr 1989). Furthermore, the $\delta^{13}C$ of the resources is reflected along the food web, with a small enrichment of 0.2 to 1.0‰ detected at each trophic level (McCutchan et al. 2003). Through this conservative transfer of $\delta^{13}C$ it is possible to identify the different resources that make up a trophic web of an ecosystem, in which food sources present different values in their isotopic compositions (DeNiro and Epstein 1978; Fry and Sherr 1989). The isotopic composition of $\delta^{15}N$, on the other hand, is consistently fractionated along a trophic web, with substantial variation in enrichment across trophic guilds. Generally, consumers with an invertebrate-based diet show an enrichment of 1.4 ± 0.21‰, while consumers with a high protein diet (such as predators/piscivores) have an enrichment of 3.3 ± 0.26‰, and consumers with an herbivorous diet (algae and plants) have an enrichment of 2.2 ± 0.30‰, per trophic level. For detritivorous and omnivorous consumers, an average enrichment of 2.3 ± 0.18‰ can be detected, as they consume a mixture of plant and animal material (McCutchan et al. 2003).

An important factor to better understand the impacts of anthropogenic activities on aquatic ecosystems is to assess the biological state of an ecosystem where the presence of human activity is minimal or non-existent (Martins et al. 2018). Such sites are referred to as reference conditions. The evaluation of reference conditions is widely used in monitoring water quality (Reynoldson et al. 1997; Davies et al. 2010; Pardo et al. 2012; Buss et al. 2015; Bouleau and Pont 2015), whereby such areas are considered to represent the best possible condition in a region (Feio et al. 2007). In addition to physical habitat and physicochemical conditions, trophic structure and ecosystem functioning are also valuable aspects to be evaluated in reference condition sites.

Consequently, the study of trophic relations and energy flow under reference conditions is fundamental for better understanding the impacts of human activities on ecosystem functioning, especially in impacted basins. In this context, the Cipó River presents an excellent opportunity for study. The river, which has its source located in the Serra do Cipó National Park, is protected across its entire length, thus presenting close to natural conditions (Alves and Pompeu 2005)), and is considered a reference for high quality aquatic environments due to the presence of many streams and springs little affected by human activities (CBH Rio das Velhas, 2016). The objective of the present study was to characterize the trophic structure of the fish community in this river (considered to be reference conditions) using stable isotopes. We tested the following hypotheses: 1) a trophic web within a reference river will exhibit a typical trophic structure; with piscivorous and parasitic fish occupying the highest trophic levels, invertivorous and omnivorous fish occupying intermediate trophic levels, and herbivorous, algivorous and detritivorous fish occupying the lower trophic levels; 2) the ^{13}C and ^{15}N isotopic composition will vary among trophic guilds, since fish consume different resources ($\delta^{13}C$ variation) and occupy different trophic levels ($\delta^{15}N$ variation); and 3) fish classified in the same trophic guild have similar $\delta^{13}C$ and $\delta^{15}N$, because they utilize the same resources. Moreover, the characterization of a river under reference conditions is expected to provide valuable information for decision-makers in bio-monitoring programs for aquatic environments impacted by anthropogenic activities.

Methodology

Study Area

This study was carried out on the Cipó River, a major tributary within the basin of the das Velhas River, which in turn, is the longest tributary of the São Francisco River, in the state of Minas Gerais, Brazil. At around 250km in length, the Cipó River begins at the meeting of the Mascate and Bocaina streams, both found within the Serra do Cipó National Park, and flows into the Paraúna River, in the municipality of Presidente Juscelino (CBH Rio das Velhas, 2016). Samples were taken at two points; the first (CP 1) is located immediately downstream of Serra do Cipó National Park and has excellent quality, cold, transparent and flowing water (18°21'11" S; 43°39'16" W, 757m elevation) (Alves and Pompeu 2010). The second point (CP 2) is located near the confluence with the Paraúna River, where the river flows over large slabs, with alternating pools and rapids (18°41'04" S; 43°59'18" W, 567 m elevation) (Alves and Pompeu 2010). Rupestrian fields are the dominant vegetation type at the National Park, but other types of vegetation are also found, including gallery forests that occur along the various watercourses (Vieira et al. 2005). High altitudes (800 - 1670m) characterize the Serra do Cipó region (Vieira et al. 2005) (Figure 1).

Fish Sampling

Two sets of fish collection were carried out at each sample point, one in the dry season (July 2015), and a second in the rainy season (October 2015). Fish were collected using gillnets (20 m long, 3–16 cm stretch measure mesh), seines (5 m long, 1 mm mesh), cast nets (3 cm stretch measure mesh), and mosquito nets (80 cm in diameter, 1 mm mesh). Gill nets were fixed in the water column for 14 h overnight. Seines were used in shallow areas or littoral zones, while the mosquito nets were employed for near-shore aquatic macrophytes (along both shorelines), undercut banks and in riffles. Cast nets were used in habitats too deep to wade. The three latter methods were employed for a period of 1-3h.

Five individuals of each species were selected for stable isotope analysis (when possible). Larger individuals had part of their muscle tissue removed in the field, and smaller individuals (<2 cm) were taken whole, having only their digestive tract removed. Samples were kept on ice in the field and subsequently frozen to avoid decomposition. In the laboratory, samples were lyophilized for 24 hours, ground to a fine and homogeneous powder using a pestle and mortar, and stored in Eppendorff tubes for later referral to a specialized laboratory for isotopic analysis. All species were classified into trophic guild according to the relevant literature (Table 1). Extra specimens of all collected species were registered in the ichthyological collection of the Federal University of Lavras (CI-UFLA).

Sampling of food resources

At each of the sampling points five samples were collected (where possible) of each one of the following baseline resources: filamentous algae; periphyton; CPOM (coarse particulate organic matter); FPOM (fine particulate organic matter); suspended matter; exotic grasses; native grasses; riparian vegetation; and bamboo.

Samples of algae, vegetation (riparian vegetation, grass and bamboo) and CPOM were randomly collected, stored in plastic pots and kept frozen until processing in the laboratory. Periphyton samples were collected by scraping stones with the aid of a brush and stored in plastic pots with distilled water. Samples of FPOM were collected by resuspending the sediment of the riverbed at each sample points and then stored in plastic pots. Suspended matter was collected using a phytoplankton net (45 µm mesh) placed in the water column for a period of three minutes at each sampling point. After collection, the liquid samples (periphyton, FPOM, and suspended material) were also immediately frozen to preserve the material. In the laboratory, samples were filtered using a vacuum pump attached to a filtration apparatus that uses calcined quartz fiber filters (Whatman® QMA quartz filters).

Following pre-treatment, all resource samples were kept in an incubator at 60° C for a minimum of 48 hours. Subsequently, they were ground to a fine and homogeneous powder, again with the aid of a pestle and mortar, and stored in Eppendorff tubes for referral to the laboratory responsible for isotopic analysis.

Data analysis

Isotopic analysis

A total of 143 individuals representing 28 fish species, and 250 samples of baseline resources were sent for isotopic analysis. Analysis took place at the Laboratório de Ecologia Isotópica (Isotopic Ecology Laboratory) of the Center for Nuclear Energy in Agriculture (CENA) at the University of São Paulo-USP, in Piracicaba. Approximately 2-5mg of dry tissue material and 5-10mg of plant samples were selected for analysis.

All samples were analysed for isotopic ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of total carbon and nitrogen content. To determine isotopic ratio, a mass spectrometer was used (Continuous flow - Isotope Ratio Mass Spectrometry - CF - IRMS) with a Carlo-Erba elemental analyzer (CHN 1110) coupled with the Delta Plus mass spectrometer from Thermo Scientific. Results were expressed by delta notation (δ), in parts per thousand (‰), as per international reference standards, calculated using the formula:

$$\delta X = [(R_{sample}/R_{Pattern}) -1] \times 10^3$$

where X is ^{13}C or ^{15}N, and R is the isotopic ratio ^{13}C / ^{12}C or ^{15}N / ^{14}N (Barrie and Prosser 1996).

Statistical analysis

To assess the trophic structure of the fish community of the Cipó River, the distribution of all species and resources were graphed as a biplot according to the isotopic signatures of carbon and nitrogen, through the package MixSIAR (Stock and Semmens 2016) in the statistical software R. Fish were included as both consumers (as separate species) and as resources (all species together), due to the presence of piscivorous and parasitic fish in the community. The Standard Ellipse Area (SEA) was estimated as a measure of the isotopic niche occupied by each trophic guild, as it represents a proxy for the richness and uniformity of consumed resources (Bearhop et al. 2004). A graphical representation of the isotopic niche was obtained using the SIBER (Stable Isotope Bayesian Ellipses) package in R (Jackson et al. 2011).

To examine whether the isotopic composition of δ^{13}C and δ^{15}N is different between trophic guilds, a Kruscal-Wallis test was performed, followed by the respective post-hoc test to identify which guilds are different from each other. To compare the isotopic composition of each species, graphs were generated using the mean, standard deviation and maximum and minimum values of δ^{13}C and δ^{15}N. For species with a large variation in isotopic compositions, a t-test was performed to examine whether this variation may have occurred between individuals collected in different seasons.

For guilds with a large variation in isotopic compositions between species (wide isotopic niche), a resource partition analysis was performed to determine the variation in the resources consumed between fish of the same guild. For this analysis we considered only the resources known to be predominantly consumed by each guild. The fractionation values considered for this analysis were 0.4 ± 1.3‰ for the carbon isotope, and 2.54 ± 1.27‰ for the nitrogen isotope (Post 2002; Vanderklift and Ponsard 2003). The partition analysis was carried out using the package MixSIAR (Stock and Semmens 2016) in R.

Results

The herbivorous species, *Myleus micans*, occupied the highest trophic level in the community, presenting more enriched δ^{13}C and δ^{15}N than the other species (Figure 2, Table 2). However, the isotopic composition of the three sampled individuals varied greatly, with one individual presenting an isotopic composition of δ^{13}C (-24.95‰) and δ^{15}N (9.77‰), notably different from the other two individuals sampled during the high-water season at the same sampling point (δ^{13}C: -16.32 and -16.69‰; δ^{15}N: 23.34 and 21.78‰). This wide variation in the isotopic composition of *M. micans* was reflected in the amplitude of the isotopic niche of the “herbivorous” guild, which showed great variation on the x- and y-axis (δ^{13}C and δ^{15}N, respectively) (Figure 3). The species *Homodiaetus* sp. (a parasitic species), was the second most enriched species in δ^{15}N (Figure 2). The piscivorous species *Acestrorhynchus lacustris* and *Hoplias intermedius* also occupied higher trophic levels, as well as the omnivorous species *Piabarcus stramineus*, and the invertivorous species, *Pimelodus fur*. The other herbivorous, omnivorous, algivorous, and invertivorous species occupied intermediate positions in the food web. The isotopic composition of δ^{13}C ($H = 29.01$; $p < 0.001$) and δ^{15}N ($H = 44.95$; $p < 0.001$) varied between trophic guilds, although large intra-guild variation was also observed (see supplementary material Figure A1).

No significant difference was observed in δ^{13}C for both algivorous and parasitic fish when compared to other trophic guilds, which was also the case for algivores in terms of δ^{15}N. The δ^{13}C isotopic composition of detritivores, invertivores and omnivores was found to differ from the δ^{13}C of herbivorous and piscivorous fish. The δ^{15}N isotopic composition of detritivores differed from other trophic guilds (with the exception of the afore-mentioned algivores). The δ^{15}N of omnivores and invertivores was also found to be distinct from herbivores. Detritivores presented the greatest intra-guild variation in the isotopic composition of δ^{13}C (≅ -35 to -22‰). Omnivores and herbivores not only presented the greatest variation in the isotopic composition of δ^{13}C (≅ -29 to -18‰ and ≅ -25 to -16‰, respectively), but also showed variation in δ^{15}N (≅ 7 to 23‰ and ≅ 9 to 23‰, respectively). For some guilds, a similar pattern in species diets was observed when comparing the values of δ^{13}C and δ^{15}N (Figure 4). The guilds of algivorous and parasitic fish were represented by only one species each, and therefore cannot offer comparison data. However, the values of δ^{13}C and δ^{15}N for the parasitic species, *Homodiaetus* sp., were similar to those of piscivorous species. The only algivorous species, *Serrapinnus piaba*, presented intermediate values of δ^{13}C and δ^{15}N when compared to species of other guilds. The two piscivorous species, *A. lacustris* and *H. intermedius* also presented similar δ^{13}C and δ^{15}N values. Among the herbivores, *Brycon orthotaenia* and *Leporinus taeniatus* also seem to feed on the same resources evidenced by the high similarity in δ^{13}C and δ^{15}N values, while *M. micans* presents greater divergence. Similarly, for invertivorous species, only a small variation in δ^{13}C and δ^{15}N was observed, with the exception of *Cichlasoma sanctifranciscense* and *P. fur*, which both presented greater variation. Despite a greater variation of δ^{13}C and δ^{15}N expected between omnivorous species, only *P. stramineus* presented a difference in isotopic compositions when compared to other species. This species also presented high intraspecific variation in δ^{13}C and δ^{15}N values, with a greater enrichment of nitrogen (15.64 ± 6.54‰) and carbon (-22.19 ± 2.55‰) observed in individuals collected during the rainy season compared to the dry season (δ^{15}N: 9.73 ± 0.43‰ e δ^{13}C: -24.81 ± 0.44‰) (Table 2). However, the observed difference between the isotopic composition of *P. stramineus* individuals recorded in each season was not found to be significant (δ^{15}N: $t = 2.017$, $p = 0.078$; δ^{13}C: $t = 2.262$, $p = 0.053$).

Species classified as detritivorous showed the greatest variation in isotopic composition, especially for δ^{13}C. The species *Harttia longipinna*, *Hypostomus* sp. and *Pareiorharphis mutuca* presented high similarity in isotopic composition of δ^{15}N and δ^{13}C, and were the species most isotopically depleted in both carbon and nitrogen. *H. francisci* and *H. margaritifer* also presented similar values of carbon and nitrogen, and are the species that occupy the highest trophic levels among the detritivores. *Steindachnerina elegans* reported intermediate values of δ^{15}N, when compared to other detritivorous species. In terms of carbon, S. elegans presented more enriched values in the rainy season (-23.07 ± 0.72‰), and more impoverished values in the dry season (-29.36 ± 1.69‰) (Figure 4, Table 2). However, it was not possible to statistically assess whether this difference was significant, due to the small number of individuals recorded in the dry season (Table 2).

Partition analysis of resources found that species classified as detritivorous vary in their diets. Although all species appear to consume the same basal resources (algae, periphyton and CPOM), the proportion of assimilation for each species varied. The species *Hypostomus* sp., *P. mutuca* and *H. longipinna* predominantly assimilated CPOM (50%, 49%, and 44%, respectively). *H. francisci* and *H. margaritifer* reported higher assimilation of periphyton (45 and 43% respectively), while *S. elegans* assimilated both algae (37%) and CPOM (38%) (Table 3).

Discussion

The expected trophic web structure was generally observed for ichthyofauna of the Cipó River. This confirms our first hypothesis, that a trophic web in a reference condition river would exhibit a typical trophic structure (piscivorous and parasitic fish occupying higher trophic levels, invertivores and omnivores occupying intermediate trophic levels, and herbivorous fish, algivores and scavengers occupying the lower trophic levels). Only δ^{13}C and δ^{15}N enrichment in *M. micans* (an herbivorous species) deviated from this expected pattern. Differences in isotopic composition of δ^{13} C and δ^{15}N were observed between most trophic guilds, confirming our second hypothesis. However, this study also reports wide variation in δ^{13}C values in species classified as detritivorous, explained by intra-guild variation in the proportion of basal resource assimilation (CPOM, algae and periphyton). This finding therefore rejects the hypothesis that fish classified in the same trophic guild present similar δ^{13}C and δ^{15}N values.

The presence of anthropogenic impacts in aquatic environments can influence the isotopic composition of aquatic organisms, such as algae (Peipoch et al. 2012; Pastor et al. 2014; Orlandi et al. 2017; Fiorentino et al. 2017), periphyton (Lee et al. 2018), macrophytes (Peipoch et al. 2012; Pastor et al. 2014), macroinvertebrates (Morrissey et al. 2013; Castro et al. 2016; Baumgartner and Robinson 2017) and fish (Carvalho et al. 2015; de Carvalho et al. 2017, 2019; Alonso et al. 2019). The notable changes are observed in δ^{15}N, which is highly impacted by nitrogen inputs from anthropogenic sources (such as agriculture, sewage and pastures, among others) (Dailer et al. 2010; Nikolenko et al. 2018). However, in a reference river such as the Cipó River, it is expected that the δ^{15}N in fish would not exhibit such interference. Although fish from most guilds presented isotopic compositions within expected values, two individuals of the species *M. Micans* revealed much more enriched δ^{15} N than is usual for an herbivorous species. Considering that there is an average enrichment of 3.0‰ between trophic levels in a food web, and that the two *M. Micans* individuals presented δ^{15} N values of 23.34 and 21.78‰, it is estimated that there are at least five trophic levels between these individuals and the least N-enriched species, *H. longipinna* (4.97‰). As an herbivorous species, thus occupying a base trophic level in the fish community, this seemingly unexpected result suggests that *M. micans* may feed in areas outside the Cipó River, perhaps by way of a large spatial range, or as a short-range migratory species. Indeed, the das Velhas River has basal resources with signatures comparable with those found in the two individuals. This river receives large amounts of domestic and industrial sewage from the Belo

Horizonte Metropolitan Region (Alves and Pompeu 2010), and is impacted by mining (Pompeu et al. 2005), agriculture (Zellhuber and Siqueira 2016), and pasture (Trajano et al. 2010). Due to the significant impact of these anthropogenic activities, food resources are enriched in nitrogen in almost all stretches of the main channel of the das Velhas River, especially autotrophic resources such as algae and periphyton (Alonso et al. 2019; de Carvalho et al. 2019).

The breadth and overlap of trophic niches also reveals much about the trophic structure of aquatic ecosystems (Layman et al. 2007; Newsome et al. 2007, 2012; Nagata et al. 2015). Wide horizontal niches (x-axis, $\delta^{13}C$) indicate large variation in resource consumption by fish of the same trophic guild, while vertically wide niches (y-axis, $\delta^{15}N$) indicate large variation in trophic position between fish of the same trophic guild. Moreover, large overlapping niches indicate that, despite being classified into different trophic guilds, fish use similar food resources. The largest variations in trophic niches were observed in the herbivore and detritivore guild, each with different explanations for the observed pattern.

The broad vertical and horizontal niche reported for herbivorous species is mainly due to the two *M. micans* individuals with highly enriched carbon and nitrogen, as explained above. The detritivorous fish guild also presented a wide horizontal niche as a result of the large variation in the $\delta^{13}C$ values of this group. This high intra-guild variation perhaps leads us to question the dependability of guild classification based on available literature, as it is generally expected that the $\delta^{13}C$ of fish in the same guild exhibit little variation. Furthermore, "detritivore" is a very broad classification, since small algae, bacteria, fungi and protozoa can be an integral part of detrital particles (Odum 1980). Consequently, within the guild of “detritivores”, one can find fish with a varied diet, feeding on algae, periphyton (biofilm), bacteria, detritus itself, among others. This makes classification difficult, and hinders the understanding of the role of each species within a trophic web, and consequently, of ecosystem functioning.

