

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

KARINE ORLANDI BONATO



**COMPOSIÇÃO DA ICTIOFAUNA, ANÁLISE ESTOMACAL E
ISOTÓPICA DE ESPÉCIES DE SILURIFORMES E DE CHARACIDAE,
EM RIACHOS SUBTROPICAIS, BRASIL**

PORTO ALEGRE

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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutor em Biologia Animal.

Área de Concentração: Biodiversidade.

Orientadora: Prof^a Dr^a Clarice Bernhardt Fialho

PORTO ALEGRE
2016

Composição da Ictiofauna, Análise Estomacal e Isotópica em Espécies de Siluriformes e de
Characidae em Riachos Subtropicais, Brasil

Karine Orlandi Bonato

Aprovada em 31 de março de 2016.

Dra. Rosilene Luciana Delariva - UNIOESTE

Dra. Lúcia Ribeiro Rodrigues - UFRGS

Dr. Marco Aurélio Azevedo – Fundação Zoobotânica do RS

*A minha mãe Márcia, meus irmãos
Gustavo e Letícia, e meu tio
Marcelo, sem eles eu nada seria.*

Adoro reticências... Aqueles três pontinhos intermitentes que insistem em dizer que nada está fechado, que nada acabou, que algo sempre está por vir! A vida se faz assim! Nada pronto, nada definido. Tudo sempre em construção. Tudo ainda por se dizer... Nascendo... Brotando... Sublimando... O que seria de nós sem a expectativa da continuação?

Nilson Furtado

AGRADECIMENTOS

A Deus, por ter me dado saúde e coragem nos momentos de dificuldade, por iluminar meu caminho fazendo com que eu conseguisse alcançar mais esse objetivo, por ter-me intuitivo até eu chegar neste laboratório que me acolheu tão bem.

A minha mãe Márcia, você é amor e dedicação. Eu agradeço todos os dias por ter você ao meu lado, por você sempre me apoiar e incentivar. Por ter assumido o compromisso de me ajudar a cada coleta, por tratar como filhos, os amigos que iam me ajudar, por cada pé-de-moleque que você fez para alegrar a galera, pelos doces de pêra para adoçar os professores. Você é meu exemplo e meu porto seguro!!

A meus irmãos, Gustavo e Letícia, eu também agradeço todos os dias por ter vocês ao meu lado. Obrigada pelo apoio e compreensão, pelas inúmeras visitas feitas que sempre me alegraram e até mesmo pelas ajudas nos trabalhos de campo (a nena me estragou dois macacões!!). Agradeço também aos meus cunhados Juliana e Gabriel (é aqui Karine??).

A meu tio Marcelo “Celo”, por ter me apoiado desde o início desta jornada. Percorreu comigo quilômetros até acharmos os pontos ideais para minhas coletas, por tua persistência, nós chegamos ao Rio dos Caixões (o mais bonito e que trouxe ótimos resultados pra minha pesquisa). Obrigada pelo teu interesse e pelo teu comprometimento.

A meus tios Selvino e Goreti, que me insentivaram muito no início desta jornada. E a todos os demais integrantes da minha enorme família, dos meus amigos de Curitiba, de Maringá e os que estão espalhados por esse mundão e que sempre torceram por mim. A minha amiga, psicoterapeuta Vania, que sempre me acolheu, me acalmou, me transmitiu luz e serenidade em todos os momentos. Ao meu psiquiatra E. Cechin, que ajudou a enfretar meus medos mais do que normais na etapa final desse processo.

A minha orientadora Profa. Dra. Clarice Bernhardt Fialho, por toda confiança que depositou em mim desde o dia que aceitou me orientar sem nem me conhecer. Obrigada por todo conhecimento que você me passou, pelas horas de conversa em sua sala, pelas boas e grandes gargalhadas e por me apoiar e incentivar sempre.

A CAPES pelas bolsas de doutorado e doutorado sanduíche concedidas.

Ao professor Dr. Luís Roberto Malabarba, por abrir as portas do laboratório de Ictiologia pra mim, por toda confiança em mim depositada, por todo o conhecimento,

sempre foi uma avalanche de aprendizagem (embora às vezes eu triasse o material bem rápido pro senhor não achar outra espécie... ahhh e os *Bryconamericus ecai*!!!). Obrigada por sempre manter a porta da sua sala aberta para tirar dúvidas, batermos um papo, pegar a chave do carro e por ter me deixado jogar fora todas aquelas tranqueiras.

A professora Dra. Maria Claudia Malabarba (popularmente conhecida como MC), MC, o final deste meu trajeto não poderia ter recebido reforço melhor, agradeço pelos ensinamentos na molecular (aquele dia foi divertido), pelos nossos papos infundáveis, por todas as vezes que você me fez rir e não foram poucas as vezes!!

A todo o pessoal do laboratório de Ictiologia que não mediram esforços para me ajudar nas coletas. Tudo ficou mais fácil e divertido com vocês, além de toda experiência e conhecimento que compartilhamos em campo, ficaram gravadas na memória muitas boas histórias. Agradeço também, ao seu Ercílio, nosso anjo da guarda em campo, pois ele era o homem do “liga/desliga” da pesca elétrica e aos demais amigos Adalberto e Bruna que prontamente atenderam meu pedido de ajuda quando precisei.

Minhas BESTS:

Alice: Alice cara de alpiste, obrigada por ser essa amiga tão sensível e meiga, ingenuamente sempre caía nas minhas brincadeiras. Obrigada por você atender meus pedidos de ajuda, por todas as conversas, de cunho pessoal e profissional, que tivemos. Você foi a única que comigo conseguiu fazer quatro pontos de coleta em um dia!!

Caroline (Carol - Fiona): As gurias da terra vermelha tem sangue nos olhos!! Obrigadaaaaa fiona, foram tantas coisas, eu te agradeço por ser minha amiga, meu anjo da guarda, por ter me ajudado desde o começo fazendo as etiquetas, pelas coletas, por cuidar das minhas plantas, do meu “ap” e de mim quando eu precisei! Agradeço-te, pelas conversas dentro do Agronomia, por escutar todos os meus desabafos e por ser minha grande companheira de limpeza e organização do laboratório.

Juliana: Obrigada por você ter ficado ao meu lado nesses quatro anos, por todas as vezes que você me ajudou, por todas as tranças que você fez no meu cabelo, pelas boas conversas, pelas idas ao shopping atras daquele sapato, pelos cineminhas, pelos segredos que nunca permaneceram segredo por muito tempo (a gente não aguenta guardar uma coisa engraçada só pra gente)!!

Priscilla: O melhor pão de queijo é ela que faz!! Obrigada guria por me acompanhar na coleta mais longa, por ficar horas comigo identificando *Astyanax*, por me apresentar ao mundo da molecular, por topar ser minha parceira profissional, e que essa parceria só nos traga mais e mais frutos, que nos leve muito além! Agradeço-te pela parceria nas comilanças, nos shows, nos papos de moda, maquiagens e afins!!

Ao Juliano, nos primeiros meses de laboratório eu disse que a parceria com ele ia render, e rendeu mesmo, uma notinha, mas rendeu. Obrigada Juliano por tudo que você me ensinou, pela paciência quando eu não via a diferença entre as espécies, pelo bom papo que sempre rendeu boas ideias, pelas gargalhadas que serão lembradas para sempre.

A Laísa, por todas as conversas sobre biologia alimentar, a troca de conhecimento e por ter me ajudado muito com as análises estatísticas.

Ao Rafael, por todas as conversas bacanas que tivemos na bancada, pelas visitas na sala 111, pra saber se estava tudo bem, pelo otimismo e incentivo de sempre.

Ao Renato, que além de ajudar em campo (na verdade muito pouco, devido à pior dor de garganta da vida dele), sempre atendeu aos meus chamados para ajudar com o programa R, o que nos rendia boas discussões sobre ecologia de peixes.

Ao professor Dr. Fernando Gertum Becker por fornecer todo o material da pesca elétrica para minhas coletas.

Ao Valerí, por muitas vezes nos “arrancar gargalhadas” com suas inusitadas teorias. Também sou grata aos motoristas da UFRGS, principalmente ao seu Luís, que sempre nos levou com muita segurança as coletas, sempre estava disposto a nos ajudar e nos tratava como filhos.

A todos os demais colegas de laboratório, e até mesmo aqueles que já não estão mais conosco, mas que estiveram presentes nas inúmeras etapas deste trabalho: Aline, Júlia, Dario, Júnior, Amanda C., Amanda, Laura, Aline, Natália, João, Andrei, Ingrid e Clayton. Aquela amiga doida que entrou pra chacoalhar a 111, Fernanda, você foi muito importante na etapa final dessa minha jornada!

A minha primeira co-orientanda Érika, você optou por trilhar outros caminhos, mas foi uma ótima IC, aprendi muito com você durante este período e tenho certeza que teu futuro será brilhante.

Ao pessoal da Auburn University, principalmente ao professor Dr. Jonathan Armbruster, por ter me aceito em seu laboratório para meu período de doutorado

sanduíche, e que no momento em que perdi minhas amostras, me ofereceu um belo plano B.

Ao Edward, que tanto me ajudou desde o meu primeiro dia em Auburn, riu das minhas trapalhadas, se esforçou para compreender meu inglês. Não tenho palavras para agradecer todo o conhecimento que você compartilhou comigo e agora estamos colhendo os frutos desta parceria.

A grande amiga que fiz em Auburn, Raise, guria de nome complicado, mas que tornou aqueles cinco meses muito mais divertidos, foram tantas conversas, chimas, desabafos, lágrimas, aventuras, corridas com sacolas de mercado na mão, só tenho a dizer: nós sobrevivemos! Também agradeço aos demais amigos que fiz lá: Lucas, a “nene” Alinne, Hilton, Thomas J., Thomas A. e Luana.

Ao meu amigo e vizinho Leandro (Lele), por todas as boas conversas que tivemos, por todo incentivo que você me deu desde o começo, por cuidar das minhas plantinhas e por todas as vezes que você me emprestou o chuveiro!!

A minha amiga Janquieli e Douglas, pelas conversas com muitos desabafos ao longo deste período.

A minha “miga” Maria Isabel, Marcio, Anita e Lia, que retornaram à minha vida para me trazer mais alegrias na etapa final deste trabalho.

A minha grande amiga pessoal e profissional Jislaine, agora com o adicional da Maria Júlia. Você está comigo desde o início Ji, eu te agradeço imensamente por sempre atender meus pedidos de socorro nas análises, por poder confiar em você, por todo conhecimento que você compartilha comigo.

A minha sempre professora, mas acima de tudo amiga Rosilene, obrigada por sempre ter uma palavra, uma ideia para dar, pelas discussões científicas.

A todas as pessoas que direta ou indiretamente contribuíram para a realização de mais esta etapa da minha vida.

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RESUMO GERAL

O objetivo deste estudo foi determinar a composição da ictiofauna de riachos da sub-bacia do Alto Jacuí, região centro-norte do Rio Grande do Sul, bem como descrever e compreender as relações que permeiam a biologia alimentar de espécies de Siluriformes e de Characidae nestes mesmos riachos. As coletas foram realizadas bimensalmente de julho de 2012 a junho de 2013, através da técnica de pesca elétrica em 10 riachos. Um total de 13.247 indivíduos foram coletados, pertencentes a 42 espécies, 10 famílias e seis ordens, das quais as que mais contribuíram foram Characiformes (36%), Cichliformes (24%) e Siluriformes (14%), com 15, dez e seis espécies respectivamente. Nós reportamos o primeiro registro de *Phalloceros spiloura* para o sistema da Laguna dos Patos e cinco espécies ficaram ao nível de gênero devido ao fato de se tratarem de novas espécies e estão sendo revisadas e descritas. O conteúdo estomacal de 1.948 indivíduos, pertencentes a três espécies (*Heptapterus* sp., *Rhamdia quelen* e *Trichomycterus poikilos*) foram analisados pelo método volumétrico, sendo 59 itens alimentares identificados. Em geral, *Heptapterus* sp. consumiu uma alta proporção de *Aegla* sp., restos de plantas terrestres e Megaloptera; *R. quelen* consumiu peixe e Oligochaeta, seguido de *Aegla* sp.; enquanto a dieta de *T. poikilos* foi baseada em Simuliidae, Ephemeroptera e Trichoptera. A segregação alimentar das espécies, bem como do fator espécie mais classe de tamanho, foram observadas. Os valores de amplitude de nicho foram altos para todas as espécies, e somente foram baixos para os maiores tamanhos de *R. quelen* e *Heptapterus* sp. No geral, as espécies mostraram baixos valores de sobreposição de nicho, e houve uma grande frequência de sobreposição alta e intermediária para as menores classes de tamanho. O modelo nulo confirmou a partição de nicho entre as espécies e uma partilha de recursos quando analisado espécie mais classe de tamanho. Posteriormente, 1.525 estômagos de 11 espécies de Characidae foram analisados. Observou-se uma diferença significativa entre a dieta dessas espécies, com plantas terrestres e aquáticas, restos de insetos terrestres e aquáticos e adultos de Hymenoptera, mostrando-se os itens mais importantes para esta diferenciação. As análises mostraram que os padrões de alimentação estão associados as características morfológicas. A posição sub-terminal da boca se correlacionou com a ingestão de itens bentônicos (insetos aquáticos e matéria orgânica); boca grande permitiu a ingestão de itens mais robustos como *Aegla* sp. e

peixes; espécies com menor variação de dentes ao longo da série externa do prémaxilar consumiram elevada proporção de plantas e insetos terrestres, assim como as espécies que apresentaram menor número de dentes na maxila. O hábito insetívoro aquático foi observado nos indivíduos com números intermediários e altos de cúspides nos dentes do maxilar. Buscando compreender a coexistência de quatro espécies sintópicas de Characidae, nós calculamos a sobreposição de nicho das espécies por período amostral e para avaliar a importância destes itens alimentares como fontes de nutrientes nós usamos análises de isótopos de carbono (^{13}C) e nitrogênio (^{14}N) para estimar a assimilação relativa. Todos os Characidae consumiram grandes proporções de plantas e insetos aquáticos. As espécies apresentaram baixa sobreposição na maioria dos períodos amostrados. Também, houve uma alta correspondência entre a análise do conteúdo estomacal e o resultado da análise isotópica. *Astyanax xiru* e *Astyanax procerus* assimilaram grandes frações de invertebrados terrestres e plantas aquáticas. *Bryconamericus iheringi* assimilou principalmente invertebrados aquáticos e secundariamente algas, enquanto *Bryconamericus* sp. assimilou principalmente invertebrados aquáticos e terrestres. Assim, nós demonstramos que as espécies congênicas têm dietas mais similares, mas que estas quatro espécies de Characidae coexistem por partição de recursos, e que a assimilação de nutrientes dessas espécies coincide com o consumo relativo dos itens alimentares ingeridos.