Additionally, the literature can be inconsistent regarding the classification of detritivorous species, with the same species often classified into different trophic guilds. The Loricariidae are commonly classified as detritivorous (Vazzoler et al. 1997; Mazzoni et al. 2010b; Villares-Junior et al. 2016) and iliophagous (Albrecht and Silveira 2001; Gomiero and De Souza Braga 2005; Mazzoni et al. 2010b; Villares-Junior et al. 2016). Yet *Hypostomus*, the largest genus in Loricariidae and the second largest genus among catfish, has been classified as detritivorous (Mazzoni et al. 2010b; Villares-Junior et al. 2016; Echevarría and González 2018; Quintela et al. 2019), iliophagous (Delariva 2001; Cataldo 2015; Lobón-Cerviá et al. 2016), periphyton-feeding (Casatti et al. 2002; Skóra et al. 2015; Teresa et al. 2016; Uieda and Fujihara 2018) and algivorous (Angermeier and Karr 1983; López-Fernández and Winemiller 2005; Ibañez et al. 2007). The Curimatidae family, which includes *S. elegans*, are also classified as detritivores (Giora and Fialho 2003), however Giora and Fialho (2003) found predominantly algae in *Steindachnerina brevipinna* stomach samples, and Teixeira and Gurgel (2004) reported essentially inorganic sediment, organic detritus and algae in the stomachs of *Steindachnerina notonota,* classifying it as iliophagous.

This limitation was reinforced by the result of the resource partition analysis in this study, which indicated that certain detritivorous species consume the same resources but in different proportions. The analysis of stable isotopes produced two distinct diet groups, one seemingly based on detritus, and the other based on periphyton. Thus, we suggest that, for the São Francisco basin, these two groups should not be grouped under the same classification ("detritivores"), as they are isotopically different with a distinct carbon origin. We instead suggest the terms detritivore for *H. longipinna*, *Hypostomus* sp. and *P. mutuca*, periphyton-feeder for *H. francisci* and *H. margaritifer,* and detritivore/algivore for *S. elegans*.

In addition to the characterization of the isotopic structure of the Cipó river fish community (as an important reference condition watercourse for the São Francisco river basin), our results indicate that species classification into very wide trophic guilds (such as the guild of the detritivores) may limit understanding of individual species' roles in the trophic structure of a community, since species may be assimilating different proportions of the same food item, or utilizing different resources. It is also important to note that the generalized classification of trophic guilds can be particularly complex due to the fact that many species change their diets according to environmental conditions and/or resource availability within their habitats (Noble et al. 2007). A further compounding factor to consider is that fish of a given species may have distinct diets according to the development stages of individuals (Winemiller 1989; Abelha et al. 2001), thus influencing trophic guild classification.

The results presented here are important for understanding the structure and functioning of aquatic ecosystems without the interference of human actions. Many, if not most aquatic environments have been impacted by anthropogenic activities (e.g. agriculture, pastures, and domestic and industrial sewage). Such activities compromise the health of these environments, and can impact the availability of food resources. This in turn can lead to a decrease in species diversity, and the extinction of specialized species (Clavel et al. 2011). These impacts can also lead to a loss of ecosystem services, such as food and water supplies (Schroter 2005). Improved knowledge of aquatic environments under reference conditions is therefore vitally important, as they can inform the design of recovery strategies for disturbed aquatic environments, since they allow us to characterise the degree of degradation and identify appropriate recovery goals within a habitat. We also highlight the importance of preservation of these reference environments, before they too are impacted by human activities.

References

Abelha MCF, Agostinho AA, Goulart E (2001) Plasticidade trófica em peixes de água doce. Acta Sci Biol … 23:425–434. https://doi.org/10.4025/actascibiolsci.v23i0.2696

Adams J (1985) The Definition and Interpretation of Guild Structure in Ecological Communities. J

Anim Ecol 54:43. https://doi.org/10.2307/4619

Albrecht MP, Silveira CM (2001) Alimentação de Loricariichthys anus nas lagoas Marcelino e Peixoto Planicie Costeira do RS.pdf. Acta Limnol. Bras. 13:7985

Alexandre CV, Esteves KE, de Moura e Mello MAM (2010) Analysis of fish communities along a rural–urban gradient in a neotropical stream (Piracicaba River Basin, São Paulo, Brazil). Hydrobiologia 641:97–114. https://doi.org/10.1007/s10750-009-0060-y

Alonso MB, de Carvalho DR, Alves CBM, et al (2019) Changes in trophic characteristics of two fish species of Astyanax (Teleostei: Characidae) in response to aquatic pollution. Zoologia 36:1–12. https://doi.org/10.3897/zoologia.36.e30445

Alves CBM, Pompeu P dos S (2010) A fauna de peixes da bacia do rio das Velhas no final do século XX. In: Alves CBM, Pompeu PS (eds) Peixes do rio das Velhas: passado e presente. ARGVMENTVM, Belo Horizonte, pp 167–189

Alves CBM, Pompeu PS (2005) Historical Changes in the Rio das Velhas Fish Fauna—Brazil. Am Fish Soc Symp 45:587–602

Alvim MCC, Peret AC (2004) Food resources sustaining the fish fauna in a section of the upper São Francisco River in Três Marias, MG, Brazil. Brazilian J Biol 64:195–202. https://doi.org/10.1590/S1519-69842004000200003

Anderson C, Cabana G, Anderson C (2007) Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. 26:273–285

Angermeier PL, Karr JR (1983) Fish communities along environmental gradients in a system of tropical streams. Environ Biol Fishes 9:117–135. https://doi.org/10.1007/BF00690857

Arias-González J, Morand S (2006) Trophic functioning with parasites: a new insight for ecosystem analysis. Mar Ecol Prog Ser 320:43–53. https://doi.org/10.3354/meps320043

Baeta A, Vieira LR, Lírio AV, et al (2017) Use of stable isotope ratios of fish larvae as indicators to assess diets and patterns of anthropogenic nitrogen pollution in estuarine ecosystems. Ecol Indic 83:112–121. https://doi.org/10.1016/j.ecolind.2017.07.062

Barrie A, Prosser SJ (1996) Mass Spectrometry of Soils. In: Mass Spectrometry of Soils. Marcel Dekker, Inc., p 536

Baumgartner SD, Robinson CT (2017) Changes in macroinvertebrate trophic structure along a land-use gradient within a lowland stream network. Aquat Sci 79:407–418. https://doi.org/10.1007/s00027-016-0506-z

Bearhop S, Adams CE, Waldron S, et al (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73:1007–1012. https://doi.org/10.1111/j.0021-8790.2004.00861.x

Bonato KO, Delariva RL, Silva JC da (2012) Diet and trophic guilds of fish assemblages in two streams with different anthropic impacts in the northwest of Paraná, Brazil. Zool 29:27–38. https://doi.org/10.1590/S1984-46702012000100004

Bouleau G, Pont D (2015) Did you say reference conditions? Ecological and socio-economic perspectives on the European Water Framework Directive. Environ Sci Policy 47:32–41. https://doi.org/10.1016/j.envsci.2014.10.012

Buss DF, Carlisle DM, Chon TS, et al (2015) Stream biomonitoring using macroinvertebrates around the globe: a comparison of large-scale programs. Environ Monit Assess 187:. https://doi.org/10.1007/s10661-014-4132-8

Carvalho DR, Castro D, Callisto M, et al (2015) Isotopic variation in five species of stream fishes under the influence of different land uses. J Fish Biol 87:559–578. https://doi.org/10.1111/jfb.12734

Casatti L, Ferreira CP, Langeani F (2009) A fish-based biotic integrity index for assessment of lowland streams in southeastern Brazil. Hydrobiologia 623:173–189. https://doi.org/10.1007/s10750-008-9656-x

Casatti L, Langeani F, Castro RMC (2002) Peixes de riacho do parque estadual Morro do Diabo, Bacia do Alto Rio Paraná, SP. Biota Neotrop 1:1–15. https://doi.org/10.1590/s1676-06032001000100005

Castro DMP, De Carvalho DR, Pompeu PDS, et al (2016) Land use influences niche size and the assimilation of resources by benthic macroinvertebrates in tropical headwater streams. PLoS One. https://doi.org/10.1371/journal.pone.0150527

Cataldo D (2015) Trophic Relationships of Limnoperna Fortunei with Adult Fishes. In: Boltovskoy D (ed) Limnoperna Fortunei. Springer International Publishing, Cham, pp 231–248

Clavel J, Julliard R, Devictor V (2011) Worldwide decline of specialist species: toward a global functional homogenization? Front Ecol Environ 9:222–228. https://doi.org/10.1890/080216

Cohen JE, Newman CM, Briand F (1985) A stochastic theory of community food webs. II. Individual webs. Proc - R Soc London, Ser B 224:449–461. https://doi.org/10.1098/rspb.1985.0043

Corrêa F (2011) Estrutura trófica da assembléia de peixes numa área de banhado do Parque Nacional da Lagoa do Peixe (RS). Universidade Federal do Rio Grande

Costa-Pereira R, Resende FR da R, De EK (2012) Estrutura Trófica da Comunidade de Peixes de Riachos da Porção Oeste da Bacia do Alto Paraná. Corumbá

Cucherousset J, Bouletreau S, Martino A, et al (2012) Using stable isotope analyses to determine the ecological effects of non-native fishes. Fish Manag Ecol 19:111–119. https://doi.org/10.1111/j.1365-2400.2011.00824.x

Dailer ML, Knox RS, Smith JE, et al (2010) Using δ15N values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai‘i, USA. Mar Pollut Bull 60:655–671. https://doi.org/10.1016/j.marpolbul.2009.12.021

Davies PE, Harris JH, Hillman TJ, Walker KF (2010) The Sustainable Rivers Audit: assessing river ecosystem health in the Murray - Darling Basin, Australia. Mar Freshw Res 61:764. https://doi.org/10.1071/MF09043

Davis AM, Blanchette ML, Pusey BJ, et al (2012) Gut content and stable isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river. Freshw Biol 57:2156–2172. https://doi.org/10.1111/j.1365-2427.2012.02858.x

de Carvalho DR, de Castro DMP, 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/s10750-017-3130-6

de Carvalho DR, Flecker AS, Alves CBM, et al (2019) Trophic responses to aquatic pollution of native and exotic livebearer fishes. Sci Total Environ 681:503–515. https://doi.org/10.1016/j.scitotenv.2019.05.092

Delariva R (2001) Relationship between morphology and diets of six neotropical loricariids. J Fish Biol 58:832–847. https://doi.org/10.1006/jfbi.2000.1499

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals.

Geochim Cosmochim Acta 42:495–506. https://doi.org/10.1016/0016-7037(78)90199-0

Dias TS, Fialho CB (2011) Comparative dietary analysis of Eurycheilichthys pantherinus and Pareiorhaphis hystrix: two Loricariidae species (Ostariophysi, Siluriformes) from Campos Sulinos biome, southern Brazil. Iheringia Série Zool 101:49–55. https://doi.org/10.1590/S0073-47212011000100006

Durães R, Pompeu P dos S, Godinho AL (2001) Alimentação de quatro espécies de Leporinus (Characiformes, Anostomidae) durante a formação de um reservatório no sudeste do Brasil. Iheringia Série Zool 183–191. https://doi.org/10.1590/S0073-47212001000100019

Echevarría G, González N (2018) Fish taxonomic and functional diversity in mesohabitats of the River Kakada, Caura National Park, Venezuela. Nat Conserv Res 3:21–39. https://doi.org/10.24189/ncr.2018.048

Eichbaum Esteves K, Alexandre CV (2011) Development of an Index of Biotic Integrity Based on Fish Communities to Assess the Effects of Rural and Urban Land Use on a Stream in Southeastern Brazil. Int Rev Hydrobiol 96:296–317. https://doi.org/10.1002/iroh.201111297

Feio MJ, Reynoldson TB, Ferreira V, Graça MAS (2007) A predictive model for freshwater bioassessment (Mondego River, Portugal). Hydrobiologia 589:55–68. https://doi.org/10.1007/s10750-006-0720-0

Feld CK, Hering D (2007) Community structure or function: effects of environmental stress on benthic macroinvertebrates at different spatial scales. Freshw Biol 52:1380–1399. https://doi.org/10.1111/j.1365-2427.2007.01749.x

Fernandes UL (2011) Ecologia trófica de quatro espécies de peixes de um rio no semiárido , Pentecoste - Ceará. UNIVERSIDADE FEDERAL DO CEARÁ

Fiorentino F, Cicala D, Careddu G, et al (2017) Epilithon δ15N signatures indicate the origins of nitrogen loading and its seasonal dynamics in a volcanic Lake. Ecol Indic 79:19–27. https://doi.org/10.1016/j.ecolind.2017.04.007

Fry B, Sherr EB (1989) δ13C Measurements as Indicators of Carbon Flow in Marine and Freshwater Ecosystems. In: Stable Isotopes in Ecological Research. pp 196–229

Gandini C V, Boratto IA, Fagundes DC, Pompeu PS (2012) Estudo da alimentação dos peixes no rio Grande à jusante da usina hidrelétrica de Itutinga, Minas Gerais, Brasil. Iheringia Série Zool 102:56–61. https://doi.org/10.1590/s0073-47212012000100008

Giora J, Fialho CB (2003) Biologia alimentar de Steindachnerina brevipinna do rio Ibicuí-Mirim, Rio Grande do Sul, Brasil. Iheringia, Sér Zool Porto Alegre 93:277–281

Gomiero LM, De Souza Braga FM (2005) Uso do grau de preferência alimentar para a caracterização da alimentação de peixes na APA de São Pedro e Analândia. Acta Sci Biol Sci 27:265–270. https://doi.org/10.4025/actascibiolsci.v27i3.1337

Hesslein RH, Hallard KA, Ramlal P (1993) Replacement of Sulfur, Carbon, and Nitrogen in Tissue of Growing Broad Whitefish (Coregonus nasus) in Response to a Change in Diet Traced by δ 34 S, δ 13 C, and δ 15 N. Can J Fish Aquat Sci 50:2071–2076. https://doi.org/10.1139/f93-230

Ibañez C, Tedesco PA, Bigorne R, et al (2007) Dietary-morphological relationships in fish assemblages of small forested streams in the Bolivian Amazon. Aquat Living Resour 20:131–142. https://doi.org/10.1051/alr:2007024

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80:595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Jepsen DB, Winemiller KO (2002) Structure of tropical river food webs revealed by stable isotope ratios. Oikos 96:46–55

Karr JR (1981) Assessment of Biotic Integrity Using Fish Communities. Fisheries 6:21–27. https://doi.org/10.1577/1548-8446(1981)006<0021:AOBIUF>2.0.CO;2

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42–48. https://doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2

Lee KY, Graham L, Spooner DE, Xenopoulos MA (2018) Tracing anthropogenic inputs in stream foods webs with stable carbon and nitrogen isotope systematics along an agricultural gradient. PLoS One 13:e0200312. https://doi.org/10.1371/journal.pone.0200312

Ligeiro R, Hughes RM, Kaufmann PR, et al (2013) Defining quantitative stream disturbance gradients and the additive role of habitat variation to explain macroinvertebrate taxa richness. Ecol Indic 25:45–57. https://doi.org/10.1016/j.ecolind.2012.09.004

Lobón-Cerviá J, Mazzoni R, Rezende CF (2016) Effects of riparian forest removal on the trophic dynamics of a Neotropical stream fish assemblage. J Fish Biol 89:50–64. https://doi.org/10.1111/jfb.12973

López-Fernández H, Winemiller KO (2005) Status of Dionda Diaboli and Report of Established Populations of Exotic Fish Species in Lower San Felipe Creek, Val Verde County, Texas. Southwest Nat 50:246–251. https://doi.org/10.1894/0038-4909(2005)050[0246:soddar]2.0.co;2

López-Rasgado FJ, Lluch-Cota SE, Balart EF, Herzka SZ (2016) Variation in isotopic trophic structure and fish diversity in mangrove systems subject to different levels of habitat modification in the Gulf of California, Mexico. Bull Mar Sci 92:399–422. https://doi.org/10.5343/bms.2015.1100

Luczkovich JJ, Ward GP, Johnson JC, et al (2002) Determining the trophic guilds of fishes and macroinvertebrates in a seagrass food web. Estuaries 25:1143–1163. https://doi.org/10.1007/BF02692212

Luz-agostinho KDG, Bini LM, Fugi R, et al (2006) Food spectrum and trophic structure of the ichthyofauna of Corumbá reservoir , Paraná river Basin , Brazil. 4:61–68

Malabarba LR, Fialhol CB, Bertaco VA, et al (2013) Peixes. In: Witt PBR (ed) Fauna e flora reserva biológica do lami José Lutzemberger, 1st edn. pp 143–184

Manetta GI, Benedito-Cecilio E (2003) Aplicação da técnica de isótopos estáveis na estimativa da taxa de turnover em estudos ecológicos : uma síntese. Acta Sci Biol Sci 25:121–129

Manna L, Rezende C, Mazzoni R (2013) Plasticity in the diet of Astyanax taeniatus in a coastal stream from south-east Brazil. Brazilian J Biol 72:919–928. https://doi.org/10.1590/s1519-69842012000500020

Martins I, Ligeiro R, Hughes RM, et al (2018) Regionalisation is key to establishing reference conditions for neotropical savanna streams. Mar Freshw Res 69:82. https://doi.org/10.1071/MF16381

Mazzoni R, Moraes M, Rezende CF, Iglesias-Rios R (2010a) Diet and feeding daily rhythm of Pimelodella lateristriga (Osteichthyes, Siluriformes) in a coastal stream from Serra do Mar - RJ. Braz J Biol 70:1123–9

Mazzoni R, Rezende C, Manna L (2010b) Feeding ecology of Hypostomus punctatus Valenciennes, 1840 (Osteichthyes, Loricariidae) in a costal stream from Southeast Brazil. Brazilian J Biol 70:569–574. https://doi.org/10.1590/S1519-69842010000300013

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

Melo CE de, Machado F de A, Pinto-Silva V (2004) Feeding habits of fish from a stream in the savanna of Central Brazil, Araguaia Basin. Neotrop Ichthyol 2:37–44. https://doi.org/10.1590/S1679-62252004000100006

Morrissey CA, Boldt A, Mapstone A, et al (2013) Stable isotopes as indicators of wastewater effects on the macroinvertebrates of urban rivers. Hydrobiologia 700:231–244. https://doi.org/10.1007/s10750-012-1233-7

Nagata R, Moreira M, Pimentel C, Morandini A (2015) Food web characterization based on δ15N and δ13C reveals isotopic niche partitioning between fish and jellyfish in a relatively pristine ecosystem. Mar Ecol Prog Ser 519:13–27. https://doi.org/10.3354/meps11071

Newsome SD, Rio CM del, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. Front Ecol Environ 5:429–436. https://doi.org/10.1890/060150.01 T

Newsome SD, Yeakel JD, Wheatley P V., Tinker MT (2012) Tools for quantifying isotopic niche space and dietary variation at the individual and population level. J Mammal 93:329–341. https://doi.org/10.1644/11-MAMM-S-187.1

Nikolenko O, Jurado A, Borges A V, et al (2018) Isotopic composition of nitrogen species in groundwater under agricultural areas: A review. Sci Total Environ 621:1415–1432. https://doi.org/10.1016/j.scitotenv.2017.10.086

Noble RAA, Cowx IG, Goffaux D, Kestemont P (2007) Assessing the health of European rivers using functional ecological guilds of fish communities: standardising species classification and approaches to metric selection. Fish Manag Ecol 14:381–392. https://doi.org/10.1111/j.1365-2400.2007.00575.x

Odum EP (1980) The Status of Three Ecosystem-Level Hypotheses Regarding Salt Marsh Estuaries: Tidal Subsidy, Outwelling, and Detritus-Based Food Chains. ACADEMIC PRESS, INC.

Orlandi L, Calizza E, Careddu G, et al (2017) The effects of nitrogen pollutants on the isotopic signal (δ 15 N) of Ulva lactuca : Microcosm experiments. Mar Pollut Bull 115:429–435. https://doi.org/10.1016/j.marpolbul.2016.12.051

Pardo I, Gómez-Rodríguez C, Wasson J-G, et al (2012) The European reference condition concept: A scientific and technical approach to identify minimally-impacted river ecosystems. Sci Total Environ 420:33–42. https://doi.org/10.1016/j.scitotenv.2012.01.026

Pastor A, Riera JL, Peipoch M, et al (2014) Temporal Variability of Nitrogen Stable Isotopes in Primary Uptake Compartments in Four Streams Differing in Human Impacts. Environ Sci Technol 48:6612–6619. https://doi.org/10.1021/es405493k

Peipoch M, Martí E, Gacia E (2012) Variability in δ 15 N natural abundance of basal resources in fluvial ecosystems: a meta-analysis. Freshw Sci 31:1003–1015. https://doi.org/10.1899/11-157.1

Peterson BJ, Fry B (1987) Stable Isotopes in Ecosystem Studies. Annu Rev Ecol Syst 18:293–320. https://doi.org/10.1146/annurev.es.18.110187.001453

Pompeu PS, Alves CBM, Callisto M (2005) The Effects of Urbanization on Biodiversity and Water Quality in the Rio das Velhas Basin , Brazil. Am Fish Soc Symp 47:11–22

Pompeu PS, Godinho HP (2003) Ictiofauna de três lagoas marginais do médio São Francisco

Post DM (2002) The long and short of food-chain length. Trends Ecol Evol 17:269–277. https://doi.org/10.1016/S0169-5347(02)02455-2

Quintela FM, Lima GP, Silveira ML, et al (2019) High arsenic and low lead concentrations in fish and reptiles from Taim wetlands, a Ramsar site in southern Brazil. Sci Total Environ 660:1004–1014. https://doi.org/10.1016/j.scitotenv.2019.01.031

Reyna SSV (2018) Aspectos ecológicos da ictiofauna de riachos de um sistema de veredas. Universidade Federal de Lavras

Reynoldson TB, Norris RH, Resh VH, et al (1997) The Reference Condition: A Comparison of Multimetric and Multivariate Approaches to Assess Water-Quality Impairment Using Benthic Macroinvertebrates. J North Am Benthol Soc 16:833–852. https://doi.org/10.2307/1468175

Rodrigues D (2013) Ecologia trófica das principais espécies de peixes do Rio Paraíba do Sul na área de influência do Aproveitamento Hidrelétrico Simplício durante o período pré- represamento. Universidade Federal de Juiz de Fora

Rybczynski SM, Walters DM, Fritz KM, Johnson BR (2008) Comparing trophic position of stream fishes using stable isotope and gut contents analyses. Ecol Freshw Fish 17:199–206. https://doi.org/10.1111/j.1600-0633.2007.00289.x

Santos F de almeida (2009) Estrutura trófica de peixes do Lago Grande, Manacapuru, AM com base nos isótopos estáveis de C e N. Universidade Federal do Amazonas

Schroter D (2005) Ecosystem Service Supply and Vulnerability to Global Change in Europe. Science (80-) 310:1333–1337. https://doi.org/10.1126/science.1115233

Simberloff D, Dayan T (1991) The Guild Concept and the Structure of Ecological Communities. Annu Rev Ecol Syst 22:115–143. https://doi.org/10.1146/annurev.es.22.110191.000555

Skóra F, Abilhoa V, Padial AA, Vitule JRS (2015) Darwin's hypotheses to explain colonization trends: evidence from a quasi -natural experiment and a new conceptual model. Divers Distrib 21:583–594. https://doi.org/10.1111/ddi.12308

Stock B, Semmens B (2016) MixSIAR GUI user manual. Version 31 1–59. https://doi.org/10.5281/zenodo.47719

Teixeira JL de A, Gurgel HDCB (2004) Dinâmica da nutrição e alimentação natural de Steindachnerina notonota (Pisces, Curimatidae), Açude de Riacho da Cruz, Rio Grande do Norte, Brasil. Rev Bras Zoociências 6:19–28

Teresa FB, Souza LS De, Silva DMA da, et al (2016) Environmental constraints structuring fish assemblages in riffles: evidences from a tropical stream. Neotrop Ichthyol 14:1–6. https://doi.org/10.1590/1982-0224-20150185

Trajano E, Secutti S, Mattox GMT (2010) Epigean and subterranean ichthyofauna in Cordisburgo karst area, eastern Brazil. Biota Neotrop 9:277–281. https://doi.org/10.1590/s1676-06032009000300026

Uieda VS, Fujihara CY (2018) Structure of the ichthyofauna of adventitious streams in the South Pantanal. Iheringia Série Zool 108:1–8. https://doi.org/10.1590/1678-4766e2018019

Vander Laan JJ, Hawkins CP, Olson JR, Hill RA (2013) Linking land use, in-stream stressors, and biological condition to infer causes of regional ecological impairment in streams. Freshw Sci 32:801–820. https://doi.org/10.1899/12-186.1

Vander Zanden, M. Jake Rasmussen JB (1999) Primary consumer d13C and d15N and the trophic position of aquatic consumers. Ecology 80:1395–1404. https://doi.org/10.1890/0012-9658(1999)080[1395:pccana]2.0.co;2

Vander Zanden MJ, Cabana G, Rasmussen JB (1997) Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ¹⁵N) and literature dietary data.

Can J Fish Aquat Sci 54:1142–1158. https://doi.org/10.1139/cjfas-54-5-1142

Vander Zanden MJ, Rasmussen JB (2001) Variation in δ 15 N and δ 13 C trophic fractionation: Implications for aquatic food web studies. Limnol Oceanogr 46:2061–2066. https://doi.org/10.4319/lo.2001.46.8.2061

Vanderklift M a, Ponsard S (2003) Sources of variation in consumer-diet 15N enrichment: a meta-analysis. Oecologia 136:169–182. https://doi.org/10.1007/s00442-003-1270-z

Vazzoler AEA de M, Agostinho AA, Hahn NS (1997) A Planície de Inundação do Alto Rio Paraná: Aspectos físicos, biológicos e socioeconômicos. EDUEM: Nupélia, Maringá

Vieira F, Santos GB, Alves CBM (2005) A ictiofauna do Parque Nacional da Serra do Cipó (Minas Gerais) e áreas adjacentes. Lundiana 6:77–87

Vilella FS, Becker FG, Hartz SM (2002) Diet of Astyanax species (Teleostei, Characidae) in an Atlantic Forest River in Southern Brazil. Brazilian Arch Biol Technol 45:223–232. https://doi.org/10.1590/S1516-89132002000200015

Villares-Junior GA, Cardone IB, Goitein R (2016) Comparative feeding ecology of four syntopic Hypostomus species in a Brazilian southeastern river. Brazilian J Biol 76:692–699. https://doi.org/10.1590/1519-6984.00915

Winemiller KO (1989) Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan ilanos. Environ Biol Fishes 26:177–199. https://doi.org/10.1007/BF00004815

Zellhuber A, Siqueira R (2016) Rio São Francisco em descaminho: degradação e revitalização. In: Cadernos do CEAS: Revista crítica de humanidades. pp 3–24

Online document

CBH Rio das Velhas, 2016. Revista Rio das Velhas: A bacia do rio Cipó e a sua importância para a qualidade das águas do rio das Velhas. 2016. Disponível em: http://cbhvelhas.org.br/noticias/a-bacia-do-rio-cipo-e-a-sua-importancia-para%E2%80%A8a-qualidade-das-aguas-do-rio-das-velhas/ Accessed May 2 2019.

Table 1. Fish species sampled in the Cipó River, Minas Gerais, and classified into trophic guilds based on the literature (cited in the column “Reference”). *species information /** genus information

Species	Trophic guilds	Reference
Acestrorhynchus lacustris (Lutken, 1875)	Piscivorous*	(Alvim and Peret 2004)
Astyanax fasciatus (Cuvier, 1819)	Omnivorous*	(Vilella et al. 2002)
Astyanax taeniatus (Jenyns, 1842)	Omnivorous *	(Manna et al. 2013)
Australoheros facetus (Jenyns, 1842)	Omnivorous *	(Corrêa 2011)
Brycon orthotaenia (Gunther, 1864)	Herbivorous*	(Pompeu and Godinho 2003)
Characidium zebra (Eigenmann, 1909)	Invertivorous*	(Costa-Pereira et al. 2012)
Cichlasoma sanctifranciscense (Kullander, 1983)	Invertivorous*	(Pompeu and Godinho 2003)
Eigenmannia virescens (Valenciennes, 1842)	Invertivorous*	(Gandini et al. 2012)
Harttia longipinna (Langeani *et al*, 2001)	Detritivorous**	(Mazzoni et al. 2010a)
Homodiaetus sp.	Parasite**	(Malabarba et al. 2013)
Hoplias intermedius (Gunther, 1864)	Piscivorous **	(Reyna 2018)
Hypostomus francisci (Lukten, 1874)	Detritivorous**	(Gandini et al. 2012)
Hypostomus margaritifer (Regan, 1908)	Detritivorous*	(Gandini et al. 2012)
Hypostomus sp.	Detritivorous*	(Melo et al. 2004)
Lepidocharax burnsi (Ferreira *et al*, 2011)	Invertivorous*	(Reyna 2018)
Leporinus amblyrhynchus (Garavello & Britski, 1987)	Invertivorous*	(Gandini et al. 2012)
Leporinus marcgravii (Lukten, 1875)	Invertivorous**	(Gandini et al. 2012)
Leporinus taeniatus (Lukten, 1875)	Herbivorous*	(Pompeu and Godinho 2003)
Megaleporinus reinhardti (Lukten, 1875)	Omnivorous **	(Durães et al. 2001)
Myleus micans (Lukten, 1875)	Herbivorous*	(Pompeu and Godinho 2003)
Pareiorhaphis mutuca (Oliveira & Oyakawa, 1999)	Detritivorous**	(Dias and Fialho 2011)
Phalloceros uai (Lucinda, 2008)	Omnivorous **	(Corrêa 2011)
Piabarcus stramineus (Eigenmann, 1908)	Omnivorous *	(Luz-agostinho et al. 2006)
Piabina argentea (Reinhardt, 1867)	Omnivorous *	(Luz-agostinho et al. 2006)
Pimelodus fur (Lukten, 1874)	Invertivorous*	(Rodrigues 2013)
Poecilia reticulata (Peters, 1859)	Omnivorous *	(Fernandes 2011)
Serrapinnus piaba (Lukten, 1875)	Algivorous*	(Pompeu and Godinho 2003)
Steindachnerina elegans (Steindachner, 1874)	Detritivorous*	(Melo et al. 2004)

Table 2. Number of samples, mean values and standard deviation of isotopic compositions of carbon $\delta^{13}C$ (‰) and nitrogen $\delta^{15}N$ (‰) for each species sampled in the Cipó River, during the dry and rainy seasons.

Species/ Trophic guilds	**Wet season**					**Dry season**				
	N	**$\delta^{15}N$ (‰)**		**$\delta^{13}C$ (‰)**		**N**	**$\delta^{15}N$ (‰)**		**$\delta^{13}C$ (‰)**	
Algivorous						**5**	**9.36**	**±0.75**	**-24.59**	**±0.69**
Serrapinnus piaba						5	9.36	±0.75	-24.59	±0.69
Detritivorous	**12**	**6.42**	**±1.71**	**-24.91**	**±3.00**	**10**	**7.52**	**±1.67**	**-28.63**	**±3.18**
Harttia longipinna						1	4.97		-30.68	
Hypostomus francisci	3	8.67	±0.95	-27.52	±0.43	1	9.46		-24.62	
Hypostomus margaritifer						3	8.97	±1.11	-26.23	±1.99
Hypostomus sp.	1	5.72		-31.78						
Pareiorhaphis mutuca						3	6.19	±0.51	-31.19	±3.32
Steindachnerina elegans	8	5.67	±1.21	-23.07	±0.72	2	7.62	±0.42	-29.36	±1.69
Herbivorous	**10**	**12.62**	**±5.50**	**-22.25**	**±3.15**	**1**	**9.20**		**-23.96**	
Brycon orthotaenia	1	9.63		-23.08						
Leporinus taeniatus	6	10.28	±2.19	-23.58	±0.97	1	9.20		-23.96	
Myleus micans	3	18.30	±7.43	-19.32	±4.88					
Invertivorous	**26**	**8.87**	**±1.29**	**-25.32**	**±1.62**	**14**	**9.67**	**±2.09**	**-25.11**	**±2.01**
Characidium zebra						1	9.35		-25.22	
Cichlasoma sanctifranciscense	4	8.71	±0.21	-22.65	±0.49					
Eigenmannia virescens	8	8.45	±1.32	-26.13	±1.43	5	8.11	±0.36	-26.64	±0.42
Lepidocharax burnsi	5	8.44	±0.30	-25.93	±0.91	5	9.53	±0.39	-25.38	±0.99
Leporinus amblyrhynchus						1	9.19		-24.38	
Leporinus marcgravii	5	8.29	±0.27	-26.34	±0.59					
Pimelodus fur	4	11.14	±1.17	-24.30	±0.58	2	14.32	±0.00	-20.90	±0.23
Omnivorous	**38**	**9.39**	**±3.35**	**-24.91**	**±2.00**	**15**	**9.30**	**±0.65**	**-25.34**	**±0.85**
Astyanax fasciatus	1	10.21		-23.74						
Astyanax taeniatus	9	8.97	±0.65	-24.18	±1.05					
Australoheros facetus	3	7.84	±0.52	-27.23	±1.25					
Piabarcus stramineus	5	15.64	±6.54	-22.19	±2.55	5	9.73	±0.43	-24.81	±0.44
Megaleporinus reinhardti	5	8.71	±0.39	-24.58	±0.90					
Phalloceros uai	5	8.08	±0.84	-26.46	±1.52					
Piabina argentea	5	8.51	±0.22	-25.81	±0.69	10	9.08	±0.65	-25.60	±0.90
Poecilia reticulata	5	7.53	±0.23	-25.64	±2.11					
Parasites						**2**	**13.40**	**±0.26**	**-23.65**	**±0.72**
Homodiaetus sp.						2	13.40	±0.26	-23.65	±0.72
Piscivorous	**9**	**11.38**	**±2.03**	**-23.04**	**±1.12**	**1**	**13.86**		**-22.87**	
Acestrorhynchus lacustris	5	12.19	±2.15	-22.44	±1.18					
Hoplias intermedius	4	10.36	±1.54	-23.78	±0.38	1	13.86		-22.87	

Table 3. Results of partition analysis (MixSIAR). Mean and standard deviation (SD) of the food source proportions in the diet of detritivorous fishes. AL = algae; CPOM = coarse particulate organic matter; PE = periphyton

Detritivorous species	AL		CPOM		PE	
	Mean	SD	Mean	SD	Mean	SD
Harttia longipinna	0.19	0.11	**0.44**	0.20	0.37	0.22
Hypostomus francisci	0.25	0.20	0.31	0.23	**0.45**	0.27
Hypostomus margaritifer	0.26	0.21	0.31	0.23	**0.43**	0.27
Hypostomus sp.	0.14	0.14	**0.50**	0.28	0.36	0.28
Pareiorhaphis mutuca	0.11	0.11	**0.49**	0.29	0.40	0.30
Steindachnerina elegans	**0.37**	0.15	**0.38**	0.15	0.25	0.20

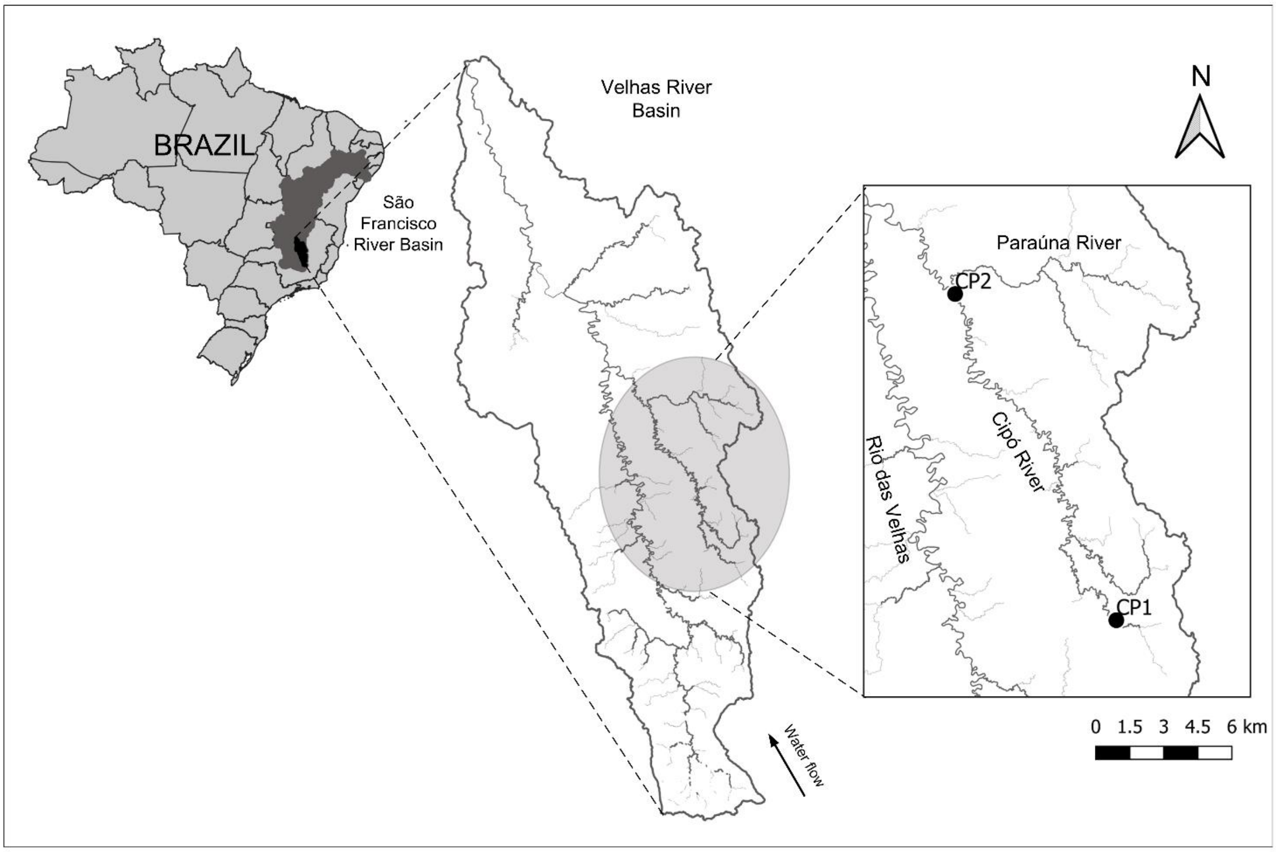

Figure 1. Sampling points along the Cipó River, a tributary of the das Velhas River basin. CP1 = sampling point 1 on the Cipó River, located downstream of the Serra do Cipó National Park; CP2 = sampling point 2 on the Cipó River, located near the confluence with the Paraúna River