Palavras-chaves: sistema Laguna dos Patos; dieta; partição de recursos; morfologia da boca e dentes; isótopos estáveis.

ABSTRACT

The objective of this study was to determine the composition of fishes of Alto Jacuí sub-basin streams, north-central region of Rio Grande do Sul, as well as describe and understand the relationships that permeate the feeding biology of species of Siluriformes and Characidae in these same streams. Samples were collected bimonthly from July 2012 to June 2013, through electrofishing technique in 10 streams. A total of 13,247 individuals were collected, belonging to 42 species, 10 families and six orders, of which the most contributed, were Characiformes (36%), Cichliformes (24%) and Siluriformes (14%), with 15, ten and six species, respectively. We report the first record of *Phalloceros spiloura* to the Laguna dos Patos system and five species were to genus level due to the fact they refer to new species and are being reviewed and described. The stomach contents of 1,948 individuals belonging to three species (*Heptapterus* sp., *Rhamdia quelen* and *Trichomycterus poikilos*) were analyzed by volumetric method, with 59 identified food items. Overall, *Heptapterus* sp. consumed a high proportion of *Aegla* sp., remains of terrestrial plants and Megaloptera; *R. quelen* consumed fish and Oligochaeta, followed by *Aegla* sp.; while *T. poikilos* diet was based on Simuliidae, Ephemeroptera and Trichoptera. Food segregation of species as well as species plus size class factors, were observed. The niche breadth values were high for all species, and only were low for the larger sizes of *R. quelen* and *Heptapterus* sp. Overall, the species showed low niche overlap values, and there was a high frequency overlay high and intermediate for smaller size classes. The null model confirmed the niche partitioning among species and a sharing of resources when analyzed species plus size class. Subsequently, 1,525 stomachs were analyzed representing 11 species of Characidae. There was a significant difference between the diet of these species, with terrestrial and aquatic plants, terrestrial and aquatic insect remains and adult Hymenoptera, being the most important items for this differentiation. The analysis showed that feeding patterns associated with many morphological features. Sub-terminal mouth positions correlated with the ingestion of benthic items (e.g., aquatic insects and organic matter); large mouths permitted ingestion of robust items such as macrocrustaceans (e.g., *Aegla*) and fish; species that have small teeth variation along the outer row of the premaxilla consumed high proportion of plants and terrestrial insects as well as those species that showed a lower number of teeth on the maxilla. Aquatic insectivory was

observed in those individuals with intermediary and high number of cusps in the maxilla teeth. To understand the coexistence of four syntopic Characidae species we calculated niche overlap of species for each sampling period, and to evaluate the importance of these food items as nutrient sources, we used carbon (^{13}C) and nitrogen (^{14}N) isotopes analyses to estimate relative assimilation. All characids consumed large proportions of plant material and aquatic insects. The species showed low dietary overlap throughout most of the sampling period. There was high correspondence between the gut content analyses and isotope mixing model estimates. *Astyanax xiru* and *Astyanax procerus* assimilated large fractions of terrestrial invertebrates and aquatic plants. *Bryconamericus iheringi* assimilated primarily aquatic invertebrates and secondarily algae, whereas *Bryconamericus* sp. assimilated primarily aquatic and terrestrial invertebrates. Therefore, we demonstrate that the congeneric species have more similar diets, but that these four Characidae species coexist by resource partitioning, and that they assimilate nutrients agree with the relative consumption of food items.

Key words: Laguna dos Patos system; diet; resource partitioning; mouth and teeth morphology teeth; stable isotopes.

INTRODUÇÃO GERAL

Os peixes são considerados o grupo mais diverso entre os vertebrados (Lowe-MacConnell, 1999), com uma estimativa de riqueza de 33.200 espécies (Froese & Pauly, 2015). A estimativa atual para a fauna de peixes na região Neotropical é cerca de 6.000 a 8.000 espécies, perfazendo um total de 13% da biodiversidade de vertebrados dos ecossistemas aquáticos do mundo, com as águas continentais brasileiras apresentando 21% da biodiversidade global existente (Schaefer, 1998; Reis *et al.*, 2003; Agostinho *et al.*, 2005).

O Brasil possui as maiores redes hidrográficas do mundo (Abell *et al.*, 2010), no entanto, muitas bacias e sub-bacias brasileiras ainda não foram amostradas (Agostinho *et al.*, 2005) ou possuem poucas informações a respeito de sua ictiofauna, principalmente quando tratado os médios e pequenos corpos d'água, como os riachos (Castro, 1999; Esteves & Aranha, 1999). Segundo Langeani *et al.* (2007), os riachos são, sem dúvida, os ambientes que apresentam o maior número de novas espécies a serem descobertas. Porém, a pequena dimensão dos riachos e ambientes de cabeceira, torna esses locais mais suscetíveis à ação antrópica, podendo assim, sofrer grandes alterações em suas populações, levando até ao desaparecimento de espécies mais sensíveis (Galves *et al.*, 2009). Toda esta situação acaba dificultando a compreensão dos processos ecológicos, biológicos, biogeográficos e também de amostragem das espécies de peixes existentes (Barletta *et al.*, 2010).

Embora a situação venha mudando nas últimas duas décadas, com o aumento de estudos em riachos, ainda há várias lacunas em algumas regiões, como por exemplo, no estado do Rio Grande do Sul. A maioria dos estudos nesse estado refere-se a rios de grande porte ou lagoas e áreas de estuário (Fialho *et al.*, 1998; Garcia & Vieira, 2001; Bastos, 2002; Garcia *et al.*, 2003; Giora, 2004; Majolo, 2005; Maltchik *et al.*, 2005; Petry & Schulz, 2006; Garcia *et al.*, 2006; Dufech & Fialho, 2006; Ribeiro & Köhler, 2007; Dufech & Fialho, 2009; Flores-Lopes *et al.*, 2010; Saccol-Pereira & Fialho, 2010). Para riachos, destacam-se Tagliani (1994), que estudou a ecologia da assembleia de três riachos na planície costeira do Estado e Becker (2002), que estudou a distribuição e abundância de peixes de corredeira e suas relações com características do habitat, a fisiografia da bacia e a posição espacial do riacho em vários riachos de Mata Atlântica, na bacia do rio Maquiné;

Bozzeti & Schulz (2004), que desenvolveram um índice de integridade biótica baseado na assembleia de peixes para riachos subtropicais, sendo o estudo realizado em cinco riachos na bacia do rio Gravataí e Sinos; Vilella *et al.* (2004), que estudaram a relação entre as variáveis ambientais e a megafauna aquática do riacho Carvão pertencente à bacia hidrográfica do Maquiné; Hirschmann (2009), que estudou a composição e estrutura da assembleia em três arroios da sub-bacia do rio Forqueta, na parte inferior da bacia hidrográfica Taquari-Antas; Silva (2009), que analisou a convergência ecomorfológica entre comunidades de peixes em riachos no alto rio Uruguai e bacia do Mampituba; Winkler-Sosinski *et al.* (2009), que descreveram a estruturação da assembleia de peixes sob a influência da introdução de espécies exóticas em três riachos da bacia do rio Silveira, que é um afluente da parte superior do rio Uruguai; Costa & Schulz (2010), utilizaram a comunidade de peixes como indicador da integridade biótica de riachos na bacia do Rio dos Sinos; e Volcan *et al.* (2011), que apresentaram informações sobre a distribuição de duas espécies anuais, *Austrolebias cyaneus* nos riachos Francisquinho, Capivari e Dom Marcos localizados na bacia do rio Jacuí e *Austrolebias juanlangi*, na bacia do rio Jaguarão.

Observa-se, com esses trabalhos, que existem poucas informações a respeito de levantamentos e estudos da ictiofauna de riachos para a bacia do rio Jacuí, sem nenhum estudo para a região superior desta bacia, denominada sub-bacia do Alto Jacuí. Os trabalhos que citam esta região são os de Malabarba (1989), que apresenta uma lista de espécies de peixes de água doce presente no sistema da laguna dos Patos, citando espécies encontradas no rio Jacuí e seus afluentes, e de Alves & Fontoura (2009), que identificaram o padrão de distribuição de peixes migradores da bacia hidrográfica do rio Jacuí, a partir de entrevistas, coleções, literatura e EIA-RIMAs desenvolvidos na região de estudo. Há ainda alguns trabalhos de revisão e descrição de novas espécies que têm sua distribuição na bacia ou em suas drenagens (Ottoni & Cheffe, 2009; Menezes & Ribeiro, 2010; Carvalho & Reis, 2011).

Ressalta-se que, para se compreender os mecanismos ecológicos que ocorrem nesses ambientes pouco explorados, deve-se utilizar inúmeras ferramentas como o levantamento ictiofaunístico e a análise da biologia alimentar das espécies encontradas, levando-se em consideração os fatores que podem influenciar tanto a assembleia quanto sua dieta.

Os riachos são ambientes altamente variáveis com porções de corredeiras rochosas até poções e pequenos remansos, mostrando assim, uma grande heterogeneidade de habitats (Winemiller *et al.*, 2008). Há muito se vem utilizando o estudo da dieta das espécies de peixes como instrumento principal para o conhecimento da dinâmica dos ecossistemas aquáticos, pois a partir destas análises pode-se aprofundar o conhecimento sobre a biologia das espécies, definir hábitos alimentares e determinar relações nas cadeias tróficas (Zavala-Camin, 1996; Polis & Winemiller, 1996; Alves *et al.*, 2011). Os peixes de riachos e de rios de grande porte, na região neotropical, apresentam um padrão alimentar baseado em recursos alóctones, apresentando uma alta plasticidade trófica (Lowe-McConnell, 1999; Abelha *et al.*, 2001; Casatti, 2002; Brandão-Gonçalves *et al.*, 2010; Mazzoni *et al.*, 2010; Tófoli *et al.*, 2010).

A capacidade de explorar variados recursos alimentares, também está relacionada com a grande riqueza de padrões morfológicos verificados para esta fauna. Isso permite que as diferentes espécies ocupem os mesmos riachos, mas explorem o habitat de maneiras distintas, seja por características morfológicas internas, externas ou comportamentais. Esses mecanismos possibilitam evitar a competição e promover a coexistência (Pianka, 1974; Hesthagen *et al.*, 2004; Townsend *et al.*, 2006; Rezende *et al.*, 2013). Quanto mais complexas são as mudanças evolutivas dos caracteres morfológicos, que resultam em mudança de padrões alimentares, mais se pode afirmar que isso segue no sentido de maior especialização (Kotrschal, 1989). Alguns autores ressaltam o poder da morfologia para delimitar grupos tróficos e compreender, por exemplo, a estrutura trófica da comunidade (Wiens & Rotenberry, 1980; Pouilly *et al.*, 2003; Mise *et al.*, 2013). Neste contexto, ao longo das últimas décadas, vários estudos demonstram forte correlação entre a ecologia e morfologia (Chao & Musick, 1977; Yamaoka, 1982; Lauder & Clark, 1984; Balon *et al.*, 1986; Motta, 1989; Winemiller *et al.*, 1995; Santos *et al.*, 2011; Dourado *et al.*, 2015).

A coexistência, no entanto, também pode ser favorecida pela abundância dos itens alimentares ofertados (Keppeler *et al.*, 2014). Assim, a disponibilidade de recursos alimentares também é um dos fatores que permitem a ocorrência de elevados números de espécies morfológicamente semelhantes no mesmo habitat (Silva *et al.*, 2012), o que é notório em riachos neotropicais (Brazil-Souza *et al.*, 2009; Cetra *et al.*, 2011; Cruz *et al.*, 2013).