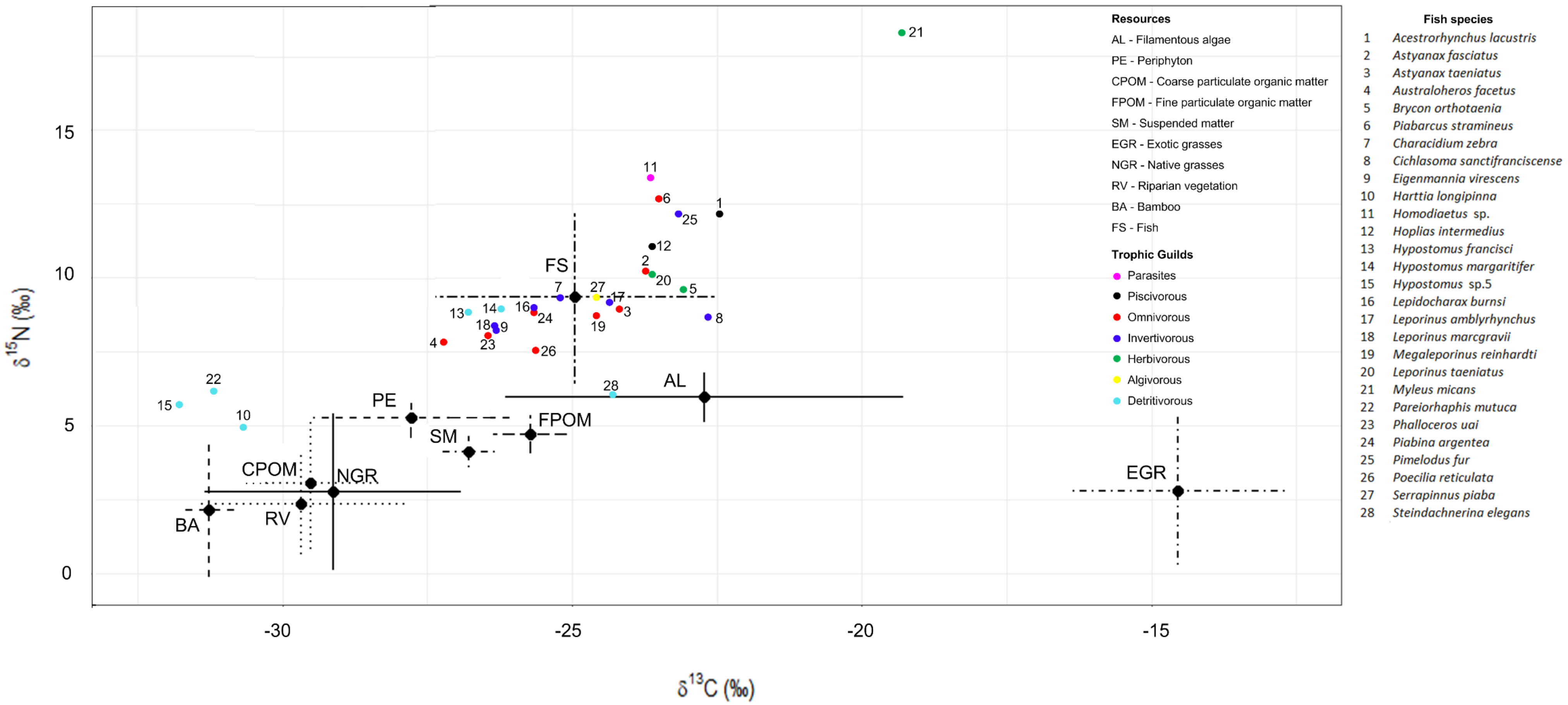

Figure 2. Graphical representation of the trophic structure of the Cipó river fish community according to the mean values of $\delta^{13}C$ and $\delta^{15}N$ of each fish species and sampled resources.

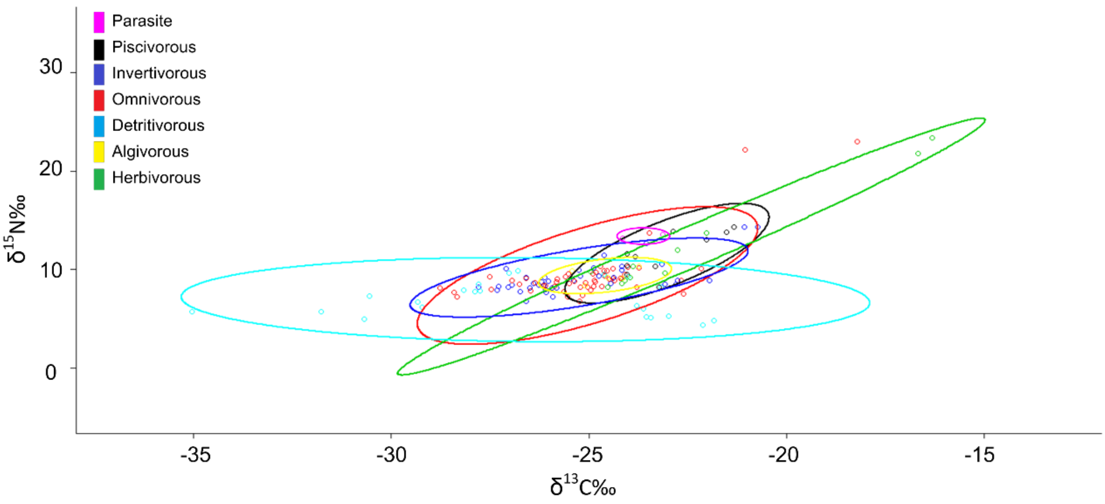

Figure 3. Ellipses (calculated using 95% confidence interval) representing the isotopic niche of fish guilds sampled in the Cipó River. Each dot represents a sampled individual

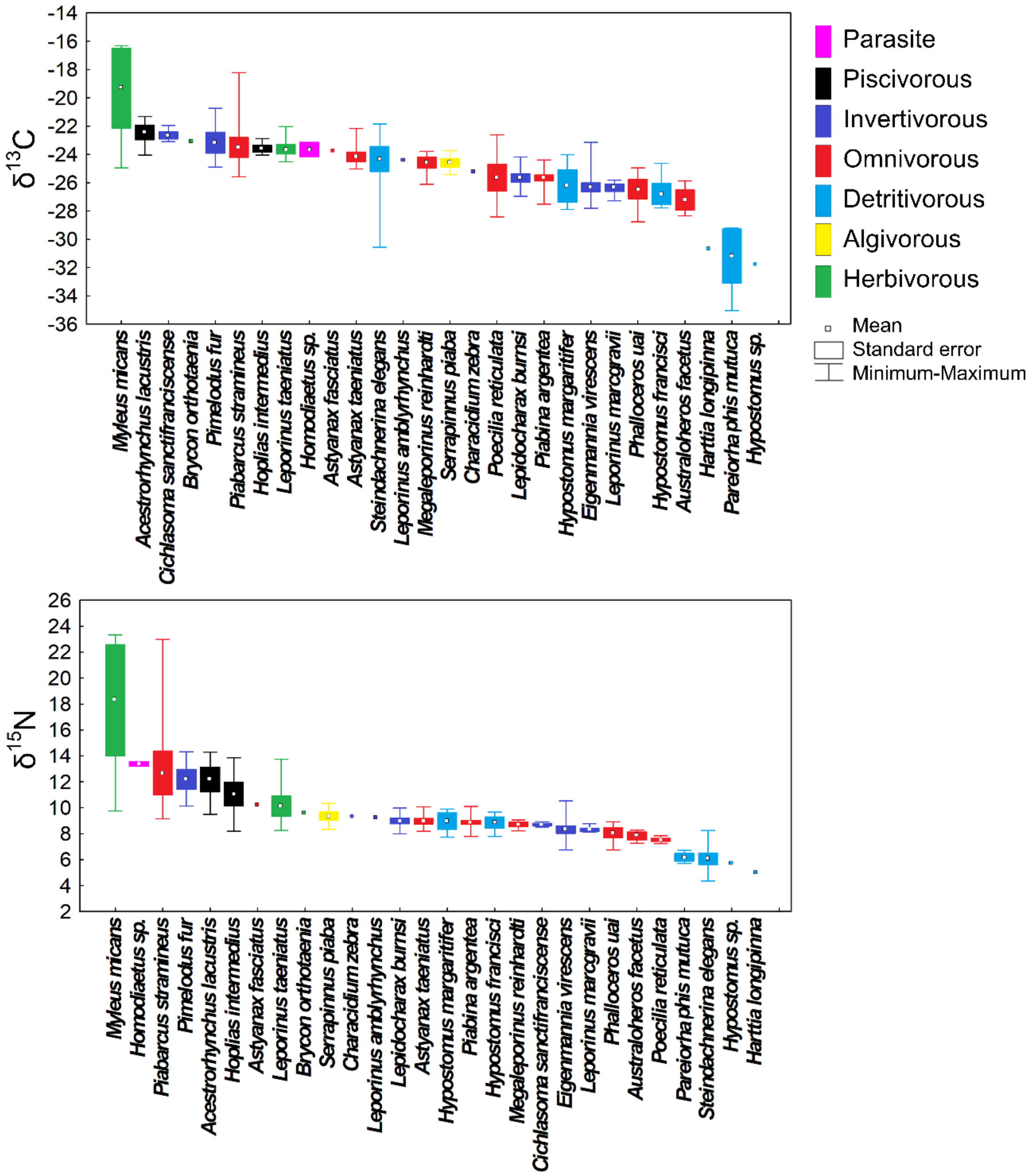

Figure 4. Variation in carbon $\delta^{13}C$ (‰) and nitrogen $\delta^{15}N$ (‰) isotopic composition in Cipó river sampled species

Supplementary material

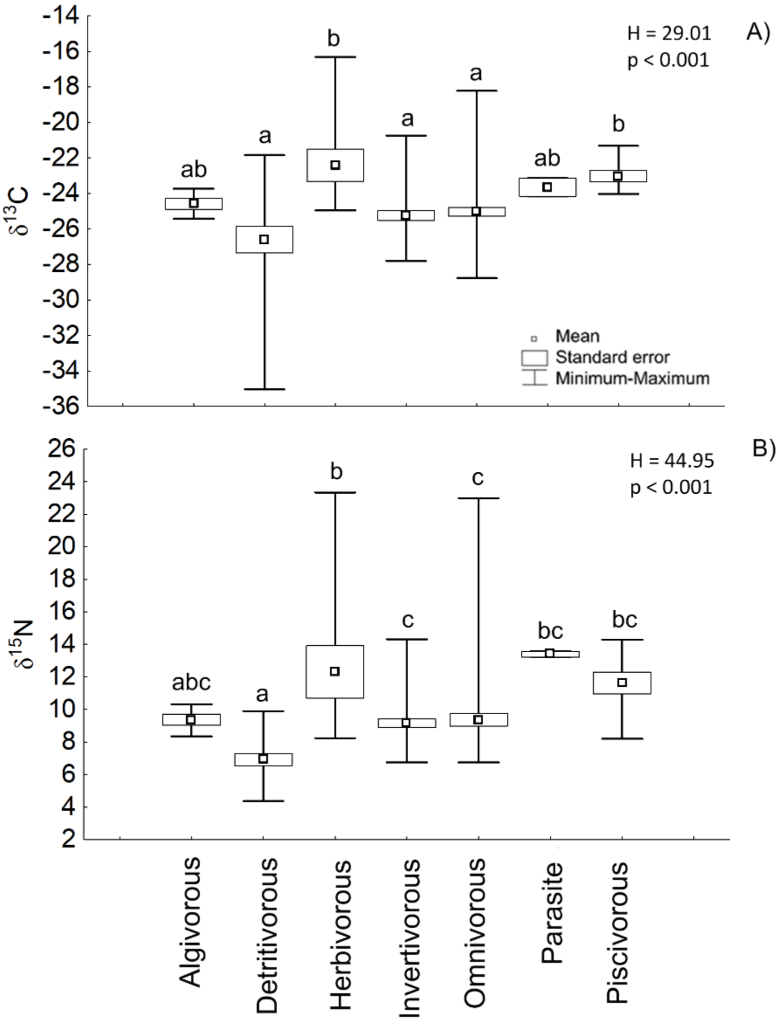

Figure A1. Variation in isotopic composition of $\delta^{13}C$ and $\delta^{15}N$ among trophic guilds of fish sampled in the Cipó River

ARTIGO VII - ***Convergent responses of fish belonging to different feeding guilds to sewage pollution***

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Manuscrito aceito para publicação na revista *Neotropical Ichthyology*

(Manuscrito redigido na língua e formato indicado para revista - número de linhas foi retirado para melhor adequação ao formato da tese)

Abstract

This study aimed to evaluate if the presence of pollutants promotes changes in feeding habits of fish species from different trophic guilds: the detritivorous species, *Hypostomus francisci*, and the piscivorous, *Hoplias intermedius*. Both species were sampled at 12 sites (with different degrees of pollution) in the Rio das Velhas basin, which is heavily polluted by domestic and industrial sewage from the Metropolitan Region of Belo Horizonte (MRBH). Stable isotope analyses of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of fish tissue and the main food resources were performed. Fishes from both trophic guilds altered their diets in degraded environments, but the detritivorous species showed greater trophic plasticity. The isotopic niche of both trophic guilds was broadest in unpolluted sites and more $\delta^{15}N$ enriched in polluted regions. The detritivorous species presented high niche-breadth in unpolluted sites, probably due to the greater variety of resources consumed. In addition, the $\delta^{15}N$ of the detritivorous was more enriched than the piscivorous species in polluted sites. In conclusion, fishes from both trophic guilds presented similar isotopic responses to environmental pollution. However, the detritivorous species was more sensitive to these alterations and therefore, is likely a better indicator of environmental condition than the piscivorous.

Resumo

Este estudo teve como objetivo avaliar se a presença de poluentes promove mudanças nos hábitos alimentares de espécies de peixes de diferentes guildas tróficas: a espécie detritívora, *Hypostomus francisci*, e a piscívora, *Hoplias intermedius*. Ambas espécies foram amostradas em 12 locais (com diferentes níveis de poluição) na bacia do Rio das Velhas, que é altamente poluída por esgoto doméstico e industrial da Região Metropolitana de Belo Horizonte (RMBH). Foram realizadas análises de isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) dos tecidos dos peixes e dos principais recursos alimentares. Ambas as espécies alteraram suas dietas em ambientes degradados, mas as espécies detritívoras apresentaram maior plasticidade trófica. O nicho isotópico de ambas as espécies foi mais amplo em locais menos perturbados e mais enriquecido em $\delta^{15}N$ em regiões poluídas. As espécies detritívoras apresentaram grande amplitude em seu nicho isotópico em locais menos perturbados, provavelmente devido à maior variedade de recursos consumidos. Além disso, o $\delta^{15}N$ da espécie detritívora foi mais enriquecido que as espécies piscívoras em locais poluídos. Em conclusão, ambas as espécies apresentaram respostas isotópicas semelhantes à poluição ambiental. No entanto, a espécie detritívora foi mais sensível a essas alterações e, portanto, é provavelmente uma melhor indicadora de condição ambiental do que a espécie piscívora.

Keywords: Detritivorous, *Hoplias intermedius*, *Hypostomus francisci*, Piscivorous, Stable isotopes.

Palavras-chave: Detritívoros, *Hoplias intermedius*, *Hypostomus francisci*, Isótopos estáveis, Piscívoros.

Running Head: Convergent trophic responses to sewage pollution

Introduction

Fish are good indicators of environmental quality and are used to assess the integrity of aquatic environments (Karr, 1981; Pompeu et al., 2005; de Carvalho et al., 2017b). The selection of these organisms for environmental biomonitoring occurs because changes in habitat can be reflected in multiple dimensions among ichthyofauna, for example, by changes in feeding habit (Carvalho et al., 2015; de Carvalho et al., 2017a), reproduction (Schulz, Martins-Junior, 2001), and community composition (Fausch et al., 1990; Cunico et al., 2006). In addition, fishes are represented at several trophic levels in aquatic food webs (Freitas, Siqueira-Souza, 2009), and many species have long-life cycles (Karr, 1981; Smith et al., 1997). Therefore, assessment of ichthyofauna to understand the effects of anthropogenic impacts on aquatic ecosystems is very important.

The discharge of domestic and industrial sewage without proper treatment into the rivers is considered as one of the main anthropogenic impacts in aquatic systems (Camargo et al., 1995; Soares et al., 2016). One of the main consequences of sewage pollution is the increased nutrient load in aquatic ecosystems, which can lead to a rapid decrease in oxygen levels through eutrophication and have catastrophic impacts on fish diversity (Smith, 2003; Silva, Fonseca, 2016).

More subtle impacts, such as changes in the food webs of aquatic ecosystems, can also be observed in polluted environments (Goulart, Callisto, 2003), related to changes in primary productivity (Delitti, 1995). In response, fish species may seek alternative resources to complement their diets in modified habitats and improve survival probability, a behaviour known as trophic plasticity (Abelha et al., 2001). Such changes in the feeding preferences are related to both seasonal and spatial differences of the supply of food (Uieda, Pinto, 2011). Although morphology may set limits to patterns of resource use, since ecomorphological traits are specific to each trophic guild (Albouy et al., 2011), these limits are broad enough to allow fishes changing their choice of prey resources to respond to local biotic and/or abiotic conditions (Ibañez et al., 2007).

Several studies suggested that certain anthropogenic activities may alter the trophic niche of aquatic organisms (e.g. Crook et al., 2015; Castro et al., 2016; de Carvalho et al., 2017a; Alonso et al., 2019). Large ranges of carbon sources exploited by benthic macroinvertebrates and fishes has been reported, for instance, in streams under influence of pasture (Castro et al., 2016; de Carvalho et al., 2017a). However, little is known about the impact of sewage pollution on the trophic niche of fish species. Furthermore, it is not known whether species of different guilds respond in the same way to stressors, such as pollutants.

The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are key tools for accessing information on feeding habits and trophic structure, since the isotopic composition of the consumers reflects the isotopic composition of their diet, providing long-term feeding information (Manetta, Benedito-Cecilio, 2003). The transfer of the carbon along the trophic web is conservative and can be used to trace the flow of energy in systems where there are several types of resources with different carbon values (Manetta, Benedito-Cecilio, 2003; Jardine et al., 2013). The ^{15}N isotope, in turn, is consistently fractionated throughout the trophic web, allowing inferences about the trophic relationships of consumers with their diet (Vander Zanden et al., 1997; Post, 2002; Vanderklift, Ponsard, 2003). Isotopic niche has been used as a proxy of the trophic niche, and represented with isotopic values ($\delta^{15}N$ and $\delta^{13}C$) as coordinates, because an animal's chemical composition is directly influenced by what it consumes as well as the habitat in which it lives (Newsome et al., 2007). A positive factor in using isotopes is that, unlike stomach contents, it is possible to analyse the main food sources of consumers over weeks (Sacramento et al., 2016; Winter et al., 2019), not just what they fed on in the moments prior to capture. Therefore, from the isotopic composition of consumers and its main food sources it is possible to evaluate the diet of captured organisms, energy flow, and the trophic dynamics of aquatic environments (Manetta, Benedito-Cecilio, 2003).

Considering that pollution can affect biota in different ways, the main objective of this work was to evaluate if the presence of sewage in aquatic environments alters the feeding habits and isotopic niche of fish species from different trophic guilds. For this, the carbon and nitrogen isotopic composition of two species – the piscivorous *Hoplias intermedius* (Günther, 1864) and the detritivorous *Hypostomus francisci* (Lütken, 1874) - were evaluated in different regions of a highly polluted Brazilian river basin. The following hypotheses were tested: 1) Species of different trophic guilds respond similarly to the presence of pollution in the aquatic environment; 2) piscivorous species are more sensitive to pollution, as they are considered more generalists (Ibañez et al., 2007) and negative effects strengthen across trophic levels and 3) both trophic guilds will present larger isotopic niches in unpolluted habitats due to a greater availability of different resources.

Material and Methods

Study area. The study was carried out in the main channel and main tributaries of the Rio das Velhas sub-basin, that belongs to the São Francisco river basin, located in the Brazilian state of Minas Gerais (Fig. 1). The headwaters of the Rio das Velhas are located in the municipality of Ouro Preto, and has an overall extension of approximately 801 km, making it the largest tributary of the São Francisco River (Machado et al., 2008). The Rio das Velhas Basin encompasses 51 municipalities of Minas Gerais, totaling almost five million inhabitants (IBGE, 2000). The river is responsible for most of the water supply in this region, presenting significant economic and social importance. Annual average temperature varies between 19 and 23 ºC, with precipitation levels between 900 and 2000mm, and well-defined seasons - dry (winter) and rainy (summer) (PDRH, 2015). The Cerrado (Brazilian Savanna) is the predominant natural vegetation in the watershed, however, approximately 90% has already been modified by human activities. Due to the diverse human activities developed in the region, this basin is in an advanced state of degradation. Main sources of pollution along the river's course include waste products from iron ore mining, and the discharge of sewage and other pollutants from the Metropolitan Region of Belo Horizonte (MRBH) in its middle portion. Despite this scenario, it is still possible to find relatively unpolluted tributaries, such as the Rio Cipó, which has its headwaters in the Serra do Cipó National Park.

Sampling design. Samples were taken at five sites along the main channel of the Rio das Velhas (RV), which were later grouped into three regions: one sampling site in the upper section of Rio das Velhas – "Upper RV" (region with the best water quality); two sample sites in the middle section of Rio das Velhas - "Middle RV" (most impacted) and two sampling sites in the lower section of Rio das Velhas – "Lower RV" (region farthest from the MRBH and near the river's mouth, presents a partial improvement in the water quality as the distance from MRBH increases). In addition, seven sites were sampled in six of the main tributaries of the Velhas River Basin, which were considered as controls due to the absence of human disturbance (Rio Jabuticatubas, Rio Taquaraçu, Rio da Onça, Rio Bicudo, Rio Curimataí and two sites in Rio Cipó). Two sewage treatment plants (STP) Arrudas and Onça - were also sampled to obtain complementary samples of the suspended material to determine the isotope signature of sewage (Tab. 1; Fig. 1). Fish collections were carried out over three campaigns in the main channel (two in the dry season and one in the rainy season) and two campaigns in tributaries (one in the dry season and one during the rainy season) (Tab. 1).

FIGURE 1

Information about the level of degradation in sampling sites (excluding Rio Taquaraçu that did not have a corresponding point) was obtained from previously published data (Feio et al., 2015). Degradation levels ranged from I (undisturbed/unpolluted) to IV (degraded/polluted) (Tab. 1).

Data on water quality, hypereutrophic condition, toxic contamination and pressure factors acting in studies sites were accessed through Instituto Mineiro de Gestão das Águas website (IGAM, 2018), which publishes quarterly monitoring reports from several points across the Rio das Velhas Basin. Values of conductivity, dissolved oxygen, ammoniacal nitrogen and total phosphorous presented in this study correspond to average values obtained from the IGAM measurements during 2015 and 2016. The hypereutrophic condition, toxic contamination and pressure factors acting in sampling sites were obtained from the report of the year 2017. Based on their geographic proximity, the IGAM monitoring sites BV010; BV136; BV139, BV141, BV144, BV147, BV149; BV150; BV151; BV162; SC33 were considered as the correspondents of CP-01, JB-01, RV-01, RV-02, ON-01, BI-01, RV-05, RV-03, RV-04, CP-02 and CU-01, respectively (Tab. 1).

TABLE 1

Fish sampling. Individuals of the detritivorous (and/or algivorous) *Hypostomus francisci* and the piscivorous *Hoplias intermedius* were collected at all sampling sites. Trophic guilds were determined from stomach content analysis previously conducted by the authors (see supplementary material Tab. 1). These species were selected because they represent different trophic guilds with resident habit (non-migratory) and because they were widely distributed and abundant throughout the Rio das Velhas basin. *Hypostomus francisci* (Siluriformes, Loricariidae), popularly known as “cascudo”, has a ventral mouth and the body is covered by bone plates. Usually, individuals of this genus inhabit rocky or sandy bottoms in places with running water, and are considered detritivorous, as they ingest large amounts of organic matter from sediments (Cardone et al., 2006). However, some authors also consider this genus as algivorous, as they scrape off algae that are adhered to rocky substrates. As a consequence, these fish are of great importance for the recycling of nutrients in aquatic environments

(Pereira, Resende, 1998; Flecker, Taylor, 2004). *Hoplias intermedius*, (Characiformes, Erythrinidae), popularly known as “trairão”, is found in both lentic and lotic environments. Adult individuals of *Hoplias* genus are mainly of predatory habit, feeding preferentially on other fishes (Carvalho et al., 2002). In addition, this species is economically important and a source of food for the local community as well.

The specimens of *H. intermedius* and *H. francisci* were collected with gillnets (20 m long, with 3–16 cm stretch measure mesh), seines (5 m long, 1 mm mesh), cast nets (3 cm stretch measure mesh), and mosquito nets (80 cm in diameter, 1 mm mesh). Gill nets were deployed in the water column for 14 h overnight. Seines were used in shallow areas or littoral zones, mosquito nets were used in near-shore aquatic macrophytes (both shorelines), undercut banks and in riffles, and cast nets were used in habitats too deep to wade. The three latter methods were employed for 1–3 h. In the field, muscle samples from adult specimens were removed and were kept frozen until laboratory processing. In laboratory, these samples were lyophilized for 24 hours and ground to a fine and homogeneous powder using a pestle and mortar and subsequently stored in *eppendorf* tubes.

Collected species were deposited in the Ichthyological Collection of the Federal University of Lavras (CI-UFLA) and the Zoology Museum of University of São Paulo (MZUSP), with the following catalogue numbers: *H. francisci* (CI-UFLA 1029; MZUSP 73724); *H. intermedius* (MZUSP 73655, MZUSP 73735, MZUSP 73839).

Resource sampling. Food resources usually consumed by *H. francisci* and *H. intermedius* were identified by analysing the stomach contents of individuals of both species (supplementary material -Tab. 1) and through information in the literature (e.g. Carvalho et al., 2002; Cardone et al., 2006). We collected five replicates (at each point) of the following resources: periphyton, filamentous algae, grasses, riparian vegetation, suspended material (raw sewage) and fish (due to piscivorous habit of *H. intermedius* – see supplementary material - Tab. 2).

Samples of algae and vegetation (grasses and riparian vegetation) were collected randomly at all sites where present, stored in plastic pots and kept frozen until laboratory processing. Periphyton were collected by scraping stones and collected material stored in plastic bottles with distilled water. To obtain the isotope signature of sewage, suspended matter in the water was sampled using a phytoplankton net (45 µm mesh) for a period of three minutes in the sewage treatment plants (STPs) Arrudas and Onça. After collection, the liquid samples (periphyton and suspended matter) were immediately frozen for preservation of the material. In the laboratory, samples were filtered using a filtration apparatus attached to a vacuum pump using calcined quartz fiber filters (Whatman® QMA quartz filters). All basal resource samples were then dried in an oven at 60°C for a minimum period of 48 hours. Afterwards, dried samples were ground to a fine and homogeneous powder using pestle and mortar and stored in *eppendorf* tubes.

Other fish species (see supplementary material - Tab. 2), which make up the assembly of each sample point and are the primary food source for *H. intermedius*, were collected with the same fishing equipment and sampling effort mentioned in the previous section. Sample processing was also done in the same manner; however, small fish individuals (with less than 3cm) were processed as a whole, without the digestive tract. To avoid contamination, all equipment and material used were washed in distilled water along the processing.

Isotopic analysis. After laboratory processing, a total of 129 fish samples (66 *H. francisci* and 63 *H. intermedius*) and 1725 resources samples (including fish from other species that served as potential resources for *H. intermedius*) were sent to the Centre for Nuclear Energy in Agriculture (CENA) at the University of São Paulo for isotopic analysis. Approximately 2-5 mg of dry material from animal tissue, and about 5-10 mg of basal resources were selected for analysis. To determine the isotopic ratio, a mass spectrometer system in the Continuous-flow (CF-IRMS) mode was used with a Carlo Erba elemental analyzer (CHN 1110) coupled to a Delta Plus mass spectrometer (Thermo Scientific). Results were expressed as the relative difference of international reference standards (air nitrogen and PeeDee Belemnite), in the delta notation (δ ‰), and calculated using the following formula:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$$

where “X” is ^{13}C or ^{15}N and “R” represents the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie, Prosser, 1996).

Statistical Analysis. Differences in the isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ of consumers and resources between the four regions were tested using one-way analyses of variance (ANOVAs) where assumptions of normality and homoscedasticity were met. The nonparametric Kruskal-Wallis test was used for data with non-normal distributions. When significant differences ($p < 0.05$) were observed, the means were compared using the post-hoc Tukey's test. These analyses were performed in the software Statistica 6.0 (Statsoft, 2004).

To evaluate the trophic structure of the detritivorous and piscivorous populations, the individuals of two species were plotted in the *bi-plot* space according to the isotopic values of carbon (x-axis) and nitrogen (y-axis) in each region. Source contributions to the detritivorous and piscivorous diet were estimated for the four regions (Upper RV, Middle RV, Lower RV, and Control) based on stable isotope data analysed through Bayesian stable isotope mixed models (Moore, Semmens, 2008; Parnell et al., 2010), using the MixSIAR package in R (Stock, Semmens, 2016). A Markov chain Monte Carlo sampling was conducted based on the following parameters: number of chains = 3; chain length = 100,000; burn in = 50,000; thin = 50 and model 4 (Resid*Process) error structure (Stock, Semmens, 2016). Diagnostic tests (Gelmin–Rubin, Heidelberger–Welch and Geweke) and trace plots were used to examine models. The fractionation values used for consumers were 0.4 ± 1.3 ‰ for C and 2.54 ± 1.27‰ for N (Post, 2002; Vanderklift, Ponsard, 2003). Both the graphical representation and the partition analysis were done using the MixSIAR package using the R software.

The isotopic niches of the detritivorous and piscivorous in both regions (Upper RV, Middle RV, Lower RV and Control) were quantified based on standard ellipse areas (SEA - expressed in ‰^{2}) through use of the Stable Isotope Bayesian Ellipses package in R – SIBER (Jackson et al., 2011). The standard ellipse area (SEA) represents the core isotopic niche space and it is a proxy of the richness and evenness of resources consumed by the population (Bearhop et al., 2004).

Results

Both the detritivorous and piscivorous showed significant variation in their carbon and nitrogen isotopic composition between study regions. For both trophic guilds, the $\delta^{13}C$ and $\delta^{15}N$ were enriched in regions under the influence of heavy pollution (middle and lower RV) (Fig. 2). Basal resources also presented extensive variation in isotopic composition of $\delta^{13}C$ and $\delta^{15}N$ between study regions, except for riparian vegetation that did not vary in $\delta^{13}C$ composition between the four sampled regions (Tab. 2).

FIGURE 2

TABLE 2

The two trophic guilds varied in their distribution in bi-plot space in unpolluted (upper RV and control) and polluted regions (middle and lower RV). In unpolluted regions, the detritivorous presented a greater amplitude in $\delta^{13}C$ and smaller amplitudes of $\delta^{15}N$ when compared to the piscivorous (Figs. 3a, d). However, in polluted regions, the detritivorous presented more enriched $\delta^{15}N$ values than the piscivorous (Figs. 3b, c).

FIGURE 3

Partition analyses revealed that the proportions of different resources assimilated by fishes varied between sample regions. The most assimilated resource by the piscivorous was fish. However, in the upper section of the Velhas river, such guild also assimilated basal resources (filamentous algae and periphyton), as well as allochthonous items from the forest in the surroundings (riparian vegetation and grasses) (Fig. 4, Supplementary material Tab. 3).

The highest variation in the proportion of assimilated resources was observed for the detritivorous. Filamentous algae were the main resource, except in lower RV sites, where the main source of carbon came from grasses. The importance of grasses as a carbon source was also observed in the upper section of the Rio das Velhas (approximately 22%). Periphyton were the second most important resource for the detritivorous in all regions (mainly in the middle section) (Fig. 4, Supplementary material Tab. 4).

FIGURE 4

Comparing the isotopic niche occupied by these species in different regions, we observed that the two fish species, despite being members of different trophic guilds, presented similar responses to the presence of pollutants (Fig. 5). In regions under high influence of sewage (middle and lower RV), both trophic guilds presented isotopic niches with values more enriched in nitrogen. In addition to influencing nitrogen levels, the presence of pollutants also altered the type and range of assimilated resources, with fishes from both trophic guilds feeding on resources with depleted $\delta^{13}C$ in unpolluted regions. Such variation in the assimilated resources was more visible for the detritivorous, as the amplitude of the x axis (carbon) was much wider in unpolluted environments.

FIGURE 5

Discussion

Using isotopic analyses, we confirm the hypothesis that fish from different trophic guilds respond similarly to the presence of pollution from domestic and industrial sewage discharge in aquatic environments. It was also possible to confirm our third hypothesis that isotopic niches occupied by fish species are wider in unpolluted environments, probably due to the consumption of a greater variety of resources. However, the greater trophic plasticity and higher nitrogen enrichment of the detritivorous diets in polluted sites, suggests that such guild may be more sensitive to variation in environmental conditions and, therefore, a better bioindicator of water quality.

We found striking evidence of nitrogen enrichment in fish species and basal resources sampled in polluted regions. Such enrichment has also been observed in aquatic environments under different anthropogenic impacts (e.g. Carvalho et al., 2015; Loomer et al., 2015; Castro et al., 2016; Orlandi et al., 2017). Although $\delta^{15}N$ values above 25‰ are rarely recorded, they have been consistently observed in fishes and resources from the Velhas basin (Alonso et al., 2019; Carvalho et al., 2019). The most extreme $\delta^{15}N$ values were observed in the region where the high discharge of pollutants occurs (middle RV) and downstream of this region (lower RV). At lower RV, despite the high $\delta^{15}N$ of fishes, the $\delta^{15}N$ was slightly less enriched than in the region close to MRBH (middle RV) likely due the sewage dilution. Such high values are probably related to eutrophication processes (Silva and Fonseca 2016), and were detected in all components of trophic webs, from autotrophic organisms (e.g. algae and periphyton) to the consumers.

In the most polluted regions (middle and lower RV), $\delta^{15}N$ of detritivorous fish were more enriched than piscivorous fish. This pattern is not expected, since, by theory, the piscivorous fishes should occupy the top of the food web. The reason why the $\delta^{15}N$ enrichment of piscivorous fishes is lower than that of detritivores is not clear. Both piscivorous and detritivorous species sampled in this study are resident, so it is unlikely piscivorous fishes are not feeding on fish from the same site where they were sampled. As detritivorous fishes usually presents bottom habits and feed in the resources located in this portion of the water bodies, probably they may be consuming $\delta^{15}N$ enriched resources (not sampled in the present study) that are not being consumed by fishes from other guilds. In addition, the detritivorous from the genus *Hypostomus* are barely predated due its morphology and adoption of defence strategies (Bruton 1996, Kirchheim and Goulart 2010), what could explain why the $\delta^{15}N$ enrichment is not propagating to piscivorous fishes. Therefore, the consume of $\delta^{15}N$ enriched unsampled resources together with the lack of predation of detritivorous fishes by piscivorous fishes (otherwise the piscivorous should be more enriched than the detritivorous) seems to be the main hypothesis to explain why detritivorous fishes are more $\delta^{15}N$ enriched than the piscivorous fishes.

The different carbon isotopic compositions between control sites (without pollution) and polluted sites (middle and lower RV), suggests that fishes from both trophic guilds are modifying their feeding habits in response to changes in the aquatic environment (Abelha et al., 2001). Such changes were reflected in the isotopic niches occupied by the consumers, and similar patterns of variation in niche amplitude, even though these species belong to different trophic guilds. Moreover, a clear homogenization in the isotopic composition of available resources in polluted areas may also explain the narrower isotopic niches of consumers in such regions (Abelha et al., 2001; Pusey, Arthington, 2003).

Fish were the most consumed resources by the piscivorous, as expected for species of the genus *Hoplias* (Pompeu, Godinho, 2001; Carvalho et al., 2002; Montenegro et al., 2013). However, other items were also assimilated by this species, which probably influenced the variation of the isotopic composition of this species between the regions. Vegetable remnants and algae/periphyton, may have been ingested together with the prey during predation events, as they are not normally part of the diet of this species (Corrêa, Piedras, 2009; Beliene et al., 2014). However, in some regions (Upper and Middle RV), this accounted for over 20% of assimilated resources, which weakens the hypothesis of accidental consumption. Another hypothesis is that these resources may have been indirectly assimilated through the ingestion of aquatic insects and their larvae, which may be herbivores/detritivorous (feeding on small algae and organic matter), or small particulate filter-feeders suspended in water. As approximately two thirds of sampled individuals were juveniles reinforces this hypothesis, as juveniles predominantly feed on aquatic invertebrates, and adults may do so also in presence of other piscivorous species (Pompeu, Godinho, 2001).

The detritivorous species assimilated a range of basal resources, including algae, periphyton and C4 grasses among the sampled regions, which consequently was reflected in the variation of the $\delta^{13}C$ isotopic composition. The detritivore/ herbivore habit has been described to representatives of the genus *Hypostomus* (Pessoa et al., 2013) and was confirmed through stomach content analysis, which explain the consumption of allochthonous items, such as plant remnants (grasses). The high consumption of algae and periphyton was expected, as this genus is also considered algivorous (Cardone et al., 2006). In addition, as this species adheres to the substrate during foraging, some items outside their normal diet can be ingested (Ross, 1986; Villares-Junior et al., 2016), such as insect larvae and pupae that are buried in sediments. Therefore, some items reflected in the isotopic composition of the detritivorous may also have been assimilated indirectly, through the accidental consumption of insects and other aquatic organisms.

It was expected that the detritivorous consumed more algae in polluted sites and more periphyton in control sites, as the presence and abundance of these resources is related to water quality (Moschini-Carlos, 1999). Greater algal growth is expected in more eutrophic sites, where there is sewage discharge (Tundisi, Matsumura, 2008). On the other hand, periphyton are more associated with undisturbed environments, where water transparency (Cetto et al., 2004), and the presence of free surfaces, such as rocks and/or submerged vegetation, favours their development (Tundisi, Matsumura, 2008). Nevertheless, the opposite was observed in the control region, with algae and periphyton contributing approximately 86% and 10% of assimilated resources, respectively. This could reflect the specific characteristics of the studied tributaries, which may have favoured the proliferation of algae rather than periphyton. However, the abundance of each resource in each sampled region was not evaluated in the present study. In addition, detritivorous may exhibit selectivity and preference for algae over periphyton, even when both resources are abundant (Bozza, Hahn, 2010).