Com o intuito de compreender esses processos biológicos e ecológicos supracitados, além da análise dos itens estomacais pelo método convencional, pode-se utilizar a análise de isótopos estáveis. Essa técnica vem sendo largamente utilizada nas últimas três décadas como complemento para obtenção de informações sobre teias tróficas, absorção de itens alimentares, origem dos itens ingeridos e especialização trófica (Phillips & Gregg, 2003; Fry, 2006; Layman *et al.*, 2012; Cucherousset & Villéger, 2015). Os isótopos mais utilizados para estudos ecológicos são os isótopos de carbono e nitrogênio (Anderson & Cabana, 2007; Connolly & Waltham, 2015). Valores de $\delta^{13}\text{C}$ são usados para traçar a origem dos diferentes recursos alimentares (DeNiro & Epstein, 1978; Vander Zanden *et al.*, 1997), enquanto os valores de $\delta^{15}\text{N}$ são utilizados para avaliar a posição trófica das espécies, considerando o fracionamento ao longo da cadeia alimentar (Minagawa & Wada, 1984; Post, 2002). Desta forma, o uso desta técnica pode trazer grande contribuição na compreensão da ecologia alimentar de peixes de riachos.

Diante da lacuna de dados e estudos em riachos para o Rio Grande do Sul, que ainda possui muitas bacias pouco exploradas cientificamente, como a sub-bacia do Alto Jacuí, que carece de informações de espécies ocorrentes e padrões biológicos e ecológicos, o objetivo deste estudo foi determinar a composição da assembleia de dez riachos da sub-bacia do Alto Jacuí, região centro-norte do Rio Grande do Sul, bem como descrever e compreender as relações que permeiam a biologia alimentar de espécies de Siluriformes e Characidae nestes ambientes. Para isso, este estudo foi dividido em cinco capítulos:

- O primeiro capítulo teve por objetivo inventariar a ictiofauna dos 10 riachos amostrados, e fornecer maiores informações sobre as espécies ocorrentes na sub-bacia do Alto Jacuí localizado no noroeste do Rio Grande do Sul;

- O segundo capítulo foi uma consequência do inventário realizado no primeiro capítulo. Assim, neste estudo foi realizado um novo registro com a extensão da distribuição de *Phalloceros spiloura* Lucinda, 2008, que até então não era registrada no sistema da Laguna dos Patos;

- O terceiro capítulo aborda a descrição e estudo da alimentação de três espécies de Siluriformes, com o objetivo de verificar diferenças na dieta das três espécies e se essas diferenças eram causadas por variações interespecíficas relacionadas com o fator ontogenético pelo qual os indivíduos das espécies passam;

- O quarto capítulo, aborda a descrição e estudo da alimentação de 11 espécies de Characidae, com o objetivo de descrever os hábitos alimentares dessas 11 espécies, verificar a existência de diferenças na dieta das espécies, bem como os itens responsáveis por essa diferenciação, e testar a relação entre a morfologia da boca e dentes e dieta das espécies;

- O quinto capítulo, traz uma abordagem isotópica em relação ao estudo da biologia alimentar de quatro espécies de Characidae no Rio dos Caixões, com o objetivo de avaliar se a assimilação relativa dos itens corresponde ao consumo relativo, como descrito pela análise convencional do conteúdo estomacal, bem como avaliar a existência de partição de recursos entre as espécies de Characidae.

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CAPÍTULO 1

Fishes of Alto Jacuí sub-basin: a poorly studied sub-basin of Northwestern Rio Grande do Sul, Brazil

Artigo publicado na revista científica Check List, volume 12, número 2,
páginas 1867-1875, doi: <http://dx.doi.org/10.15560/12.2.1867>
ISSN 1809-127X, Abril de 2016.

Karine O. Bonato & Clarice B. Fialho

Fishes of Alto Jacuí sub-basin: a poorly studied sub-basin of northwestern Rio Grande do Sul, Brazil

Karine O. Bonato* and Clarice B. Fialho

Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Zoologia, Programa de Pós-Graduação em Biologia Animal, CEP 91501-970, Porto Alegre, RS, Brazil

* Corresponding author. E-mail: kakabio2005@yahoo.com.br

Abstract: The streams in the state of Rio Grande do Sul (mainly in the Alto Jacuí sub-basin belonging to the Laguna dos Patos system) have scarce information about their ichthyofauna. Thereby for providing information about stream species, the purpose of the present study was to inventory the ichthyofauna of the streams of the Alto Jacuí sub-basin, located in northwestern state of Rio Grande do Sul. The samples were taken bimonthly from June 2012 to June 2013 using electrofishing technique in 10 streams. A total of 13,247 specimens were collected belonging to 42 species, 10 families and six orders. We report the occurrence of five new species that have not yet been described by researchers.

Key words: stream; Alto Jacuí sub-basin; Laguna dos Patos system; ichthyofauna inventory

INTRODUCTION

Fish are considered the most diverse group of vertebrates (Lowe-MacConnell 1999), with an estimated richness of 32,900 species (Froese and Pauly 2014). By December 2013, Pelayo-Villamil et al. (2014) had found 14,782 described species of fish that occur only in freshwater. Although there is a lack of a complementary information, current estimates of the ichthyofauna in the Neotropical region are that there are about 6,000 to 8,000 species, totaling 13% of the vertebrate biodiversity in aquatic ecosystems worldwide, with Brazilian continental waters showing 21% of global diversity (Reis et al. 2003; Agostinho et al. 2005).

There is still a lack of knowledge of fish richness, mainly in South America, Africa and Asia, which is due to a lack of sampling and databasing (Pelayo-Villamil et al. 2014). Brazil has the largest river networks in the world (Galves et al. 2009); however, many Brazilian basins and sub-basins have not yet been sampled (Agostinho et al. 2005), or there exists little information about

their fish fauna, especially with medium-sized and small water bodies such as streams (Castro 1999). According to Langeani et al. (2007) streams are the environments that have the highest number of new species still to be discovered. But the small size of streams and headwater environments makes these places more susceptible to anthropogenic action and they may experience significant change in their population structure, leading to the disappearance of the most sensitive species (Galves et al. 2009). This situation makes it difficult to understand ecological, biological and biogeographical processes (Barletta et al. 2010).

Although the situation has been changing in the last two decades with an increase of studies (biological, ecological and systematic studies) in streams, there is a lack of knowledge in some regions such as in southern Brazil. Most studies in the state of Rio Grande do Sul refer to large rivers, lagoons or estuarine areas (Fialho et al. 1998; Garcia and Vieira 2001; Bastos 2002; Garcia et al. 2003; Majolo 2005; Maltchik et al. 2005; Dufech and Fialho 2006; Garcia et al. 2006; Petry and Schulz 2006; Ribeiro and Köhler 2007; Dufech and Fialho 2009; Flores-Lopes et al. 2010; Saccol-Pereira and Fialho 2010). The only stream environment studies from Rio Grande do Sul are: Tagliani (1994), Becker (2002), Bozzeti and Schulz (2004), Vilella et al. (2004), Hirschmann (2009), Silva (2009), Winkler-Sosinski et al. (2009), Costa and Schulz (2010) and Volcan et al. (2011).

Little information are available about sampling and studies of the ichthyofauna in streams for the Jacuí river basin and no studies of the upper region of the basin, called the Alto Jacuí sub-basin. Malabarba (1989) showed a list of freshwater fish present in the Laguna dos Patos system and cited species found in Jacuí River and its tributaries. Alves and Fontoura (2009) identified the distributive pattern of migratory fish of the Jacuí River basin, but the data were obtained through interviews, collections, literature and technical studies (EIA-RIMA

Estudo e Relatório de Impacto Ambiental) developed in the study region. Additionally there are some taxonomic reviews and descriptions of new species that are distributed on this drainage (Ottoni and Cheffe 2009; Menezes and Ribeiro 2010; Carvalho and Reis 2011).

We emphasize that to understand the ecological mechanisms in these little-explored environments we must use many tools, including ichthyofaunal studies. Streams are highly heterogeneous environments (Winemiller et al. 2008) and this allows for the establishment of numerous species of fish. Further, more studies of streams in south Brazil are necessary because some basins are not as well explored as the Alto Jacuí sub-basin. Therefore, the aim of this study is to inventory and provide more information about distribution and species richness of ichthyofauna in the Alto Jacuí sub-basin located in northwestern Rio Grande do Sul.

MATERIALS AND METHODS

Study site

The Alto Jacuí sub-basin belongs to the large Laguna dos Patos system and is located in the state of Rio Grande do Sul in the northwestern Middle Plateau and Central Depression region. The Alto Jacuí has its headwaters located in the municipality of Passo Fundo and occupies an area of 16,062 km² with its rivers flowing into the Lago Guaíba (COAJU 2009). The basin's vegetation consists of Seasonal Deciduous Forest and some areas of

Subtropical Ombrophilous Forest. The economy is based on agriculture (soybeans, corn, wheat and rice) and livestock. The basin is drained by the Jacuí, Jacuí-Mirim, Jacuizinho, Caixões, Ivaí and Soturno rivers (SEMA 2010). The Jacuí River is the main tributary of the basin and it is responsible for 85% of the waters forming the Lago Guaíba (FEPAM 2011).

Thus, this study was conducted in 10 streams (Figures 1 and 2–11) in northwestern Rio Grande do Sul, which corresponds to the Alto Jacuí sub-basin (Table 1). All streams flow into the Jacuí River, which is one of the main tributaries to the Laguna dos Patos system.

Data collection

Fish samples were collected with authorization number 34940 from register number 3196382 from Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). This study was approved by the Ethics Committee on Animal Use of the Universidade Federal do Rio Grande do Sul (permit number 24434) and was conducted in accordance with protocols in their ethical and methodological aspects for the use of fish.

The fish were collected in June, August, October and December 2012; February, April and June 2013. Each sampling event lasted four days. For the sampling, we used electrofishing with three stages of 30 min each, in stretches of 50 m per sampling stream. After sampling, fish were euthanized with 10% eugenol (Vidal et al. 2008; Lucena et al. 2013a), fixed in 10% formalin

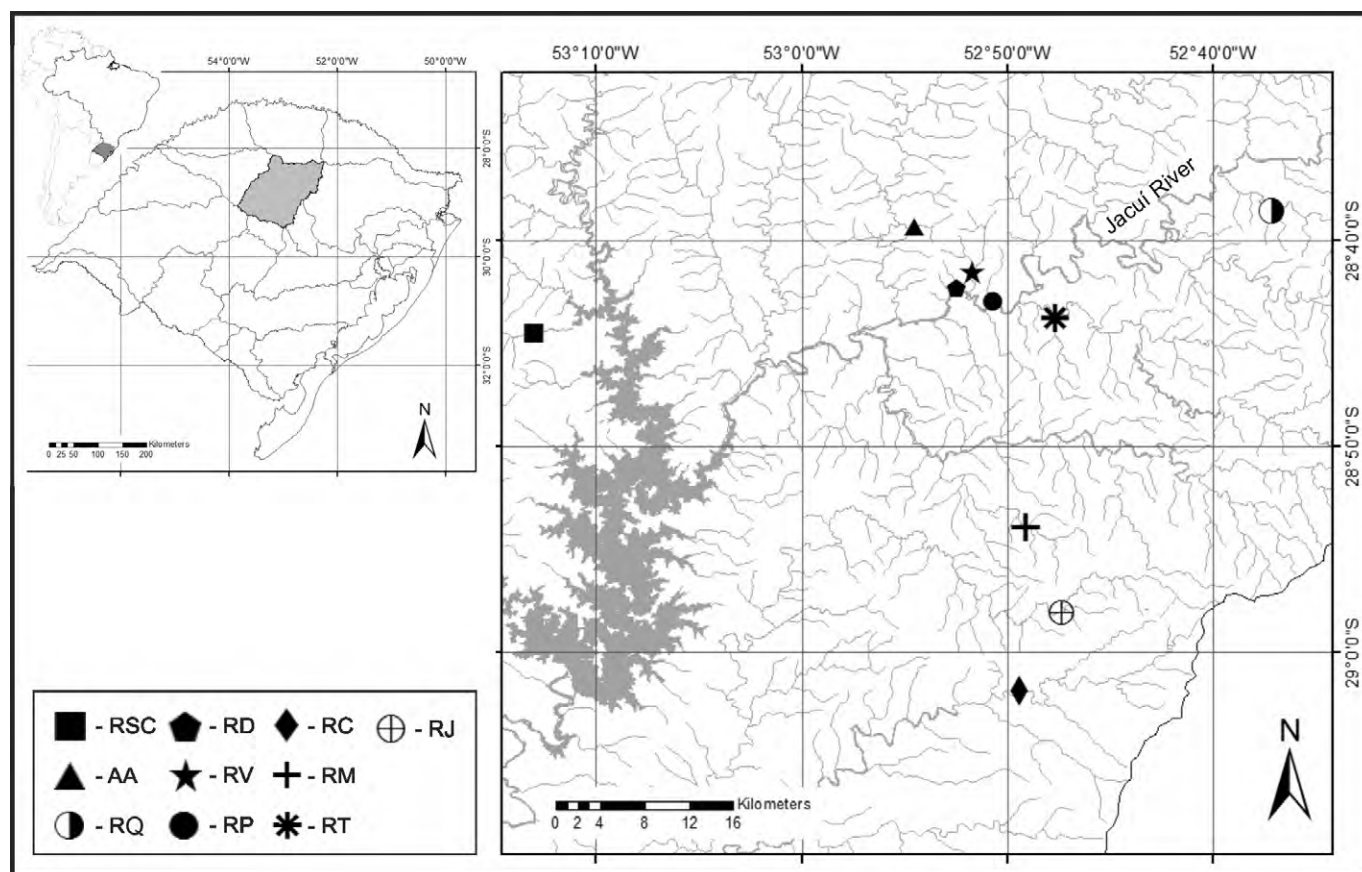


Figure 1. Sampling streams in the Alto Jacuí sub-basin. For stream code see Table 1.

and then transferred to 70% alcohol for conservation. The taxonomic identification was carried out in the laboratory using Rodriguez and Reis (2008), Bertaco and

Lucena (2010), Ferrer and Malabarba (2013), Lucena et al. (2013b), Lucena and Soares (2016) and additional literature cited herein. Classification and nomenclature follows Reis et al. (2003), with additional changes made by Thomaz et al. (2015) for Characidae. The nomenclature for Cichlidae followed the new classification of bony fishes proposed by Betancur et al. (2013) that include this family in the order Cichliformes. The voucher specimens were deposited in the fish collection of the Departamento de Zoologia at Universidade Federal do Rio Grande do Sul (UFRGS; Table 2).