It is interesting to note that carbon from grasses contributed 22% of the isotopic composition of detritivorous in the upper RV and 51% in the lower RV. This high assimilation of grasses was not expected, as it is not considered as a primary energy source for detritivorous fish (Araujo-Lima et al., 1986). In addition, C4 grasses are generally consumed by fish in small quantities because they contain fewer nutrients and are difficult to digest. However, recent studies have suggested that the contribution of C4 plants can be substantial to aquatic communities (Hoeinghaus et al., 2007; Ferreira et al., 2012), and herbivorous fish species can feed on this resource in impacted environments (Carvalho et al., 2015). In addition, we also need to consider the indirect assimilation of C4 sources through debris consume (Garcia et al., 2016), since it is composed by a mix of autochthonous (e.g. algae, macrophytes and periphyton) and allochthonous material (e.g. leaves from trees and grasses) provided by river banks.

Through the results it was possible to observe that the detritivorous species showed great variation in the proportion of assimilated resources in different regions and high enrichment in the isotopic composition of nitrogen in polluted sites. These results suggest that resident detritivorous species may be more sensitive to environmental changes than migratory or predatory taxa, probably because they are primary consumers and feed exclusively on resources available in a restricted area. Detritivorous species are of great importance for the functioning of aquatic ecosystems (Pereira, Resende, 1998) and usually comprise a significant portion of the total community (Vasconcelos Filho et al., 2009). Therefore, changes in the environment, and subsequent alterations in the feeding habits of this species, may have implications for ecosystem functioning in aquatic habitats.

We can conclude that the use of carbon and nitrogen isotopes contributes greatly to our understanding of the trophic ecology of fish species in impacted aquatic environments. We observed that fishes from both trophic guilds exhibited high food plasticity, varying the proportion of resources consumed among regions. Moreover, pollution promoted nitrogen enrichment in resources and consumers which, consequently, was reflected in the isotopic niches occupied by them. Such changes may have major impacts on ecosystem functioning in aquatic systems, especially when important changes are observed at different levels of trophic webs (e.g. top predators and detritivorous). In addition, for future biomonitoring and assessment of the biotic integrity, we suggest the use of first-level consumers rather than species that occupy higher trophic levels (predators), as they could be more sensitive to changes in environmental conditions. Finally, it is important to emphasise that our results are based on the evaluation of only two species in a large area. Therefore, whole fish community evaluations should be encouraged.

Acknowledgments

We thank the Agência Peixe Vivo and the Comitê de Bacia Hidrográfica do Rio das Velhas (CBH - Rio das Velhas) for the project financial support and the Projeto Manuelzão for the logistical support. Thanks to Aline J. Grossi (Federal University of Lavras/ UFLA) and Luiza Hoehne (Federal University of Minas Gerais/ UFMG) for the support on processing of samples, and Lucas Grossi (Projeto Manuelzão) for the map elaboration. Thanks also to the Benthos Ecology Laboratory (UFMG) and the Laboratory of Fish Ecology (UFLA) that allowed processing of samples and infrastructure, and to the Centre for Nuclear Energy in Agriculture (CENA) for their support and partnership in the isotopic analysis. Paulo S. Pompeu received a research grant and a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (303548/2017-7) and from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (00608/2015). Débora R. Carvalho was financed in part by the Coordenação de Aperfeiçoamento Pessoal de Nível Superior - Brasil (CAPES) – Finance code 001.

References

Abelha M, Agostinho A, Goulart E. Plasticidade trófica em peixes de água doce. Acta Sci. Biol. Sci. 2001; 23(2):425–434.

Albouy C, Guilhaumon F, Villéger S, Mouchet M, Mercier, L, Culioli JM, Tomasini JA, Le Loch'H F, Mouillot, D. Predicting trophic guild and diet overlap from functional traits: statistics, opportunities and limitations for marine ecology. Mar Ecol Prog Ser. 2011; 436:17-28. https://doi.org/10.3354/meps09240

Alonso MB, Carvalho DR, Alves CBM, Moreira MZ, Pompeu PS. Changes in trophic characteristics of two fish species of Astyanax (Teleostei: Characidae) in response to aquatic pollution. Zoologia. 2019; 36: 1-12.

Araujo-Lima CARM, Forsberg BR, Victoria R, Martinelli L. Energy sources for detritivorous fish in the Amazon. Science. 1986; 234(4781):1256–1258. doi:10.1126/science.234.4781.1256

Barrie A, Prosser SJ. Automated Analysis of Light- Element Stable Isotopes by Isotope Ratio Mass Spectrometry. In: Boutton TW, Yamasaki SI, editors. Mass Spectrometry of Soils. New York: Marcel Dekker; 1996. p.1-46.

Bruton MN. Alternative life-history strategies of catfishes. Aquatic Living Resources. 1996; 9:35–41.

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H. Determining trophic niche width: a novel approach using stable isotope analysis. J. Animal Ecol. 2004; 73(5):1007–1012. doi:10.1111/j.0021-8790.2004.00861.x

Beliene GH, Rocha ARM, Souza CMM. Food Parameters Hoplias malabaricus as Environmental Analysis Tool in Lake Ugly, RJ, Brazil. Engineering and Science. 2014; 1(1):1–8.

Bozza AN, Hahn NS. Uso de recursos alimentares por peixes imaturos e adultos de espécies piscívoras em uma planície de inundação neotropical. Biota Neotrop. 2010; 10(3):217–226. doi:10.1590/S1676-06032010000300025

Camargo AFM, Bini LM, Schiavetti A. Avaliação dos impactos provocados pelas descargas de esgotos orgânicos em alguns corpos d'água do município de Rio Claro. In: Esteves FA, editor. Estrutura, Funcionamento e Manejo de Ecossistemas Brasileiros. Rio de

Janeiro: Oecologia Brasiliensis; 1995. p.395–406.

Cardone IB, Lima-Junior SE, Goitein R. Diet and capture of Hypostomus strigaticeps (Siluriformes, Loricariidae) in a small Brazilian stream: relationship with limnological aspects. Braz. J. Biol. 2006; 66(1A):25–33. doi:10.1590/S1519-69842006000100005

Carvalho DR, Castro D, Callisto M, Moreira MZ, Pompeu PS. Isotopic variation in five species of stream fishes under the influence of different land uses. J. Fish Biol. 2015; 87(3): 559–578. doi:10.1111/jfb.12734

Carvalho DR, Flecker A, Alves CBM, Sparks J, Pompeu PS. Trophic responses to aquatic pollution of native and exotic livebearer fishes. Science of the Total Environment. 2019; 681: 503-515.

Carvalho LN, Fernandes CHV, Moreira VSS. Alimentação de Hoplias malabaricus (Bloch, 1794) (Osteichthyes, Erythrinidae) no rio Vermelho, Pantanal Sul Mato-Grossense. Rev. Bras. Zoociências. 2002; (4):227–236.

Castro DMP, Carvalho DR, Pompeu PS, Moreira MZ, Nardoto GB, Callisto M. Land Use Influences Niche Size and the Assimilation of Resources by Benthic Macroinvertebrates in Tropical Headwater Streams. PLoS One. 2016; 11(3): e0150527. doi:10.1371/journal.pone.0150527

Cetto JM, Leandrini JA, Felisberto SA, Rodrigues L. Comunidade de algas perifíticas no reservatório de Irai, Estado do Paraná, Brasil. Acta Sci. Biol. Sci. 2004; 26(1):1–7. doi:10.4025/actascibiolsci.v26i1.1645

Corrêa F, Piedras SRN. Alimentação de Hoplias aff. malabaricus (Bloch, 1794) e Oligosarcus robustus Menezes, 1969 em uma lagoa sob influência estuarina, Pelotas, RS. Biotemas. 2009; 22(3):121–128.

Crook DA, Lowe WH, Allendorf FW, Erős T, Finn DS, Gillanders BM, et al. Human effects on ecological connectivity in aquatic ecosystems: Integrating scientific approaches to support management and mitigation. Sci. Total Environ. 2015; 534(April):52–64. doi:10.1016/j.scitotenv.2015.04.034

Cunico AM, Agostinho AA, Latini JD. Influência da urbanização sobre as assembléias de peixes em três córregos de Maringá, Paraná. Rev. Bras. Zool. 2006; 23(4):1101–1110.

de Carvalho DR., Castro, DMP, Callisto M, Moreira MZ, Pompeu PS. The trophic structure of fish communities from streams in the Brazilian Cerrado under different land uses: an approach using stable isotopes. Hydrobiologia. 2017; 795(1):199-217. doi:10.1007/s10750-017-3130-6

de Carvalho DR, Leal CG, Junqueira NT, Castro MA, Fagundes DC, Alves CBM., et al. A fish-based multimetric index for Brazilian savanna streams. Ecol. Indic. 2017; 77(January):386–396. doi:10.1016/j.ecolind.2017.02.032

Delitti WBC. Estudos de ciclagem de nutrientes: instrumentos para a análise funcional de ecossistemas terrestres. In: Esteves FA, editor. Estrutura, Funcionamento e Manejo de Ecossistemas Brasileiros. Rio de Jeneiro: Oecologia Brasiliensis; 1995. p.469–486

Fausch KD, Lyons J, Karr JR, Angermeier PL. Fish Communities as Indicators of Environmental Degradation. Am. Fish. Soc. Symp. 1990; 8(April):123–144.

Feio MJ, Ferreira WR, Macedo DR, Eller AP, Alves CBM, França JS, Callisto M. Defining and Testing Targets for the Recovery of Tropical Streams Based on Macroinvertebrate Communities and Abiotic Conditions. River Res. Appl. 2015; 31(1):70–84. doi:10.1002/rra.2716

Ferreira A, Cyrino JEP, Duarte-Neto PJ, Martinelli LA. Permeability of riparian forest strips in agricultural, small subtropical watersheds in south-eastern Brazil. Mar. Freshwater Res. 2012; 63(12):1272–1282. doi:10.1071/MF12092

Flecker AS, Taylor BW. Tropical Fishes as Biological Bulldozers: Density Effects on Resource Heterogeneity and Species Diversity. Ecology. 2004; 85(8):2267–2278.

Freitas CEC, Siqueira-Souza FK. O uso de peixes como bioindicador ambiental em áreas de várzea da bacia amazônica. Rev. Agrogeoambiental. 2009; (1):39–45. doi:10.1016/j.chemosphere.2015.04.064

Garcia AM, Claudino MC, Mont'Alverne R, Pereyra PER, Copertino M, Vieira JP. Temporal variability in assimilation of basal food sources by an omnivorous fish at Patos Lagoon Estuary revealed by stable isotopes (2010-2014). Marine Biology Research. 2016; 13(1):98-107, doi: 10.1080/17451000.2016.1206939.

Goulart MDC, Callisto M. Bioindicadores de Qualidade de Água como Ferramenta em Estudos de Impacto Ambiental. Revista da FAPAM. 2003; 2(2):153–164.

Hoeinghaus DJ, Winemiller KO, Agostinho AA. Landscape-Scale Hydrologic Characteristics

Differentiate Patterns of Carbon Flow in Large-River Food Webs. Ecosystems. 2007; 10(6): 1019–1033. doi:10.1007/s10021-007-9075-2

Ibañez C, Tedesco PA, Bigorne R, Hugueny B, Pouilly M, Zepita C, Zubieta J, Oberdorff T. Dietary-morphological relationships in fish assemblages of small forested streams in the Bolivian Amazon. Aquatic Living Resources. 2007; 20(2), 131-142.

Jackson AL, Inger R, Parnell AC, Bearhop S. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. J. Anim. Ecol. 2011; 80(3):595–602. doi:10.1111/j.1365-2656.2011.01806.x

Jardine TD, Hunt RJ, Faggotter SJ, Valdez D, Burford MA, Bunn SE. Carbon from periphyton supports fish biomass in waterholes of a wet – dry tropical river. River Res. Applic. 2013; 29(5):560–573. DOI: 10.1002/rra.2554

Karr JR. Assessment of biotic integrity using fish communities. Fisheries. 1981; 6(6):21–27.

Kirchheim PD, Goulart E. Ecomorfologia de predação e antipredação em Siluriformes (Osteichthyes). Oecologia Australis. 2010; 14:550–568.

Loomer HA, Oakes KD, Schiff SL, Taylor WD, Servos MR. Use of Stable Isotopes to Trace Municipal Wastewater Effluents into Food Webs within a Highly Developed River System. River Res. Appl. 2015; 31(9):1093–1100. doi:10.1002/rra.2826

Machado ATM, Alves CBM, Callisto M. Projeto Manuelzão: metodologia e resultados. In: Lisboa AH, Goulart EMA, Diniz LFM, editors. Projeto Manuelzão. A história da mobilização que começou em torno de um rio. Belo Horizonte: Instituto Guaicuy - SOS Rio das Velhas; 2008. p.39–54.

Manetta GI, Benedito-Cecilio E. Aplicação da técnica de isótopos estáveis na estimativa da taxa de turnover em estudos ecológicos: uma síntese. Acta Sci. Biol. Sci. 2003; 25(1):121–129.

Montenegro AKA, Vieira ACB, Cardoso MML, Souza JERT, Crispim MC. Piscivory by Hoplias aff. malabaricus (Bloch, 1974): a question of prey availability? Acta Limnol. Bras. 2013; 25(1):68–78.

Moore JW, Semmens BX.Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett. 2008; 11(5):470–480. doi:10.1111/j.1461-0248.2008.01163.x

Moschini-Carlos V. Importância, estrutura e dinâmica da comunidade perifítica nos ecossistemas aquáticos continentais. In: Pompêo MLM, editor. Perspectivas na Limnologia no Brasil. São Luís: Grafica e Editora Uniao; 1999. p.1-11.

Newsome SD, Martinez del Rio C, Bearhop S, Phillips L. A niche for isotopic ecology. Frontiers in Ecology and the Environment. 2007; 5: 429–436.

Orlandi L, Calizza E, Careddu G, Carlino P, Costantini ML, Rossi L. The effects of nitrogen pollutants on the isotopic signal (Δ15N) of Ulva lactuca: Microcosm experiments. Mar. Pollut. Bull. 2017; 115(1–2): 429–435. doi:10.1016/j.marpolbul.2016.12.051

Parnell AC, Inger R, Bearhop S, Jackson AL. Source partitioning using stable isotopes: coping with too much variation. PLoS One. 2010; 5(3): e9672. doi:10.1371/journal.pone.0009672

Pereira RAC, Resende EK. Peixes detritívoros da planície inundável do rio Miranda, Pantanal, Mato Grosso do Sul Brasil. Corumbá: Empresa Brasileira de Pesquisa Agropecuária – Embrapa; 1998.

Pessoa EKR, Silva NB, Chellappa NT, Souza AA, Chellappa S. Morfologia comparativa do trato digestório dos peixes Hoplias malabaricus e Hypostomus pusarum do açude Marechal Dutra , Rio Grande do Norte , Brasil . Biota Amazônia. 2013; 3(1):48–57. doi:10.18561/2179-5746/biotaamazonia.v3n1p48-57

Pompeu PS, Godinho AL. Mudança na dieta da traíra Hoplias malabaricus (Bloch) (Erythrinidae, Characiformes) em lagoas da bacia do rio Doce devido à introdução de peixes piscívoros. Rev. Bras. Zool. 2001; 18(4):1219–1225. doi:10.1590/S0101-81752001000400016

Pompeu PS, Alves CBM, Callisto M. The Effects of Urbanization on Biodiversity and Water Quality in the Rio das Velhas Basin, Brazil. Am. Fish. Soc. Symp. 2005; (47):11–22.

Post D. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology. 2002; 83(3):703–718.

Pusey BJ, Arthington AH. Importance of the riparian zone to the conservation and management of freshwater fish: a review. Mar. Freshwater Res. 2003; 54(1):1-16. doi:10.1071/MF02041

Ross ST. Resource Partitioning in Fish Assemblages: A Review of Field Studies. Copeia.

1986; (2): 352-388. doi:10.2307/1444996

Sacramento PA, Manetta GI, Benedito E. Diet-tissue discrimination factors (Δ 13C and Δ 15N) and turnover rate in somatic tissues of a neotropical detritivorous fish on C3 and C4 diets. Journal of fish biology. 2016; 89(1), 213-219.

Schulz UH, Martins-Junior H. Astyanax fasciatus as bioindicator of water pollution of Rio dos Sinos, RS, Brazil. Braz. J. Biol. 2001; 61(4):615–622. doi:10.1590/S1519-69842001000400010

Silva AR, Fonseca ALD. Eutrofização dos recursos hídricos como ferramenta para a compreensão das doenças de vinculação hídrica Introdução. Geosul. 2016; 31(62):247–270.

Smith VH. Eutrophication of freshwater and coastal marine ecosystems a global problem. Environ. Sci. Pollut. Res. 2003; 10(2):126–139. doi:10.1065/espr2002.12.142

Smith WS, Barrella W, Cetra M. Comunidades de peixes como indicadoras de poluição ambiental. Rev. Bras. Ecol. 1997; 1(December):67–71.

Soares AFS., Araújo AS, Rodrigues NUA, Cunha NC (2016). Environmental compensation due to lack of treatment of domestic wastewater in the municipality of Campanha (MG). Ambiência. 2016; (12):831–839. doi:10.5935/ambiencia.2016.Especial.08

Stock BC, Semmens BX. Unifying error strucctures in commonly used biotracer mixing models. Ecology. 2016; 97(3):576–582. doi:10.1002/ecy.1517

Stock B, Semmens B. MixSIAR GUI user manual. Version 3.1. 2016; (March):1–59. doi:10.5281/zenodo.47719

Tundisi G, Matsumura T. Limnology. CRC Press; 2008.

Uieda VS, Pinto TLF. Feeding selectivity of ichthyofauna in a tropical stream: space-time variations in trophic plasticity. Community Ecology. 2011; 12(1), 31-39.

Vander Zanden MJ, Cabana G, Rasmussen JB. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ($\delta^{15}N$) and literature dietary data. Can. J. Fish. Aquat. Sci. 1997; 54(5):1142–1158. doi:10.1139/cjfas-54-5-1142

Vanderklift M, Ponsard S. Sources of variation in consumer-diet delta 15N enrichment: a meta-analysis. Oecologia. 2003; 136(2):169–82. doi:10.1007/s00442-003-1270-z

Vasconcelos Filho AL, Neumann-Leitão S, Eskinazi-Leça E, Porto-Neto FF. Hábitos alimentares de consumidores primários da ictiofauna do sistema estuarino de Itamaracá (Pernambuco - Brasil). Rev. Bras. Enga. Pesca. 2009; 4(1):21–31.

Villares-Junior GA, Cardone IB, Goitein R. Comparative feeding ecology of four syntopic Hypostomus species in a Brazilian southeastern river. Braz. J. Biol. 2016; 76(3): 692–699. doi:10.1590/1519-6984.00915

Winter ER, Nolan ET, Busst GM, Britton JR. Estimating stable isotope turnover rates of epidermal mucus and dorsal muscle for an omnivorous fish using a diet-switch experiment. Hydrobiologia. 2019; 828(1), 245-258.