Figures 2–11. General view of the sampled streams in the Alto Jacuí sub-basin, Rio Grande do Sul, Brazil: **2:** AA; **3:** RP; **4:** RD; **5:** RC; **6:** RJ; **7:** RM; **8:** RQ; **9:** RSC; **10:** RT; **11:** RV. For stream code see Table 1.

RESULTS

A total of 13,247 specimens belonging to 42 species, ten families and six orders (Table 2 and Figures 12–32) were collected. The most significant orders were Characiformes (36%), Cichliformes (24%) and Siluriformes (14%), with 15, 10 and six species, respectively. Cyprinodontiformes was represented by two species, and both Gymnotiformes and Synbranchiformes were represented by only one species. The predominant families were Characidae (12 species, 29%), Loricariidae (nine species, 21%), Cichlidae (seven species, 17%) and Heptapteridae (four species, 10%), followed by Crenuchidae, Poeciliidae and Trychomycteridae with two species (5% each). Erythrinidae, Pimelodidae, Gymnotidae and Synbranchidae showed one species, corresponding to 2% each of the total richness.

Five species are identified only to genus level and correspond to undescribed species: *Australoheros* sp. (Rícan and Kullander 2008), *Bryconamericus* sp. b (Silva 1998), *Heptapterus* sp. (Bockmann 1998), *Ituglanis* sp. (J. Ferrer, personal communication) and *Bryconamericus* sp. a which also seem to be a new species but, could not be described because it may just be a variation (as color and body shape) of *Bryconamericus iheringii*. According to Bonato and Ferrer (2013), the individuals of *Phalloceros spiloura* Lucinda, 2008 collected in the Alto Jacuí sub-basin during the present study represent the first record of this species to the Laguna dos Patos system.

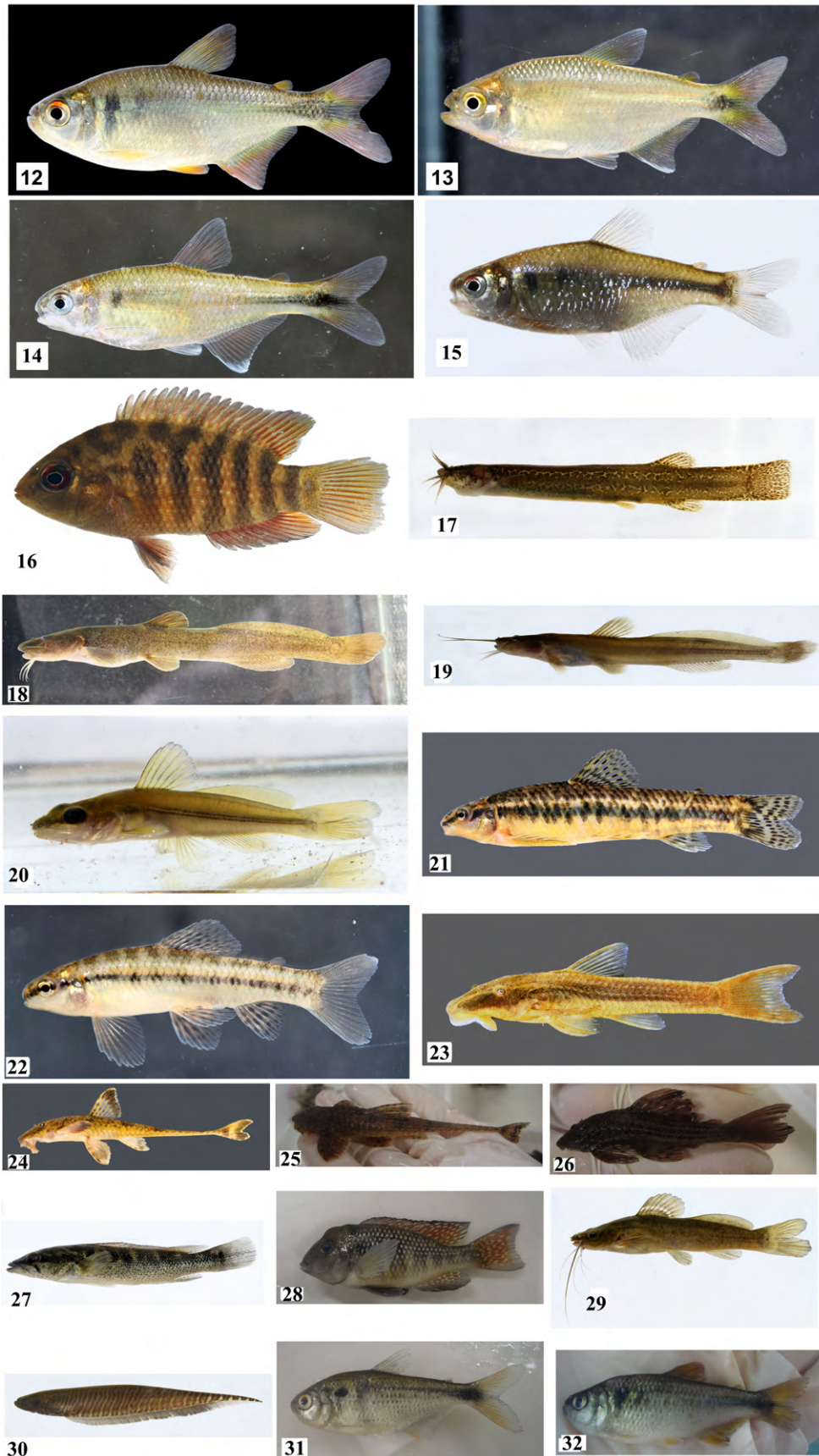
The highest species richness was found in RP, RT, RC and RQ with 28 species, 27 species, 25 species and 21 species, respectively. RM and RSC showed the lowest species richness with only 15 and 14 sample species.

Table 1. Geographic coordinates, elevation and localization of the sampled streams and their respective codes in the Alto Jacuí sub-basin.

Stream	Code	Geographic coordinates	Elevation (m)	Locality
Caixões River	RC	29°01'54.4" S, 052°49'25.1" W	420	Guanxuma, Espumoso
Jacuizinho River	RJ	28°58'02.9" S, 052°47'20.3" W	513	Depósito, Espumoso
Morcego River	RM	28°53'55.0" S, 052°49'05.6" W	461	São Domingos, Espumoso
Turvo River	RT	28°43'47.0" S, 052°47'40.4" W	351	Santo Antônio, Espumoso
Quati River	RQ	28°38'31.8" S, 052°37'07.9" W	439	Mormaço
Santa Clara River	RSC	28°44'30.1" S, 053°13'03.0" W	439	Santa Clara, XV de Novembro
Valoroso Stream	RV	28°41'32.0" S, 052°51'41.5" W	376	Teutônia, Tapera
Divinéia Stream	RD	28°42'16.7" S, 052°52'25.9" W	350	Teutônia, Tapera
Paz Stream	RP	28°42'57.3" S, 052°50'41.7" W	378	Vila Paz, Tapera
Angico Stream	AA	28°39'17.9" S, 052°54'31.1" W	368	São Rafael, Tapera

Table 2. List of fish species collected at each sampled stream in the Alto Jacuí sub-basin. See Table 1 for stream names. Asterisk indicates the endemic species to Laguna dos Patos system.

Taxa	Streams										Voucher (UFRGS)
	RC	RJ	RM	RV	RD	AA	RSC	RQ	RP	RT	
CHARACIFORMES											
Characidae											
<i>Astyanax lacustris</i> (Lütken, 1875)	X			X	X	X		X	X	X	19977
<i>Astyanax laticeps</i> (Cope, 1894)					X		X			X	19327
<i>Astyanax obscurus</i> (Hensel, 1870) *	X						X		X		19329
<i>Astyanax procerus</i> Lucena, Castro & Bertaco, 2013 *	X	X	X	X	X	X	X	X	X	X	19323
<i>Astyanax xiru</i> Lucena, Castro & Bertaco, 2013	X		X		X	X			X	X	19325
<i>Bryconamericus iheringii</i> (Boulenger, 1887)	X	X	X	X	X	X	X	X	X	X	19974
<i>Bryconamericus</i> sp. a	X	X									19975
<i>Bryconamericus</i> sp. b *	X										19980
<i>Diapoma alburnus</i> (Hensel, 1870)	X	X	X					X	X	X	19976
<i>Diapoma dicropotamicus</i> (Malabarba & Weitzman, 2003) *			X					X			19952
<i>Oligosarcus jacuiensis</i> Menezes & Ribeiro, 2010	X	X			X	X	X	X	X	X	19978
<i>Oligosarcus jenynsii</i> (Günther, 1864)				X		X		X	X	X	19979
Crenuchidae											
<i>Characidium orientale</i> Buckup & Reis, 1997	X										19962
<i>Characidium pterostictum</i> Gomes, 1947	X	X	X	X	X	X	X	X	X	X	19973
Erythrinidae											
<i>Hoplias malabaricus</i> (Bloch, 1794)		X	X			X			X	X	19961
SILURIFORMES											
Heptapteridae											
<i>Rhamdia quelen</i> (Quoy & Gaimard, 1824)	X	X	X	X		X	X	X	X	X	19265
<i>Rhamdella eriarcha</i> (Eigenmann & Eigenmann, 1888)	X										19951
<i>Heptapterus mustelinus</i> (Valenciennes, 1835)	X	X					X				19967
<i>Heptapterus</i> sp.	X	X	X	X	X	X	X	X	X	X	19266
Trichomycteridae											
<i>Ituglanis</i> sp.									X		19949
<i>Trichomycterus poikilos</i> Ferrer & Malabarba, 2013 *	X	X	X	X	X	X	X	X	X	X	19267
Loricariidae											
<i>Ancistrus brevipinnis</i> (Regan, 1904)	X	X	X	X	X	X	X	X	X	X	19984
<i>Eurycheilichthys limulus</i> Reis & Schaefer, 1998	X	X	X	X	X	X	X	X	X	X	19985
<i>Hemiancistrus punctulatus</i> Cardoso & Malabarba, 1999 *	X	X		X	X	X		X	X	X	19986
<i>Hisonotus armatus</i> Carvalho, Lehmann, Pereira & Reis, 2008 *	X										19957
<i>Hisonotus brunneus</i> Carvalho & Reis, 2011 *		X						X			19959
<i>Hypostomus commersoni</i> (Valenciennes, 1836)					X				X		19958
<i>Rineloricaria baliola</i> Rodriguez & Reis, 2008		X	X	X	X	X	X	X	X	X	19982
<i>Rineloricaria cadeae</i> (Hensel, 1868) *				X		X		X	X	X	19983
<i>Rineloricaria microlepidogaster</i> (Regan, 1904)	X	X	X								19981
Pimelodidae											
<i>Pimelodus pintado</i> Azpelicueta, Lundberg & Loureiro, 2008									X	X	19960
GYMNOTIFORMES											
Gymnotidae											
<i>Gymnotus</i> aff. <i>carapo</i> Linnaeus, 1758	X							X	X	X	19966
CICHLIFORMES											
Cichlidae											
<i>Australoheros</i> sp.	X	X	X					X		X	19968
<i>Crenicichla lepidota</i> Heckel, 1840				X	X				X	X	19969
<i>Crenicichla punctata</i> Hensel, 1870	X			X	X			X	X	X	19972
<i>Geophagus brasiliensis</i> (Quoy & Gaimard, 1824)				X			X		X	X	19965
<i>Gymnogeophagus gymnogenys</i> (Hensel, 1870)									X	X	19964
<i>Gymnogeophagus labiatus</i> (Hensel, 1870)	X										19954
<i>Gymnogeophagus rhabdotus</i> (Hensel, 1870)								X		X	19955
CYPRINODONTIFORMES											
Poeciliidae											
<i>Phalloceros caudimaculatus</i> (Hensel, 1868)				X		X			X	X	19963
<i>Phalloceros spiloura</i> Lucinda, 2008				X	X	X					17827
SYNBRANCHIFORMES											
Synbranchidae											
<i>Synbranchus marmoratus</i> Bloch, 1795									X		19953
Total of Species (n= 42)	25	18	15	18	17	18	14	21	28	27	



Figures 12–32. Some species of fishes found in Alto Jacuí sub-basin, Rio Grande do Sul. Photos by L.R. Malabarba. **12:** *Astyanax xiru*, **13:** *Astyanax procerus*, **14:** *Bryconamericus* sp. b (UFRGS 17931), **15:** *Bryconamericus iheringii* (UFRGS 17992), **16:** *Australoheros* sp.(UFRGS 17989), **17:** *Trichomycterus poikilos*, **18:** *Heptapterus* sp. (UFRGS 17933), **19:** *Heptapterus mustelinus*, **20:** *Rhamdella eriarcha* (UFRGS 17984), **21:** *Characidium pterostictum* (UFRGS 17826), **22:** *Characidium orientale*, **23:** *Eurycheilichthys limulus* (UFRGS 17986), **24:** *Rineloricaria baliola* (UFRGS 17991), **25:** *Rineloricaria cadeae*, **26:** *Hyposotomus commersoni*, **27:** *Crenicichla punctata* (UFRGS 17990), **28:** *Gymnogeophagus gymnogenys*, **29:** *Rhamdia quelen*, **30:** *Gymnotus* aff. *carapo* (UFRGS 17989), **31:** *Astyanax laticeps*, **32:** *Astyanax obscurus*.

DISCUSSION

According to Pelayo-Villamil et al. (2014) an average of 240.2 species of fishes were described per year in the last ten years worldwide. The five new species uncovered by this inventory (*Australoheros* sp., *Heptapterus* sp., *Bryconamericus* sp. a and b, and *Ituglanis* sp.) support the importance of this type of study. In addition, inventories are important in extending the distributional range of some species, such as *Phalloceros spiloura* that was previously only known from the coastal drainages of states of Rio Grande do Sul and Santa Catarina, Iguaçú and Uruguay river basins, and as part of this study, was found in the Alto Jacuí sub-basin representing a new record for the Laguna dos Patos system (Bonato and Ferrer 2013).

Malabarba (1989) registered 25 species of the 42 species sampled in this study to Laguna dos Patos system. The most recent literature indicates a total of 160 species to the Laguna dos Patos system (Malabarba et al. 2009) including 35 species that were new species and yet not described in 2009. Of these 35 species listed by Malabarba et al. (2009), we have sampled five species that were described in recent years (*Oligosarcus jacuiensis* Menezes & Ribeiro, 2010; *Hisonotus brunneus* Carvalho & Reis, 2011; *Astyanax procerus* Lucena, Castro & Bertaco, 2013; *Astyanax xiru* Lucena, Castro & Bertaco, 2013; *Trychomycterus poikilos* Ferrer & Malabarba, 2013), indicating that a representative amount of the ichthyofauna of the upper Jacuí River was described in recent years. There are no comparable studies for the Alto Jacuí sub-basin. We can only make comparisons with other basins belonging to the Laguna dos Patos system. For stream environments Bozzetti and Schulz (2004) found 57 species in the Gravataí and Sinos sub-basins, Hirschmann (2009) found 55 species in the Forqueta sub-basin (Taquari-Antas basin), and Becker et al. (2013) found 119 species for the Taquari-Antas basin but in respect to the last study, the high number of captured species is likely due to their larger sampling of 519 sites.

The number of species found in this study is lower compared to those cited by other studies mainly because it was conducted in streams from a headwater region and many of the streams (of lower species richness) are first-order. The highest occurrence of the orders Characiformes, Cichliformes and Siluriformes in studies is also well documented for the Laguna dos Patos system and for the Neotropical region (Castro 1999; Garcia et al. 2003; Buckup et al. 2007; Lévêque et al. 2008; Costa and Schulz 2010). Headwater streams do not have an exclusive fish fauna but, species that form populations residing in streams and also that occur in larger bodies of water with different characteristics (Castro 1999). The fish fauna of streams is based on small species and according to Castro (1999) it seems to be the only

general pattern with real diagnostic value for stream environments. In this study, the streams with lower species richness are the first-order streams, which have a habitat of lower complexity as RD, RV and AA streams (see Table 1 for stream codes). This situation is expected in accordance with the River Continuum Theory (Vannote et al. 1980). Thus, the larger streams, with greater width between banks, areas with and without shading, and more heterogeneous environment showed the highest species richness (Ferreira and Casatti 2006; Suárez and Petrere-Junior 2005) as occurred in the RT, RP and RC streams.

Despite the fact that we did not evaluate the degree of anthropic influences in the sampled streams, all streams sampled here showed some kind of human interference. Most streams are very close to agricultural areas with the presence of dairy cattle or pig livestock. In stream RP there was a considerable amount of waste coming from homes and sometimes we found dead animals within the stream. Probably the residents of the region slaughter animals for their own consumption and discard the remains of the animal in the river. However, this stream had considerable marginal vegetation and a heterogeneous environment with changing pools and areas of rapids, which led to the high amount of richness observed.

Due to a lack of data for streams of the studied sub-basin it is difficult to say that the number of species found is representative of the streams belonging to Laguna dos Patos system. The checklist showed 42 species representing 26% of the species mentioned for the Laguna dos Patos system. This study is an important record for the region of the Alto Jacuí sub-basin due to the lack of extensive collecting effort in the region. The expansion of the sampled streams in Jacuí River basin may increase the records of species and information about endemic species.

ACKNOWLEDGEMENTS

We thank the colleagues of the Ichthyology Lab at the Universidade Federal do Rio Grande do Sul for field work; MSc. J. Ferrer, MSc. P.C. Silva for help in identifying the species studied and Dr. L.R. Malabarba to provide the species figures; the Orlandi and Bonato families for help and support in field work; and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Proc. 1104786, to KOB) for financial support. We also thank to Adam J. Taylor for reviewing the English of this manuscript.

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Author contributions: KOB collected data, verified the specimens and wrote the text. CBF wrote the text.

Received: 17 November 2015

Accepted: 17 March 2016

Academic editor: Tiago P. Carvalho

CAPÍTULO 2

**New record and distribution extension of *Phalloceros
spiloura* Lucinda, 2008 (Cyprinodontiformes: Poeciliidae)**

Artigo publicado na revista científica Check List, volume 9, número 6,
páginas 1545-1547, Dezembro de 2013.

Karine O. Bonato & Juliano Ferrer

New record and distribution extension of *Phalloceros spiloura* Lucinda, 2008 (Cyprinodontiformes: Poeciliidae)

Karine Orlandi Bonato* and Juliano Ferrer

Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Zoologia, Avenida Bento Gonçalves 9500, bloco IV, prédio 4343-5. CEP 91501-970. Porto Alegre, RS, Brazil.

* Corresponding author. E-mail: kakabio2005@yahoo.com.br

ABSTRACT: *Phalloceros spiloura* Lucinda, 2008 is known from the coastal drainages of Rio Grande do Sul and Santa Catarina States, Iguaçú and Uruguai river basins. Its geographic distribution is herein extended to a new basin, the Laguna dos Patos system, an isolated costal drainage from Southern Brazil.

The monophyletic genus *Phalloceros* Eigenmann, 1907 comprises 22 species broadly distributed throughout southern and southeastern river basins in South America, being diagnosed by the preopercular canal partially closed and the presence of paired appendix in tip of the third gonopodial ray (Lucinda 2008). This genus was recently revised by Lucinda (2008), who recognized and described 21 new species in addition to *Phalloceros caudimaculatus* (Hensel, 1868).

Phalloceros species are small viviparous poeciliids, being omnivorous, herbivorous, and detritivorous and commonly found in areas with reduced riparian vegetation (Teixeira 1989; Oliveira and Bennemann 2005; Gomiero and Braga 2008; Mazzoni *et al.* 2010; Bonato *et al.* 2012).

Phalloceros spiloura Lucinda, 2008 is diagnosed from its congeners by the following autapomorphies: a rounded spot located on the lower half of the caudal peduncle close to the base of the lowest caudal-fin rays, a patch of dark pigmentation on the last anal-fin ray of females, and the halves of gonopodial paired appendix straight and perpendicular to ray 3 (Lucinda 2008). Furthermore, *Phalloceros spiloura* can be distinguished from its congeners by the gonapophysis of vertebra 14 straight in adult males and the anterior orbital bone present (Lucinda 2008).

The only published recording of its distribution is in the original description of the species, where it was known to occur in the coastal drainages of Rio Grande do Sul and Santa Catarina States, including the Tramandaí, Mampituba, Tubarão, Itajaí-Açu, Itapocu, Cubatão river

basins, and the inland basin of Iguaçú river in Paraná State (Lucinda 2008). In the non-type material of *Phalloceros spiloura* Lucinda (2008) also cited a single record for upper Uruguai river basin, which is not mentioned in the “distribution” section.

In June 2012, 22 specimens of *Phalloceros spiloura* (Figure 1) were captured through electrofishing in two small streams tributaries of rio Jacuí, Laguna dos Patos system (Appendix 1), an isolated coastal drainage in Rio Grande do Sul, Brazil and northeast of Uruguay (Figure 2, red circle). Even with the extensive sampling that resulted in a large number of material deposited in two fish collections from southern Brazil (Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul and Departamento de Zoologia, Universidade Federal do Rio Grande do Sul; UFRGS) previous records of *P. spiloura* in this drainage are not known, at least are not cited in the literature. As well, recent checklists from different portions of Laguna dos Patos system (*e.g.* Malabarba *et al.* 2009; 2013; Luz-Agostinho *et al.* 2010; Carvalho *et al.* 2012; Becker *et al.* 2013), did not reported *Phalloceros spiloura*.

The specimens collected were examined and identified as *Phalloceros spiloura* due to the presence of three autapomorphies cited by Lucinda (2008), namely: the rounded spot located on the lower half of the caudal peduncle close to the base of lowest caudal-fin rays present in all specimens (Figure 1), a patch of dark pigmentation on the last anal-fin rays present in all females, and the halves of gonopodial paired appendix straight and perpendicular



FIGURE 1. *Phalloceros spiloura*, male, 19.5 mm, UFRGS 17827, Divinéia stream, Municipality of Tapera, Rio Grande do Sul State, Brazil.

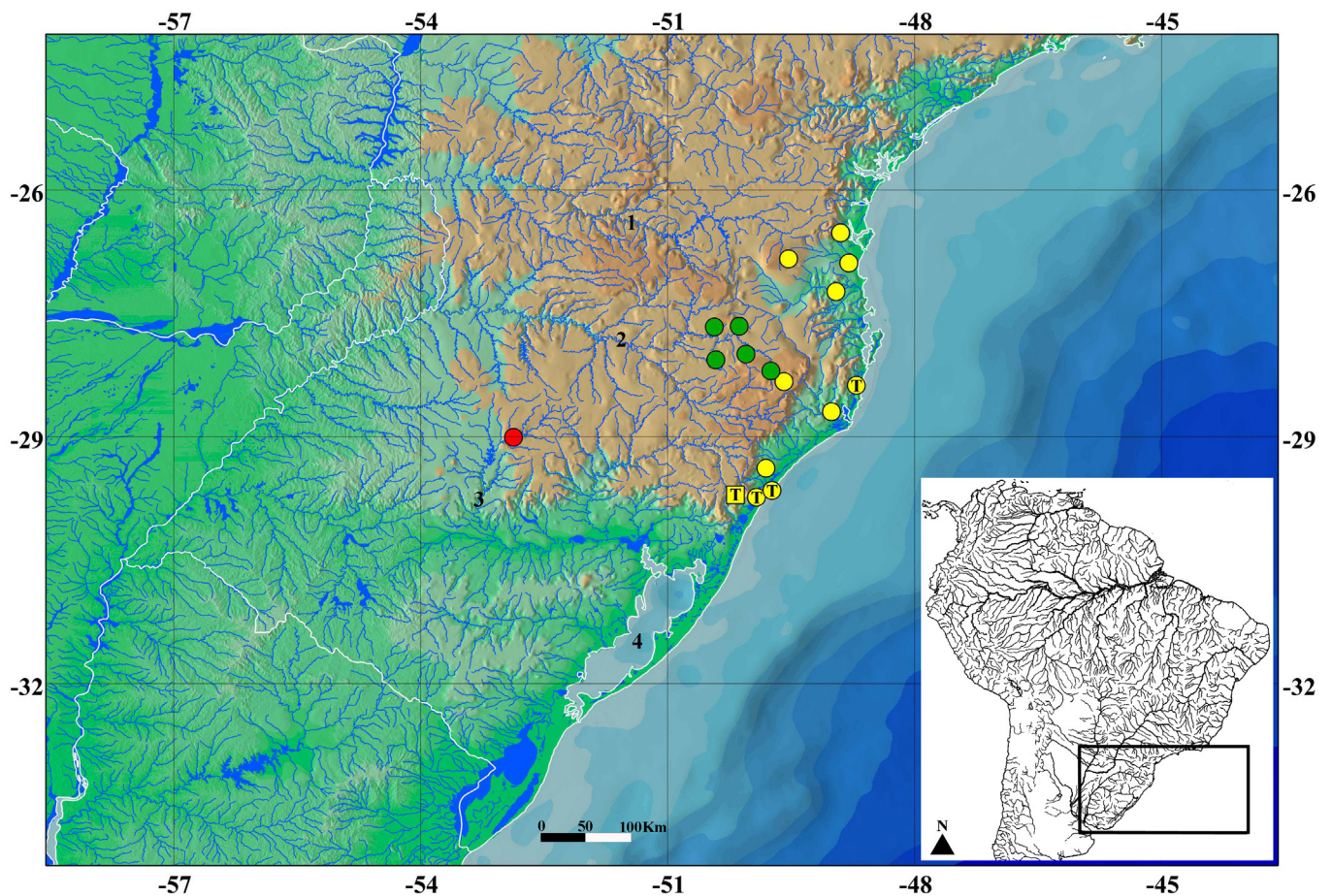


FIGURE 2. *Phalloceros spiloura* currently known distribution. Symbols may represent more than one locality. Yellow circles are localities from types (T) and non-types cited by Lucinda (2008); square represent type locality. Red circle is the new record from Laguna dos Patos system. Green circles are new records from the rio Uruguai basin. Abbreviations: 1, rio Iguaçu; 2, rio Uruguai; 3, rio Jacuá; 4, Laguna dos Patos.

to third gonopodial ray observed in one male cleared and stained (c&s) following the method proposed by Taylor and Van Dyke (1985). Moreover, all examined female specimens exhibited the urogenital papillae curved to right (Figure 3) and the c&s male specimen examined possesses the gonapophysis of vertebra 14 straight, which are additional characters useful in recognizing *Phalloceros spiloura*.