Tab. 1. Geographic location (in degrees/minutes/seconds and UTM, date, altitude and municipality) and water quality of the sampling sites sampled in the main channel and tributaries of Rio das Velhas. Cond.= Condutivity (µS/cm); D.O.= Dissolved oxygen (mg/l); Am. Nitr. = Ammoniacal nitrogen (mg/l); Phosp. = Total phosphorus (mg/l); Tox. Contam. = Toxic contamination; Deg. Level = degradation level ranging from I (undisturbed/unpolluted) to IV (degraded/polluted) (Feio et al., 2015). *Sites with hypereutrophic condition according IGAM.

Characteristics of sampling sites						**Water Quality**						
Region	**Sampling sites**	**Date of sampling**	**Coordinates**	**Altitude (m)**	**Municipality**	**Cond.**	**D.O.**	**Am. Nitr.**	**Phosp.**	**Tox. Contam.**	**Deg. Level**	**Pressure Factors**
Upper RV	RV-01	19/08/2015 20/01/2016 9/06/2016	20˚01'10.7"S 43˚49'45.4"W 23K 622454 E 7785916 W	729	Nova Lima	73.21	7.54	0.12	0.08		I	
Middle RV	RV-02	10/08/2015 11/01/2016 31/05/2016	18˚48'19.2"S 44˚09'09.2"W 23K 589298 E 7920498 W	567	Curvelo	287.20	7.25	0.92	0.41	Arsenic and total ammoniacal nitrogen	III*	Gold metallurgy and discharge of domestic sewage
Middle RV	RV-03	11/08/2015 12/01/2016 1/06/2016	18˚25'33.2"S 44˚11'10.9"W 23K 585926 E 7962502 W	552	Corinto	203.23	7.30	0.23	0.22	Arsenic	II	Agriculture
Lower RV	RV-04	13/08/2015 13/01/2016 3/06/2016	17˚51'55.4"S 44˚32'57.4"W 23K 547752 E 8024649 W	495	Lassance	162.21	7.63	0.21	0.17	Arsenic	II	Discharge of domestic sewage and agriculture (sugar cane)
Lower RV	RV-05	12/08/2015 14/01/2016 2/06/2016	17˚12'25.9"S 44˚48'49.8"W 23K 519793 E 8097515 W	464	Várzea da Palma	153.00	8.35	0.14	0.11	Arsenic	II	Discharge of domestic sewage
Control	Rio Taquaraçu (TQ)	19/06/2015 23/10/2015	19º36’03.1”S 43º44’09.9”W 23K 632551 E 7832196 N	677	Taquaraçu de Minas	67.55	7.49	0.20	0.08		-	

Control	Rio Jaboticatubas (JB)	16/06/2015 22/10/2015	19º27’51.4”S 43º54’18.0”W 23K 614931 E 7847433 N	651	Jaboticatubas	102.20	7.55	0.14	0.06	I
Control	Rio Cipó (CP1)	15/06/2015 21/10/2015	19º20’01.1”S 43º39’17.0”W 23K 641315 E 7861707 N	757	Santana do Riacho	18.13	6.70	0.13	0.03	I
Control	Rio da Onça (ON)	11/07/2015 18/10/2015	19º02’48.1”S 44º15’15.9”W 23K 578454 E 7893841 N	632	Cordisburgo	312.75	7.85	0.22	0.05	II
Control	Rio Cipó (CP2)	10/07/2015 13/10/2015	18º41’07.0”S 43º59’48.4”W 23K 605789 E 7933699 N	567	Presidente Juscelino	68.18	6.90	0.13	0.05	I
Control	Rio Bicudo (BI)	06/07/2015 17/10/2015	18º21’30.7”S 44º35’30.8”W 23K 543117 E 7970099 N	564	Corinto	72.77	6.22	0.11	0.07	II
Control	Rio Curimataí (CU)	08/07/2015 15/10/2015	17º59’33.4”S 44º10’47.5”W 23K 586829 E 8010438 N	518	Augusto de Lima	55.10	6.75	0.12	0.06	I
Sewage - MRBH	STP Arrudas	20/07/2016 25/01/2017			Sabará					
Sewage - MRBH	STP Onça	20/07/2016 18/01/2017			Belo Horizonte					

Tab. 2. Variation in the carbon and nitrogen isotopic composition of the resources sampled in the four regions of the Rio das Velhas Basin, Minas Gerais, Brazil. Letters (a, b, c and d) indicate significant differences according to post-hoc Tukey's test

	Regions	Algae			Periphyton			Grasses			Riparian vegetation			Fish			Sewage		
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
δ^{13}C	Control	53	-25.02	±6.33**a**	44	-25.85	±1.88**a**	70	-22.09	±8.31**a**	70	-29.52	±1.75**a**	474	-24.68	±2.62**a**	40	-23.16	±0.40
	Upper RV	5	-21.78	±2.16**ab**	15	-24.09	±2.22**ab**	5	-14.33	±0.99**ab**	5	-30.94	±1.00**a**	109	-20.65	±2.32**b**			
	Middle RV	18	-11.64	±8.47**b**	30	-21.62	±3.45**b**	10	-15.25	±4.51**b**	10	-29.99	±1.45**a**	278	-20.16	±2.18**b**			
	Lower RV	15	-6.41	±1.21**b**	30	-18.91	±3.68**c**	10	-17.46	±6.71**ab**	10	-29.27	±1.51**a**	280	-18.24	±2.82**c**			
	p	<0.01			<0.01			0.02			0.24			<0.01					
δ^{15}N	Control	53	7.05	±2.13**a**	44	6.34	±1.06**a**	70	3.65	±2.23**a**	70	2.40	±2.18**a**	474	10.23	±2.11**a**	40	1.50	±0.45
	Upper RV	5	8.63	±0.69**ac**	15	6.12	±1.10**a**	5	-0.93	±0.78**b**	5	0.50	±2.33**a**	109	9.19	±1.30**b**			
	Middle RV	18	22.64	±3.73**b**	30	20.45	±5.28**b**	10	5.95	±2.97**c**	10	5.17	±2.75**b**	278	22.82	±3.33**c**			
	Lower RV	15	13.26	±2.71**bc**	30	14.21	±3.26**b**	10	9.76	±3.05**d**	10	7.94	±2.32**b**	280	19.43	±3.35**d**			
	p	<0.01			<0.01			<0.01			<0.01			<0.01					

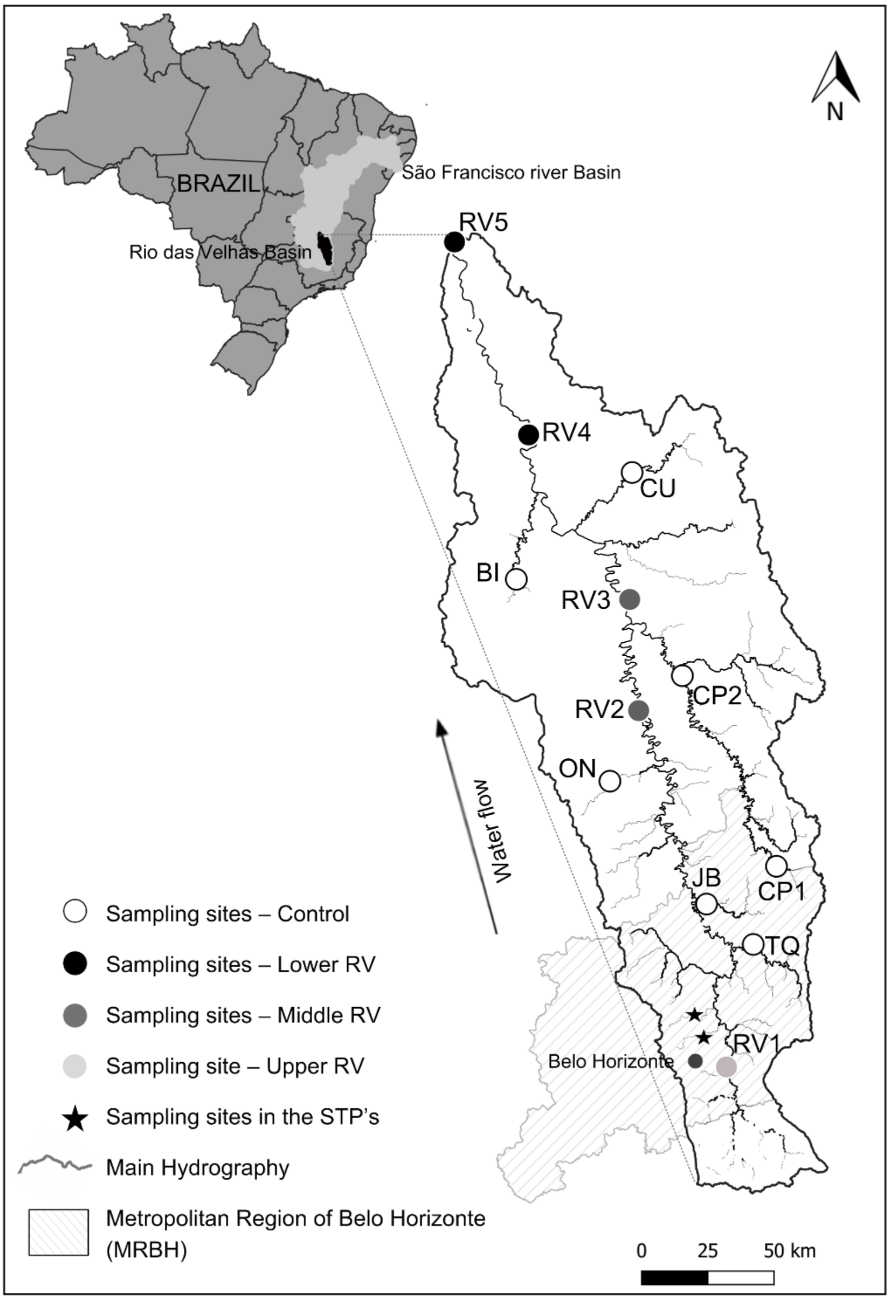

Fig. 1. Sampling network in the Rio das Velhas Basin, Minas Gerais, Brazil. Sampling sites at Rio das Velhas main stem (RV-01 to RV-05), Rio Taquaraçu (TQ); Rio Jaboticatubas (JB); Rio Cipó (CP1 and CP2); Rio da Onça (ON); Rio Bicudo (BI); Rio Curimataí (CU); and Sewage Treatment Plants (STP's).

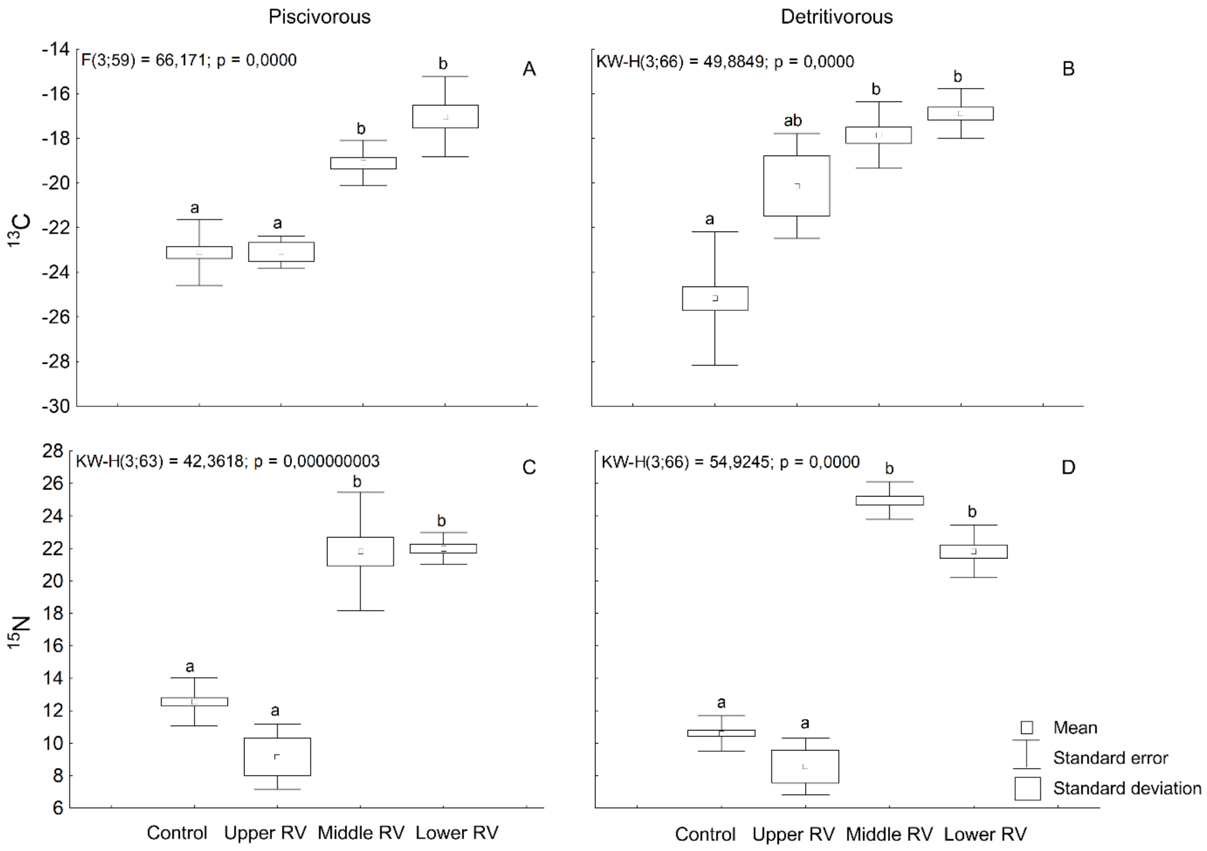

Fig. 2. Variation in the isotopic composition of carbon (**a** and **c**) and nitrogen (**c** and **d**) in the piscivorous species *Hoplias intermedius* (**a** and **c**) and the detritivorous species *Hypostomus francisci* (**b** and **d**) among the studied regions.

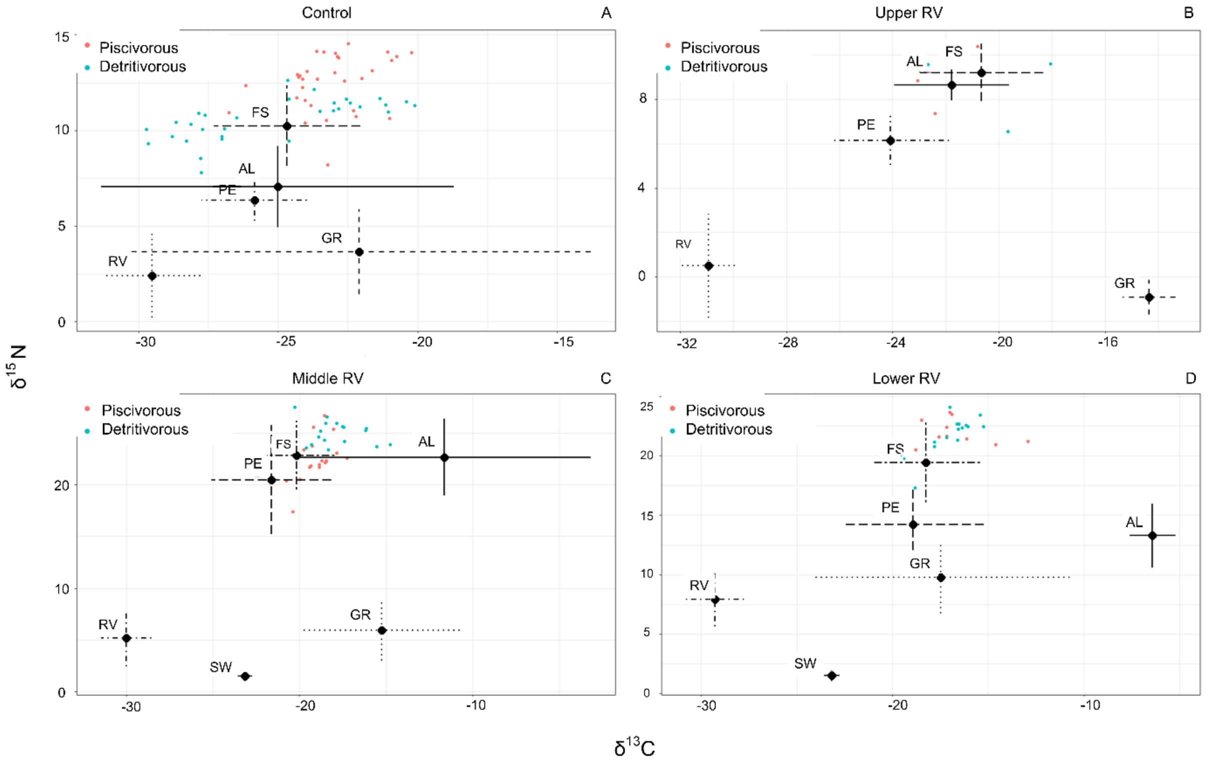

Fig. 3. Distribution of the piscivorous species *Hoplias intermedius* (red points) and the detritivorous species *Hypostomus francisci* (blue points) in the bi-plot space by study regions: **a.** Upper RV, **b.** Middle RV, **c.** Lower RV and **d.** Control sites. Resources: GR = Grasses; RV= Riparian vegetation; PE = periphyton; SW = Sewage (before treatment); AL = filamentous algae and FS = Fish.

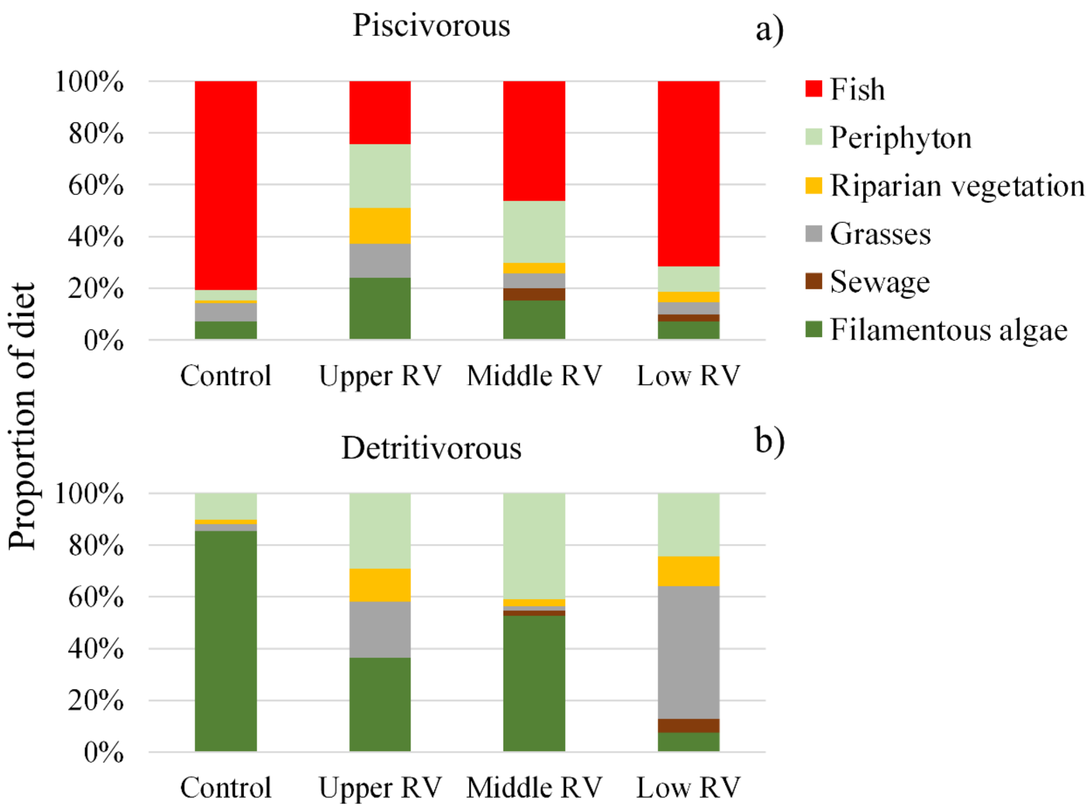

Fig. 4. Mean proportion of each resource assimilated by the species *Hoplias intermedius* (**a**) and *Hypostomus francisci* (**b**) at each study region (MixSIAR results). AL = filamentous algae; SW = Sewage (before treatment); GR = Grasses; RV= Riparian vegetation; PE = periphyton and FS = Fish.