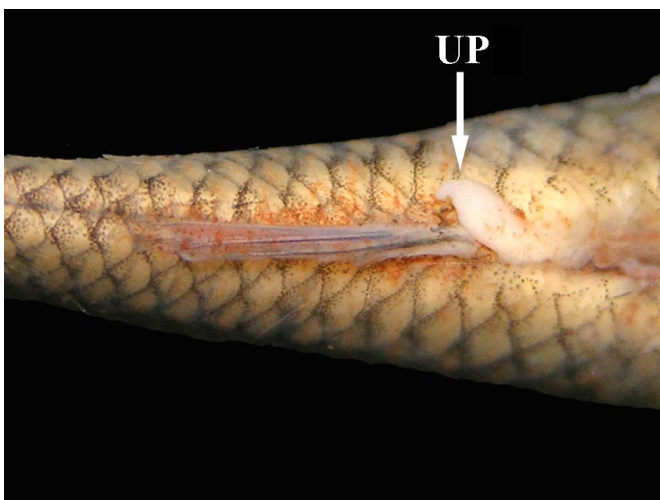


FIGURE 3. Urogenital papilla (UP) right curved of *Phalloceros spiloura*, 33.4 mm, UFRGS 17827, Divinéia stream, Municipality of Tapera, Rio Grande do Sul State, Brazil.

The specimens of *Phalloceros spiloura* were captured syntopically with *P. caudimaculatus* in streams of approximately 2 m wide, 10 to 60 cm depth having margins associated with floating vegetation (Figure 4). Both streams have different microhabitats as pools and rapids and bottom composed by rocky, gravel and mud (fine sediment). Its margins are eroded with little or no riparian vegetation, and surrounded by agricultural areas (mainly soybean). A preliminary analysis of stomach contents according to the volumetric method (Hyslop 1980) found a predominance of aquatic insect larvae (Chironomidae, Ephemeroptera and immature Lepidoptera) (21.30%) and detritus (76.22%).

The presence of *Phalloceros spiloura* in the rio Uruguai basin is also herein confirmed through a revisionary examination of *Phalloceros* specimens housed at UFRGS fish collection (Appendix 1). Besides the unique record cited by Lucinda (2008), nine additional holdings of *Phalloceros* specimens were recognized as *P. spiloura*. Such specimens were collected in rio Canoas and rio Caveiras in the upper portion of the rio Uruguai basin (Appendix 1; Figure 3, green circles).

These new records demonstrate that *Phalloceros spiloura* is widely distributed in the upper reaches of Laguna dos Patos system, Uruguai and Iguaçu river basins as well as in small coastal drainages from Rio Grande do Sul and Santa Catarina States.



FIGURE 4. Angico stream (A) and Divinéia stream (B), Municipality of Tapera, Rio Grande do Sul State, Brazil.

ACKNOWLEDGMENTS: We thank the financial support from Coordenação de Aperfeiçoamento de Pessoal de nível Superior (CAPES, Proc. 1104786, to KOB) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Proc. 142010/2012-0, to JF).

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RECEIVED: August 2013

ACCEPTED: October 2013

PUBLISHED ONLINE: December 2013

EDITORIAL RESPONSIBILITY: Pedro Holanda Carvalho

APPENDIX 1. Material Examined. *Phalloceros spiloura*. All from Brazil. Laguna dos Patos system: Rio Grande do Sul State: UFRGS 17827, 18 (1 female c&s), Municipality of Tapera, Angico stream, Jacuí river basin, 28°39'18" S, 52°54'31" W. Col. K. O. Bonato, J. Ferrer, C. Vogel, L. Cavalheiro, 20/VI/2012; UFRGS 17828, 4 (1 male c&s), Municipality of Tapera, Divinéia stream, Jacuí river basin, 28°42'16" S, 52°52'26" W. Col. K. O. Bonato, J. Ferrer, C. Vogel, L. Cavalheiro, 20/VI/2012; Uruguay river basin: Santa Catarina State: UFRGS 8140, 1, Municipality of Bocaina do Sul, Macacos river, 27°41'25" S, 50°03'07" W, J. Anza, J. Ferrer, M. Azevedo, 19/II/2004; UFRGS 8141, 1, Municipality of Ponte Alta, Águas Pretas river, 27°41'26" S, 50°09'51" W, Col. J. Anza, J. Ferrer, M. Azevedo, 18/XII/2004; UFRGS 8142, 1, Municipality of Palmeira, Ribeirão das Palmeiras stream tributary to Rio dos Índios river, 27°33'53" S, 50°09'58" W, Col. J. Anza, J. Ferrer, M. Azevedo, 19/XII/2004; UFRGS 8143, 2, Municipality of Otacílio Costa, unnamed stream tributary to Águas Pretas river, 27°21'13" S, 50°08'04" W, Col. J. Anza, M. Azevedo, J. Ferrer, 18/XII/2004; UFRGS 8144, 57, Municipality of Rio Rufino, unnamed stream tributary to Canoas river, 27°53'43" S, 49°44'48" W, Col. L. Malabarba, J. Anza, J. Ferrer, G. Neves, 01/X/2004; UFRGS 8145, 4, Municipality of Ponte Alta, Rio dos Cachorros river, 27°21'34" S, 50°25'59" W, Col. J. Anza, J. Ferrer, M. Azevedo, 17/XII/2004; UFRGS 8146, 14, Municipality of Palmeira, swamp on road BR 116, Canoas river basin, 27°35'26" S, 50°07'53" W, Col. J. Anza, M. Azevedo, J. Ferrer, 19/XII/2004; UFRGS 8250, 7, Municipality of Ponte Alta, Ponte Alta river, 27°28'34" S, 50°22'45" W, Col. J. Anza, M. Azevedo, J. Ferrer, 17/XII/2005; UFRGS 8251, 3, Municipality of Lages, Amolafaca river, 27°45'40" S, 50°25'03" W. Col. J. Anza, M. Azevedo, J. Ferrer, 19/XII/2004.

CAPÍTULO 3

Evidence of niche partitioning under ontogenetic influences among three morphologically similar Siluriformes in small subtropical streams

Artigo publicado na revista científica PLoS One, volume 9, número 10, doi:
10.1371/journal.pone.0110999, Outubro de 2014.

Karine O. Bonato & Clarice B. Fialho



Evidence of Niche Partitioning under Ontogenetic Influences among Three Morphologically Similar Siluriformes in Small Subtropical Streams

Karine Orlandi Bonato*, Clarice Bernhardt Fialho

Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Zoologia, Programa de Pós-Graduação em Biologia Animal, CEP 91501-970, Porto Alegre, Rio Grande do Sul, Brazil

Abstract

Ontogenetic influences in patterns of niche breadth and feeding overlap were investigated in three species of Siluriformes (*Heptapterus* sp., *Rhamdia quelen* and *Trichomycterus poikilos*) aiming at understanding the species coexistence. Samplings were conducted bimonthly by electrofishing technique from June/2012 to June/2013 in ten streams of the northwestern state of Rio Grande do Sul, Brazil. The stomach contents of 1,948 individuals were analyzed by volumetric method, with 59 food items identified. In general *Heptapterus* sp. consumed a high proportion of *Aegla* sp., terrestrial plant remains and Megaloptera; *R. quelen* consumed fish, and Oligochaeta, followed by *Aegla* sp.; while the diet of *T. poikilos* was based on Simuliidae, Ephemeroptera and Trichoptera. Specie segregation was observed in the NMDS. Through PERMANOVA analysis feeding differences among species, and between a combination of species plus size classes were observed. IndVal showed which items were indicators of these differences. Niche breadth values were high for all species. The niche breadth values were low only for the larger size of *R. quelen* and *Heptapterus* sp. while *T. poikilos* values were more similar. Overall the species were a low feeding overlap values. The higher frequency of high feeding overlap was observed for interaction between *Heptapterus* sp. and *T. poikilos*. The null model confirmed the niche partitioning between the species. The higher frequency of high and intermediate feeding overlap values were reported to smaller size classes. The null model showed resource sharing between the species/size class. Therefore, overall species showed a resource partitioning because of the use of occasional items. However, these species share resources mainly in the early ontogenetic stages until the emphasized change of morphological characteristics leading to trophic niche expansion and the apparent segregation observed.

Citation: Bonato KO, Fialho CB (2014) Evidence of Niche Partitioning under Ontogenetic Influences among Three Morphologically Similar Siluriformes in Small Subtropical Streams. PLoS ONE 9(10): e110999. doi:10.1371/journal.pone.0110999

Editor: Ben J. Mans, Onderstepoort Veterinary Institute, South Africa

Received: May 23, 2014; **Accepted:** September 26, 2014; **Published:** October 23, 2014

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: This research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Proc. 1104786 to the first author. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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

* Email: kakabio2005@yahoo.com.br

Introduction

According to the competitive exclusion principle [1], species cannot coexist because competing for resources could lead to the exclusion of one or the other species or a population decrease. For coexistence to be possible, a niche differentiation would be required [2,3].

This niche differentiation is known as resource partitioning that, according to [4], is any substantial difference in resource use between coexisting species. This resource partitioning would be the maintainer mechanisms of species biodiversity [5]. However, there is a neutral theory whose precept is that the diversity of species is the result of stochastic factors such as ecology drift, speciation, selection and dispersal acting at local and regional scale [6,7]. Thus, in this theory is assumed that species have similar ecological needs and there is not a competitively superior species [6,8]. Hubbell's neutral model thus assumes that limited dispersal, rather than niche specialization, is the main explanation for spatial structure across ecological communities [9].

Other classic affirmation that tries to explain the involvement of interspecific competition in coevolution and complements the niche theory is “the ghost of competition past” [10]. Partitioning of resources can also be a consequence of competition past because in the past the species had a negative interaction and, during the evolutionary process, eventually developed distinct morphological and physiological characteristics that segregated it [2,3]. Thereby, differences in trophic morphology, distinct habitat use, activity periods and tactical capture all minimize the effect of overlap [11,12,13].

The partitioning of resources may be influenced by factors such as time, space and ontogeny [4,14,15]. Therefore, these factors should be considered when we want to understand the mechanisms of fish species coexistence in streams [16]. Studies have demonstrated that the ontogenetic process may also be involved in resource partitioning by coexisting species [17,18,19]. Thus in order to segregation occur, differentiations in item consumption are necessary and these differences are related to size-dependent morphology, physiology and behavior [20,21,22]. The differences between sizes and stages of life are not restricted only to the

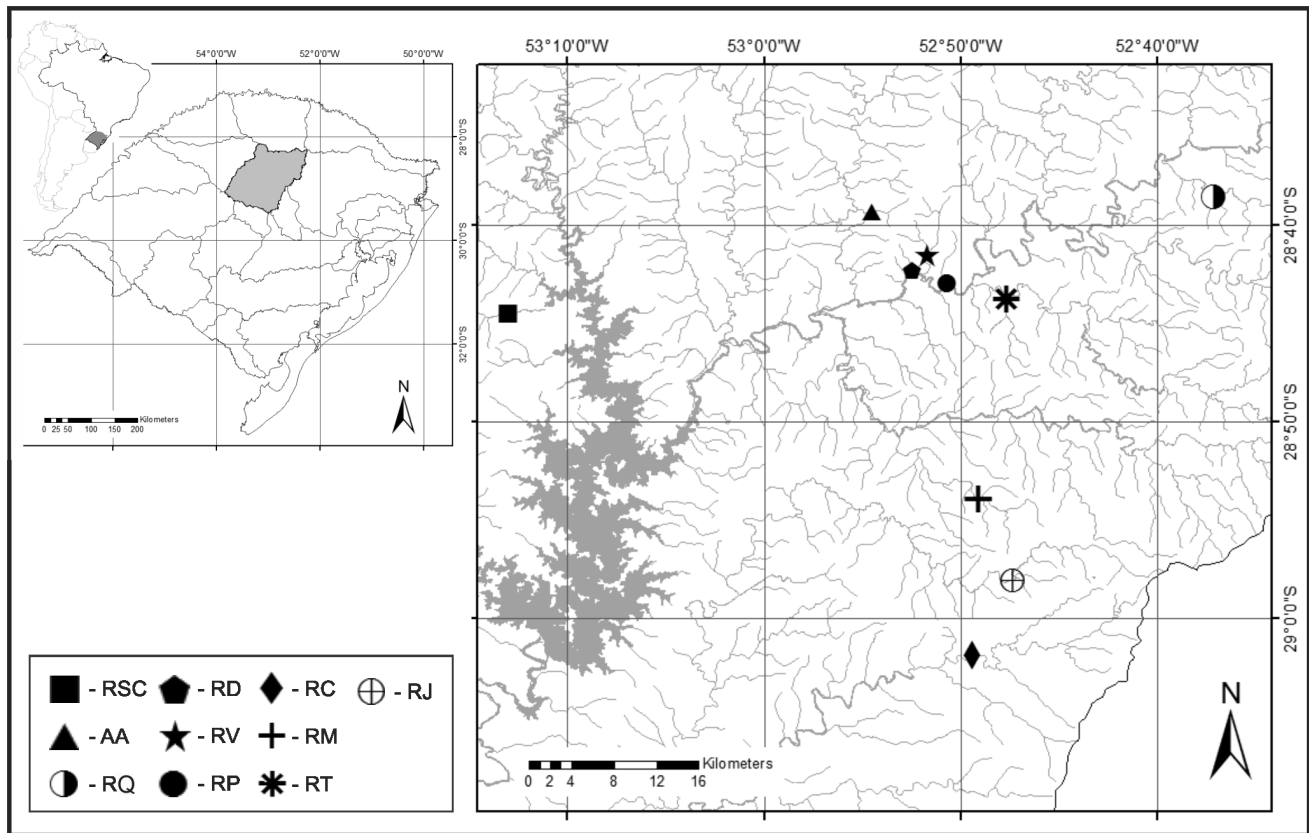


Figure 1. Sampling streams. Sampling streams in the Alto Jacuí sub-basin, state of Rio Grande do Sul, Brazil. For stream code see Material and Methods.

doi:10.1371/journal.pone.0110999.g001

features mentioned above, but also to energy requirement. This can lead individuals mainly the adults to use larger prey to maximize their energy intake [23].

Phylogenetically related species sharing morphological features especially in some stage of ontogenetic development. These related species tend to show ecological similarities [24] and, can be great instrument for studying the influence of ontogenetic factor in the coexistence of species. There is still a lack of studies that address a broader community context using three or more species [4,19].

The Siluriformes are considered one of the most basal groups of fish and have 2,867 freshwater species and they have a diverse morphology, usually with benthic habits [25,26]. Within the family Heptapteridae there are numerous genus including *Rhamdia* and *Heptapterus* [25,26]. *Rhamdia quelen* is an opportunistic benthic species that can live in the midst of rocks, in deep wells, forages at night and near the margins searching for larger benthic macroinvertebrates and small fish [27,28,29,30]. Species of the genus *Heptapterus* also live in crevices formed in rocky bottoms and in rapids, occupying low and medium depths, and are benthic [25]. The *Heptapterus* sp. used in this work is a new specie that is being described. *Trichomycterus poikilos* belongs to Trichomycteridae family and is a recently described species [31]. The species of *Trichomycterus* genus can feed during the day or at night revolving the substrate surface. They usually inhabit small water courses, strong currents and clear waters. Most of the species of the genus are *Trichomycterus* reported to live in streams with high circulation and bottom mainly composed of small stones and well oxygenated streams [32,33,34]. By having a relatively thin, elongated and depressed body they can explore the small spaces among rocks

very well [35]. These three catfish have body elongate, trunk roughly cylindrical, head depressed, mouth wide and subterminal with small teeth distributed in three rows in the premaxilla and lower jaw (*T. poikilos*) or teeth in both jaws (*Heptapterus* sp.) or terminal mouth with small teeth inserted in dentigerous plates (*R. quelen*); they have maxilla and nasal barbels [32,36,37]. Therefore due to phylogenetic relationship, similar morphological and living habits are good tools for the study of coexistence of species.

Thus, this study was developed to test the hypothesis that three similar species of Siluriformes have a feed segregation influenced by ontogenetic process that allows the coexistence of species. Specifically, we tested the existence of dietary differences among three species and these differences are caused by interspecific variations related to the ontogenetic process by which individuals of these species spend. Therefore these variation combined with the use of non-limiting resources allows the coexistence of these species.

Materials and Methods

Ethics statement

Fish samples were collected with authorization n° 34940 from register n° 3196382 from Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). IBAMA is the federal agency responsible for the environment in Brazil, and as such is responsible for emitting licenses to collect fish specimens according to Law N° 7,735 of February 22, 1989, in Brazil. This study was approved by Ethics Committee on Animal Use of the Universidade Federal do Rio Grande do Sul (Permit Number:

Table 1. Taxonomic position of three species in the Alto Jacuí sub-basin.

Order/Family/Specie	Specie Code	Specie/Size Class Code	Size Classes (cm)
SILURIFORMES			
Heptapteridae			
<i>Heptapterus</i> sp.	H	H1	1 = 1.16–5.99;
		H2	2 = 6.00–10.99;
		H3	3 = 11.00–14.99;
		H4	4 = 15.00–19.74;
		H5	5 = 20.00–28.00
<i>Rhamdia quelen</i> (Quoy & Gaimard, 1824)	R	R1	1 = 1.42–4.99;
		R2	2 = 5.00–9.90;
		R3	3 = 10.00–15.90;
		R4	4 = 16.00–27.50
Trichomycteridae			
<i>Trichomycterus poikilos</i> Ferrer & Malabarba, 2013	T	T1	1 = 1.26–2.99;
		T2	2 = 3.00–4.99;
		T3	3 = 5.00–6.99;
		T4	4 = 7.00–8.93

Specie code of the three species studied, specie/size class code by each size class and size variation for each size class.
doi:10.1371/journal.pone.0110999.t001

24434) and was conducted in accordance with protocols in their ethical and methodological aspects, for the use of fish. The committee follows National and International Norms and Guidelines, especially law 11.794 from November 8th, 2008 which disciplines the raising and use of animals for educational and research purposes. The study data presented herein are available as Table S1 and S2 files (e.g. stomach content analysis).

Sampling

The study was conducted in ten streams (Figure 1) in northwest Rio Grande do Sul, which correspond to the Alto Jacuí sub-basin – Caixões River (RC) (S29° 01' 54.4"/W 52° 49' 25.1"); Jacuizinho River (RJ) (S 28° 58' 02.9"/W 52° 47' 20.3"); Morcego River (RM) (S 28° 53' 55.0"/W 52° 49' 05.6"); Turvo River (RT) (S 28° 43' 47.0"/W 52° 47' 40.4"), Valoroso Stream (RV) (S 28°

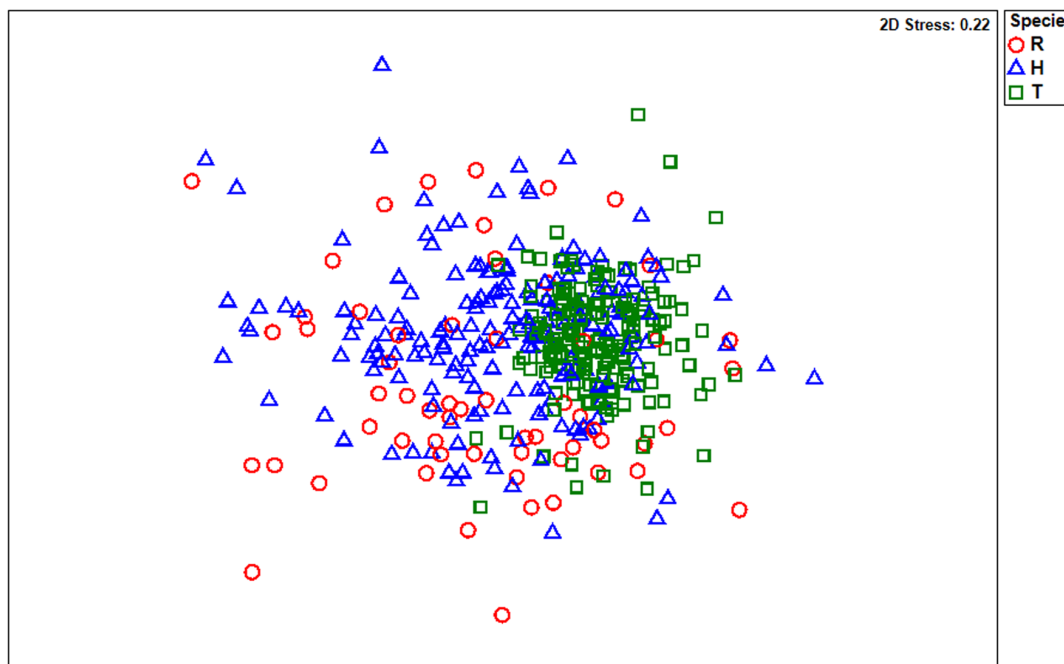


Figure 2. Two-dimensional plot of three fish species analyzed in Alto Jacuí sub-basin. The ordination resulting of the NMDS of the three species. For specie code see Table 1.
doi:10.1371/journal.pone.0110999.g002

Table 2. Permutational Multivariate Analysis of Variance results of each sampling stream in the Alto Jacuí sub-basin.

Stream	Specie Factor		Specie/Size Class Factor	
	F	p	F	p
RC	9.17	0.00	7.27	0.00
RJ	5.55	0.00	9.67	0.00
RM	8.33	0.00	3.00	0.01
RV	13.06	0.00	9.52	0.00
RD	16.19	0.00	9.36	0.00
AA	10.52	0.00	16.32	0.00
RSC	21.96	0.00	7.44	0.01
RQ	3.32	0.01	9.64	0.00
RP	21.78	0.00	13.34	0.00
RT	15.50	0.00	27.19	0.00

F and p values of specie and specie/size class factors. For stream code see Material and Methods.
doi:10.1371/journal.pone.0110999.t002

41° 32.0"/W 52° 51' 41.5"); Divinéia Stream (RD) (S 28° 42' 16.7"/W 52° 52' 25.9"); Arroio Angico (AA) (S 28° 39' 17.9"/W 52° 54' 31.1"); Paz Stream (RP) (S 28° 42' 57.3"/W 52° 50' 41.7"); Santa Clara River (RSC) (S 28° 44' 30.1"/W 53° 13' 03.0"); and Quati River (RQ) (S 28° 38' 31.8"/W 52° 37' 07.9"). All streams flow into the Jacuí River, which is one of the main tributaries to the Laguna dos Patos system and the streams are considered headwater streams.

Fish were collected in June, August, October and December 2012; February, April and June 2013. Each sampling event lasted four days. For the sampling, we used electrofishing with three stages of 30 min each, in stretches of 50 m per sampling stream. After sampling, fish were euthanized with 10% eugenol [38,39], fixed in 10% formalin and then transferred to 70% alcohol for conservation. Fish were identified in the laboratory with identification keys and voucher specimens were deposited in the fish collection of the Departamento de Zoologia at the Universidade Federal do Rio Grande do Sul (*Rhamdia quelen* - UFRGS 19263, *Heptapterus* sp. - UFRGS 19266, *Trichomycterus poikilos* - UFRGS 19267). Individuals were dissected for stomach removal, which were then conserved in 70% alcohol.

Diet Composition and Factors-Influence

Stomach contents were identified under optical and stereoscopic microscopes set to the lowest taxonomic level possible. Food items were identified using the identification keys [40,41,42] for invertebrates. They were then quantified according to the volumetric method (*i.e.*, the total volume of a food item consumed by the fish population given as a percentage of the total volume of all stomach contents [43]) using graduated test tubes and a glass counting plate [44].

The Nonmetric Multidimensional Scaling (NMDS) were used to verify the possible diet differentiation between the species. The NMDS consisting of an ordination technique that shows the distance between objects considered in accordance with a previously calculated dissimilarity matrix (Bray-Curtis) [45,46]. After we used Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations [47] based on a dissimilarity Bray-Curtis matrix [46] to confirm statistically the existence of the difference between the species' diets and the influence of the factor specie plus size classes within each stream. This analysis was based on data volume. If a difference was found,

the Indicator Value Index (IndVal) [48] was applied to get the food item indicators for each species and specie plus size classes also within each stream. The IndVal is based on a comparison of relative abundances and relative frequencies of the factors that are being tested in different groups selected *a priori* [49]. The greater specificity and fidelity of an item to a particular group, the greater the value of the indicator; and this method proves robust to differences within the group, sample sizes, and differences in abundance between the groups [50]. All analyzes were performed using R software [51] with the Vegan package [52].