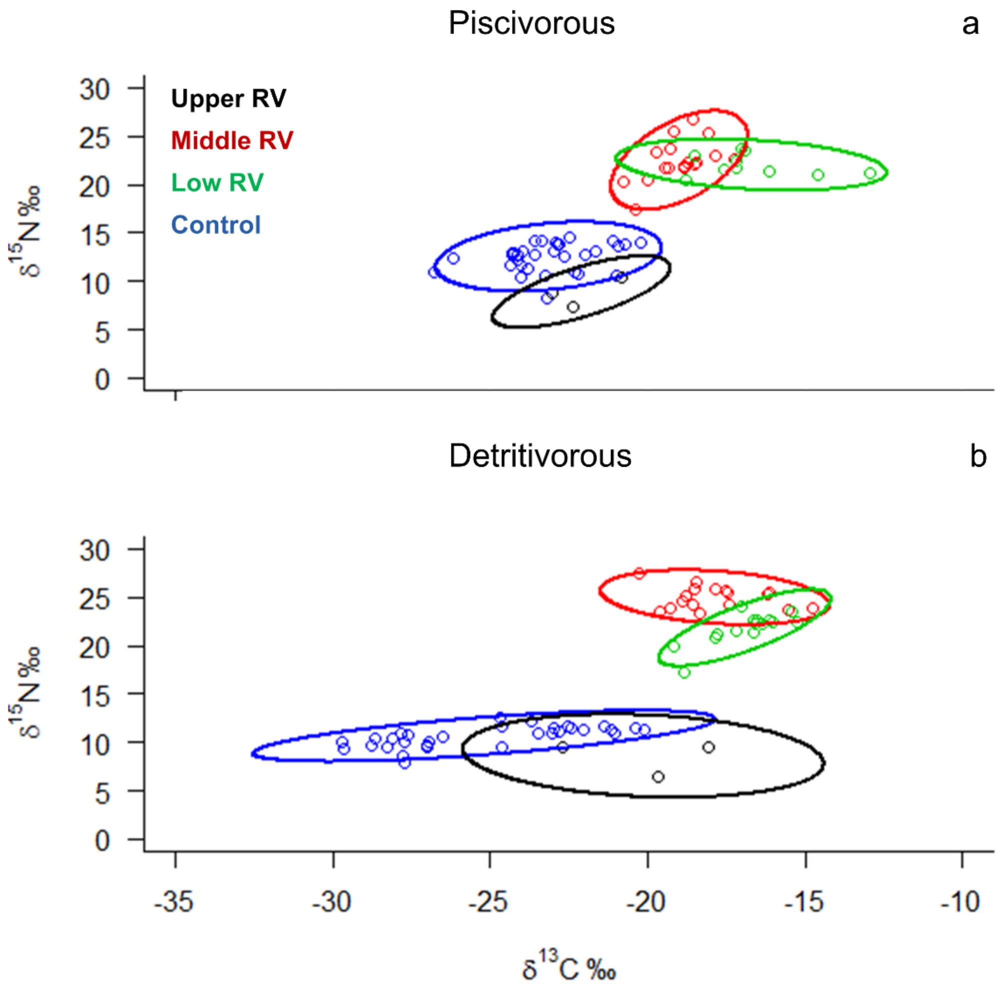

Fig. 5. Isotopic niche (measured by ellipse area with 95% confidence interval) occupied by **a.** *Hoplias intermedius* (piscivorous) **b.** *Hypostomus francisci* (detritivorous) in different regions of the Rio das Velhas Basin, Minas Gerais, Brazil.

Supplementary material

Supplementary material Tab. 1. Stomach contents results used to determine the trophic guilds of *Hoplias intermedius* and *Hypostomus francisci*. AI = Food index of each food item found in the stomachs of *Hoplias intermedius* and *Hypostomus francisci* in each sampled region of the Rio das Velhas Basin, Minas Gerais, Brazil. No stomach content analyses were performed for the species *Hoplias intermedius* at Upper RV due to the small number of individuals sampled.

Item	AI *Hoplias intermedius*				AI *Hypostomus francisci*			
	Control	Upper RV	Middle RV	Lower RV	Control	Upper RV	Middle RV	Lower RV
Detritus	0.000	0.000	0.000	0.000	0.376	0.691	0.239	0.553
Plant remnants	0.000	0.000	0.000	0.001	0.004	0.006	0.389	0.284
Seeds and Fruits	0.000	0.000	0.000	0.000	0.086	0.303	0.008	0.164
Algae/Periphyton	0.000	0.000	0.000	0.000	0.533	0.000	0.364	0.000
Digested Matter	0.006	0.000	0.015	0.003	0.000	0.000	0.000	0.000
Fish	0.984	0.000	0.976	0.945	0.000	0.000	0.000	0.000
Diptera larvae and pupae	0.000	0.000	0.000	0.051	0.001	0.000	0.000	0.000
Insects remnants	0.009	0.000	0.004	0.000	0.000	0.000	0.000	0.000
Scales	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000

Supplementary material Tab. 2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of fish species sampled at Rio das Velhas Basin used as food sources to the piscivorous species *Hoplias intermedius*

Species	Control					Upper RV					Middle RV					Lower RV				
	N	Mean+SD $\delta^{13}C$		Mean+SD $\delta^{15}N$		N	Mean+SD $\delta^{13}C$		Mean+SD $\delta^{15}N$		N	Mean+SD $\delta^{13}C$		Mean+SD $\delta^{15}N$		N	Mean+SD $\delta^{13}C$		Mean+SD $\delta^{15}N$	
Acestrorhynchus lacustris	5	-22.44	1.18	12.19	2.15						3	-18.89	1.33	21.64	2.96	1	-19.74		20.92	
Anchoviella vaillanti											5	-20.58	1.13	22.77	1.08	7	-21.24	2.49	19.97	2.28
Apareiodon hasemani						5	-19.77	0.56	8.03	1.30	5	-18.95	0.70	25.22	0.52	10	-15.78	2.56	17.17	0.94
Apareiodon ibitiensis											6	-21.29	1.62	24.32	2.41	6	-17.26	1.33	19.78	2.17
Astyanax eigenmanniorum	5	-23.87	1.60	9.45	0.98															
Astyanax fasciatus	10	-23.85	2.26	9.96	0.76											1	-21.49		17.04	
Astyanax lacustris	11	-22.87	1.14	11.12	3.25	7	-24.13	2.26	9.77	1.00	14	-20.13	1.24	20.86	3.34	16	-20.12	2.75	15.89	3.63
Astyanax rivularis						1	-20.10		9.58											
Astyanax taeniatus	32	-23.56	1.48	9.53	0.82	13	-21.15	0.87	8.54	1.53	12	-20.15	1.75	23.38	2.40	11	-19.62	1.13	15.58	1.19
Australoheros facetus	4	-27.07	1.07	8.52	1.43															
Bergiaria westermanni											1	-18.37		23.88						
Brycon orthotaenia	1	-23.08		9.63												4	-21.70	1.88	14.24	4.08
Characidium zebra	1	-25.22		9.35												1	-19.26		16.15	
Cichlasoma sanctifranciscense	5	-23.08	1.06	8.87	0.41	4	-22.66	0.35	9.93	1.40	15	-20.60	1.38	21.77	3.06	10	-18.54	1.43	19.49	1.68
Coptodon rendalli	5	-21.78	0.24	11.48	0.18						10	-20.98	1.10	24.49	3.61	9	-12.82	0.47	20.99	0.77
Crenicichla lepidota											1	-20.36		22.47						
Curimatella lepidura											11	-20.13	2.08	20.29	2.40	10	-20.57	1.47	18.89	1.64
Eigenmannia virescens	42	-25.40	2.19	10.08	1.59						16	-20.38	1.53	22.86	2.23	6	-17.93	1.34	20.76	1.41
Gymnotus carapo											1	-20.44		24.70						
Harttia longipinna	8	-29.04	1.09	8.74	1.59															
Harttia torrenticola	13	-27.81	1.35	9.83	1.51															
Hemigrammus marginatus						4	-23.79	1.55	9.21	1.36	1	-19.64		21.39		6	-21.80	1.38	16.16	1.08

Hisonotus sp.	9	-28.49	1.53	9.89	1.04															
Homodiaetus sp.	2	-23.65	0.72	13.40	0.26															
Hoplosternum littorale	1	-21.48		10.34							4	-20.14	0.42	18.87	4.44					
Hypostomus auroguttatus	8	-22.99	3.65	11.32	0.72															
Hypostomus commersoni											3	-22.12	0.14	25.58	1.73					
Hypostomus francisci	31	-25.18	3.00	10.61	1.07	3	-20.13	2.35	8.56	1.76	17	-17.85	1.49	24.95	1.15	15	-16.89	1.12	21.81	1.62
Hypostomus lima	3	-26.32	0.40	10.61	0.23	15	-18.89	1.36	9.35	1.20						7	-18.73	1.98	19.79	1.72
Hypostomus macrops	1	-21.99		11.77							7	-22.33	1.84	28.14	1.21					
Hypostomus margaritifer	3	-26.23	1.99	8.97	1.11											2	-15.57	0.29	22.51	0.41
Hypostomus sp.	5	-25.02	3.8434	10.621	2.7477															
Knodus moenkhausii						5	-20.46	0.90	9.59	0.48	5	-20.23	1.31	25.56	1.33					
Lepidocharax burnsi	54	-24.43	1.53	10.56	1.13	10	-21.96	0.91	8.48	0.92										
Leporellus vittatus																6	-17.97	1.56	23.46	0.85
Leporinus amblyrhynchus	1	-24.38		9.19																
Leporinus marcgravii	8	-26.28	0.61	8.86	0.88															
Leporinus taeniatus	17	-25.05	2.06	10.46	1.70						3	-22.91	0.93	27.79	1.06					
Lophiosilurus alexandri																1	-18.00		21.44	
Megaleporinus obtusidens	1	-25.07		11.22							2	-20.26	1.58	24.77	2.41					
Megaleporinus reinhardti	7	-25.25	1.40	8.75	0.46						4	-19.14	0.67	18.52	4.93	5	-20.33	0.93	18.28	2.22
Moenkhausia costae											7	-21.63	2.41	22.30	1.59	2	-17.50	0.22	23.66	3.74
Myleus micans	3	-19.32	4.88	18.30	7.43						1	-19.98		20.71		6	-17.73	0.80	19.60	1.27
Oreochromis niloticus											8	-19.45	1.88	20.02	1.49	9	-16.91	3.26	16.60	1.89
Orthospinus franciscensis											1	-19.18		21.42		3	-24.46	3.41	12.92	2.69
Pachyurus francisci											1	-16.95		24.96		2	-15.87	1.70	22.72	0.26
Pareiorharphis mutuca	3	-31.19	3.32	6.19	0.51															
Phalloceros uai	15	-23.68	2.35	9.99	1.53	8	-18.69	2.42	10.31	1.32	12	-18.73	1.39	21.64	3.34	18	-15.14	1.94	19.80	1.80
Phenacorhamdia tenebrosa	5	-25.05	0.93	10.25	0.38															
Piabarcus stramineus	21	-23.77	1.75	12.01	3.67						5	-20.53	1.91	22.46	1.40	15	-17.92	2.23	19.28	2.52
Piabina argentea	54	-23.90	2.09	10.49	1.36	8	-20.00	1.58	8.96	1.13	10	-22.23	3.84	29.13	2.00	20	-17.31	1.79	22.19	2.92
Pimelodus fur	10	-23.10	3.20	13.59	3.77						2	-19.71	1.28	20.50	4.03	5	-19.10	1.15	21.04	1.71

Pimelodus maculatus											2	-19.81	0.52	22.80	1.48	1	-21.60		16.87	
Pimelodus pohli											12	-20.00	2.52	21.25	2.91	7	-18.86	2.13	20.98	2.10
Poecilia reticulata	15	-24.69	2.01	9.59	1.56	6	-17.15	0.81	9.96	1.44	11	-19.13	3.13	25.01	4.52	5	-19.26	0.61	15.23	0.36
Prochilodus costatus	2	-29.60	0.33	10.71	0.09															
Pygocentrus piraya											3	-19.10	1.86	20.85	3.29	7	-18.17	1.65	22.87	1.54
Rhamdia quelen	5	-25.59	1.37	11.19	0.82	11	-20.82	0.46	9.88	0.96										
Rhinelepis aspera											1	-19.88		22.48						
Roeboides xenodon											3	-19.73	3.12	21.83	3.01	1	-17.16		25.22	
Salminus franciscanus	1	-25.12		13.62							2	-29.19	1.95	14.70	0.81	2	-25.64	1.88	13.63	0.25
Schizodon knerii											1	-19.92		21.44		2	-16.39	0.17	18.31	3.69
Serrapinnus heterodon						7	-22.41	3.47	8.26	0.71	6	-18.66	1.15	22.73	0.54	1	-16.01		23.60	
Serrapinnus piaba	5	-24.59	0.69	9.36	0.75											5	-20.98	2.08	14.11	0.95
Serrasalmus brandtii											17	-19.69	1.43	22.03	1.52	10	-18.21	1.21	22.29	2.35
Stegophilus insidiosus																5	-16.16	0.52	23.72	0.37
Steindachnerina elegans	42	-24.89	3.62	8.47	1.93	2	-19.01	0.09	8.55	0.62	8	-20.01	2.27	21.84	2.44					
Synbranchus marmoratus																1	-20.97		18.54	
Tetragonopterus chalceus											2	-21.13	3.16	21.76	0.39	2	-23.37	4.42	13.31	3.81
Trachelyopterus galeatus											3	-21.49	0.87	21.68	2.63	2	-19.28	0.57	17.63	0.27
Triportheus guentheri											14	-20.65	1.78	21.49	1.54	15	-18.19	2.90	21.17	2.94

Supplementary material Tab. 3. MixSIAR summary statistics - Proportion of each resource assimilated by the piscivorous species *Hoplias intermedius*

MixSIAR results *Hoplias intermedius*									
Control									
Resources	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Algae	0.07	0.059	0.002	0.005	0.024	0.055	0.103	0.185	0.217
Grasses	0.069	0.039	0.005	0.009	0.038	0.067	0.097	0.138	0.152
Riparian vegetation	0.013	0.012	0	0.001	0.004	0.009	0.018	0.037	0.045
Periphyton	0.04	0.039	0.001	0.002	0.013	0.028	0.055	0.118	0.145
Fish	0.807	0.057	0.682	0.705	0.773	0.813	0.848	0.89	0.903
Upper RV									
Resources	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Algae	0.24	0.157	0.012	0.022	0.114	0.219	0.342	0.531	0.583
Grasses	0.131	0.084	0.009	0.015	0.068	0.118	0.18	0.29	0.328
Riparian vegetation	0.138	0.092	0.008	0.016	0.066	0.127	0.194	0.312	0.355
Periphyton	0.247	0.161	0.013	0.025	0.118	0.228	0.353	0.546	0.601
Fish	0.244	0.153	0.015	0.027	0.119	0.228	0.346	0.52	0.577
Middle RV									
Resources	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Algae	0.153	0.069	0.034	0.049	0.107	0.147	0.193	0.273	0.312
Grasses	0.059	0.042	0.002	0.004	0.025	0.053	0.088	0.138	0.152
Riparian vegetation	0.042	0.033	0.002	0.003	0.015	0.035	0.06	0.106	0.121
Periphyton	0.239	0.164	0.006	0.016	0.103	0.22	0.347	0.548	0.598
Fish	0.463	0.174	0.081	0.143	0.349	0.483	0.601	0.713	0.742
Sewage	0.044	0.033	0.002	0.003	0.018	0.036	0.064	0.107	0.12
Lower RV									
Resources	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Algae	0.07	0.053	0.003	0.006	0.029	0.059	0.099	0.17	0.2
Grasses	0.048	0.048	0.001	0.002	0.013	0.032	0.066	0.147	0.179
Riparian vegetation	0.039	0.039	0.001	0.002	0.011	0.026	0.053	0.117	0.146
Periphyton	0.099	0.097	0.003	0.005	0.028	0.07	0.14	0.298	0.36
Fish	0.719	0.151	0.355	0.417	0.637	0.753	0.833	0.901	0.915
Sewage	0.026	0.028	0.001	0.001	0.007	0.016	0.033	0.083	0.107

Supplementary material Tab. 4. MixSIAR summary statistics - Proportion of each resource assimilated by the detritivorous species *Hypostomus francisci*

MixSIAR results *Hypostomus francisci*									
				Control					
Resources	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Algae	0.855	0.109	0.59	0.674	0.816	0.881	0.924	0.964	0.973
Grasses	0.023	0.022	0.001	0.001	0.007	0.017	0.033	0.068	0.082
Riparian vegetation	0.02	0.019	0.001	0.001	0.006	0.014	0.028	0.057	0.07
Periphyton	0.102	0.11	0.002	0.005	0.031	0.07	0.136	0.292	0.381
				Upper RV					
Resources	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Algae	0.364	0.188	0.033	0.056	0.22	0.366	0.504	0.668	0.709
Grasses	0.218	0.126	0.019	0.036	0.124	0.207	0.298	0.447	0.507
Riparian vegetation	0.126	0.1	0.005	0.009	0.05	0.104	0.175	0.327	0.384
Periphyton	0.292	0.191	0.016	0.027	0.136	0.269	0.419	0.643	0.708
				Middle RV					
Resources	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Algae	0.526	0.161	0.25	0.283	0.402	0.514	0.637	0.816	0.852
Grasses	0.021	0.02	0.001	0.001	0.007	0.015	0.03	0.061	0.074
Riparian vegetation	0.023	0.021	0.001	0.001	0.007	0.017	0.033	0.066	0.079
Periphyton	0.413	0.177	0.047	0.085	0.289	0.429	0.549	0.673	0.705
Sewage	0.017	0.017	0	0.001	0.005	0.013	0.025	0.05	0.06
				Lower RV					
Resources	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Algae	0.075	0.065	0.002	0.004	0.024	0.058	0.108	0.202	0.24
Grasses	0.513	0.233	0.013	0.026	0.418	0.563	0.678	0.81	0.845
Riparian vegetation	0.115	0.083	0.004	0.009	0.048	0.1	0.167	0.273	0.309
Periphyton	0.246	0.23	0.006	0.01	0.071	0.171	0.346	0.754	0.823
Sewage	0.052	0.051	0.002	0.003	0.015	0.037	0.071	0.152	0.187