Trophic Niche Breadth and Feeding Overlap

Inferences about the level of specialization of overall species and they along their ontogenetic process were used the Levin's measure [53] that was calculated for each species in each stream and to specie-class size in each stream using volume data. The Hurlbert's formula [54] was applied to standardize the trophic niche measure (ranging from 0 to 1).

We used the Pianka's index [55] to estimate the species and size classes' feeding overlap within each of the spatial-temporal units (seven samples months in each site). Overlap values range from zero to one, where zero indicates overlap absence and one indicates complete overlap. We established three categories to improve understanding of the overlap results, high overlap is given by overlap values >0.6, intermediate values overlap between 0.4–0.6 and low overlap values <0.4 [56]. Only groups represented by five or more individuals were used to comparisons or groups that contained similar numbers of individuals. To evaluate the significance of Pianka's index [57] we used the null model with RA3 algorithm [58]. The null model performed 1,000 Monte Carlo randomizations for we can compare the created patterns (mean niche overlap values for all group pairs) with the those in the real data. In this model mean overlap values that are significantly lower than those expected by chance might indicate food partitioning, however values higher than those expected by chance might indicate food sharing [59]. Both the null model analysis and feeding overlap were computed using EcoSim 7.0 [57].

Table 3. Indicator Value (IndVal), *p* value and Frequency of food items consumed by three species analyzed, discriminated among species.

Stream	Indicator Item	Specie	IndVal	<i>p</i>	Frequency
RC	Aquatic Lepidoptera larvae	R	0.704	0.05	2
	Bivalve	R	0.427	0.02	2
	Hymenoptera	R	0.380	0.02	2
	Ephemeroptera	H	0.679	0.03	193
RJ	Coleoptera larvae	R	0.645	0.00	10
	Adult Coleoptera	R	0.577	0.01	4
	Terrestrial insect remains	R	0.525	0.00	6
	Gastropoda	R	0.413	0.00	2
	Testae Amoebae	R	0.385	0.00	12
	Simuliidae	T	0.581	0.02	48
RM	Oligochaeta	R	0.816	0.00	8
	Coleoptera larvae	R	0.729	0.05	6
	Terrestrial plant remains	R	0.586	0.04	30
	Gastropoda	R	0.578	0.02	23
	Testae Amoebae	R	0.350	0.04	12
RV	Terrestrial plant remains	R	0.760	0.00	11
	Terrestrial insect remains	R	0.662	0.03	4
	Diptera Pupae	R	0.429	0.01	1
	<i>Aegla</i> sp.	H	0.974	0.01	7
	Megaloptera	H	0.964	0.02	3
RD	Oligochaeta	R	0.933	0.02	4
	<i>Aegla</i> sp.	R	0.918	0.00	12
	Terrestrial plant remains	R	0.855	0.00	24
	Odonata nymph	R	0.828	0.00	9
	Adult Coleoptera	R	0.822	0.00	5
	Terrestrial insect remains	R	0.765	0.03	13
	Trichoptera	R	0.727	0.00	67
	Animal organic matter	R	0.622	0.04	6
	Coleoptera larvae	R	0.595	0.00	8
	Hymenoptera	R	0.517	0.03	3
	Scale	R	0.504	0.03	5
	Simuliidae	H	0.701	0.00	85
	Chironomidae	H	0.575	0.00	92
	AA	Oligochaeta	R	0.994	0.00
Animal organic matter		R	0.800	0.02	2
Aquatic insects remains		R	0.555	0.02	9
Adult Coleoptera		H	0.511	0.05	4
Simuliidae		T	0.711	0.02	106
RSC	Fish	R	0.999	0.02	1
	Oligochaeta	R	0.970	0.01	2
	Terrestrial Lepidoptera larvae	R	0.902	0.02	1
	Terrestrial insect remains	R	0.798	0.03	5
	Ephemeroptera	H	0.853	0.01	60
	Odonata nymph	H	0.813	0.04	3
	Sediment	H	0.380	0.03	11
RQ	Terrestrial insect remains	R	0.627	0.00	3
	Scale	R	0.518	0.00	7
	Adult Coleoptera	R	0.494	0.02	3
	Animal organic matter	R	0.481	0.02	2

Table 3. Cont.

Stream	Indicator Item	Specie	IndVal	<i>p</i>	Frequency
	Terrestrial plant remains	R	0.408	0.04	14
	Gastropoda	R	0.390	0.00	5
RP	Fish	R	0.988	0.04	4
	Terrestrial insect remains	R	0.474	0.00	5
RT	Nematoide	R	0.795	0.03	9
	Orthoptera	R	0.713	0.04	3
	Scale	R	0.650	0.03	9
	Coleoptera larvae	R	0.638	0.04	6
	Adult Coleoptera	R	0.623	0.04	4
	Diplopoda	R	0.476	0.03	1
	Terrestrial Hemiptera	R	0.462	0.04	2
	Detritus	R	0.339	0.04	1
	Megaloptera	H	0.921	0.04	6
	<i>Aegla</i> sp.	H	0.895	0.04	26
	Odonata nymph	H	0.509	0.04	8

Only items with significant values $p < 0.05$ are listed. For stream and specie code see Material and Methods and Table 1.
doi:10.1371/journal.pone.0110999.t003

Results

Diet Composition and Factors-Influence

The contents of 1,984 stomachs (Table S1) belonging to three species (Table 1) were analyzed. We recorded 59 food items wherein *Heptapterus* sp. consumed a high proportion of *Aegla* sp. (34.7%), terrestrial plant remains (14.8%) and Megaloptera (12.8%) and *Rhamdia quelen* consumed fish (40.2%), and Oligochaeta (30.9%) followed by *Aegla* sp. (9.8%). The diet of *Trichomycterus poikilos* was based on Simuliidae (31.4%), Ephemeroptera (25.2%) and Trichoptera (18.7%) (Table S2).

The NMDS analysis (Figure 2) showed the existence of a differentiation pattern among the species. In all streams the PERMANOVA analysis corroborated the presence of species' differentiation and the influence of factor class size (Table 2). The most significant food items that contributed to species and species plus size class's differentiation were indicated by IndVal (Table 3, 4). The items indicated for IndVal for the species were the occasional items and in the major were indicated to *R. quelen* and *Heptapterus* sp. that have a more varied diet. The same occurred for the size class where the indicators items appeared most for larger classes by the use of occasional items.

Trophic Niche Breadth and Feeding Overlap

Niche breadth values were high (>0.61) for all species in all streams. The mean values were higher for *T. poikilos* ($Ba: 0.777$) and *R. quelen* ($Ba: 0.727$) than *Heptapterus* sp. ($Ba: 0.693$) (Figure 3). Overall for size classes 92.7% of niche breadth values were high and the few intermediate (0.4–0.61) as R4, H4 and H5. There is an abrupt niche breadth reduction in the larger size classes for *R. quelen* and *Heptapterus* sp. However *T. poikilos* maintain a more uniform niche breadth (Figure 4).

Feeding overlap values for species were mostly low (0–0.4) in all spatial-temporal units. *Rhamdia quelen* and *T. poikilos* only had low feeding overlap values. However the interaction between *R. quelen* with *Heptapterus* sp. showed larger quantitative of low overlap, and about 12% were of high and intermediate values. The largest number of high feeding overlap were the interaction

between *Heptapterus* sp. and *T. poikilos* (Figure 5). Most of observed values (84%) were not significantly higher than those expected by chance. This result indicates a resource partitioning among the species.

Investigating the feeding overlap values for species and their class size we observed that the most of feeding overlaps remains were low. However high and intermediate feeding overlaps were in largest frequency only in smaller size classes independently of these size are equivalents (Figure 6, 7). Observed values were significantly higher than those expected by chance in 60% of the feeding overlap interactions, showing the major of the spatial-temporal units for size class is occurring resource sharing.

Discussion

The partitioning resource found for the three species of Siluriformes in all streams sampled is relates with the differential use of resources by the species. Food items that were responsible for these differences varied from a stream to another, but overall the items were used in greater quantity or frequency by species. The indicator items for *R. quelen* and *Heptapterus* sp. were of the occasional use, higher frequency and larger size. The indicator item for *T. poikilos* appeared rarely and when appeared was Simuliidae larva that was widely used.

The high consumption of fish, Oligochaeta and *Aegla* sp. by *R. quelen* indicate a carnivorous/piscivorous habit. This habit is described in the literature [27,60,61]. *Heptapterus* sp., which was considered an invertivorous species, ingested large amount of *Aegla* sp., terrestrial plant remains, and Megaloptera- though in smaller proportions (but with higher frequency) fed on aquatic insects such as Ephemeroptera and Trichoptera. *Heptapterus* sp. was also reported as invertivorous but as ingesting a much larger amount aquatic larvae invertebrates [60] and two species of *Heptapterus* are classified within the guild of those that mainly eat items from their aquatic environment [62]. *Trichomycterus poikilos* was shown to be insectivorous, eating mostly aquatic larvae of Simuliidae, Ephemeroptera and Trichoptera. This insectivorous diet with ingestion of benthic larvae is well reported in the

Table 4. Indicator Value (IndVal), *p* value and Frequency of food items consumed by three species analyzed, discriminated among species/size classes.

Stream	Indicator Item	Specie/Size Class	IndVal	<i>p</i>	Frequency
RC	Bivalve	R3	0.249	0.01	2
	Hymenoptera	R3	0.174	0.01	2
	Fish	H1	0.907	0.03	6
	Megaloptera	H1	0.848	0.02	9
	Aquatic insects remains	H4	0.738	0.01	12
	Aquatic Hemiptera	H4	0.381	0.05	3
	Scale	H4	0.156	0.02	6
RJ	Adult Coleoptera	R3	0.438	0.01	4
	<i>Aegla</i> sp.	H4	0.949	0.02	12
	Fish	H4	0.836	0.03	1
	Scale	H5	0.542	0.01	4
	Rvegsu	H5	0.438	0.02	17
	Simuliidae	T4	0.704	0.01	48
RM	Terrestrial plant remains	R3	0.493	0.01	30
	Adult Coleoptera	R3	0.490	0.02	6
	Gastropoda	R3	0.376	0.04	23
	Animal organic matter	H3	0.732	0.01	10
	Rvega	H3	0.164	0.02	6
RV	Diptera Pupae	R4	0.143	0.03	1
	Fish	H3	0.815	0.04	1
	Adult Coleoptera	H3	0.143	0.04	1
	Ephemeroptera	T4	0.585	0.03	70
RD	Terrestrial plant remains	H4	0.787	0.01	24
	Aquatic insects remains	H4	0.654	0.04	12
	Coleoptera larvae	H4	0.308	0.05	8
	Simuliidae	T4	0.469	0.00	85
AA	Oligochaeta	R2	0.985	0.03	8
	Animal organic matter	R2	0.584	0.03	2
	Aquatic insects remains	R2	0.283	0.03	10
	Megaloptera	H3	0.938	0.04	4
	<i>Aegla</i> sp.	H5	0.984	0.01	4
	Rit	H5	0.349	0.03	3
RSC	Fish	R4	0.997	0.02	1
	Oligochaeta	R4	0.942	0.01	2
	Terrestrial Lepidoptera larvae	R4	0.818	0.02	1
	Terrestrial insect remains	R4	0.656	0.00	5
	Ephemeroptera	H3	0.649	0.05	61
	Sediment	H3	0.148	0.04	11
	Simuliidae	T4	0.641	0.04	64
RQ	Scale	R2	0.289	0.05	7
	Terrestrial insect remains	R3	0.445	0.04	3
	<i>Aegla</i> sp.	H3	0.739	0.04	11
	Odonata nymph	H4	0.249	0.01	2
RP	Fish	R4	0.990	0.02	5
	Nematoide	R4	0.375	0.04	4
	Chironomidae	T4	0.222	0.02	70
RT	Detritus	R1	0.106	0.00	1
	Scale	R2	0.301	0.05	9
	Coleoptera larvae	R2	0.296	0.04	6

Table 4. Cont.

Stream	Indicator Item	Specie/Size Class	IndVal	p	Frequency
	Diplopoda	R2	0.162	0.05	1
	Terrestrial Hemiptera	R2	0.153	0.04	2
	Nematoide	R3	0.776	0.01	9
	Orthoptera	R3	0.654	0.02	3
	Sediment	H5	0.488	0.04	47

Only items with significant values $p < 0.05$ are listed. For stream and specie/size class code see Material and Methods and Table 1.
doi:10.1371/journal.pone.0110999.t004

literature for other species of the genus *Trichomycterus* [63,64,65,66,67].

The diet differentiation were observed in the ontogenetic level when we analyzed the specie plus size class factor. In this case over again the indicator items were those used occasionally by the larger size classes. The influence of this factor has been studied in Neotropical streams and is connected to the innumerous features that change with the fishes development, be they morphological and physiological characteristics (e.g. increasing individual size, mouth gap, changes in the digestive tract) or even behavioral habits (e.g. locomotion ability) [68,69,70]. The more features that shift over development and that differentiate these species are related to the size that can achieve *R. quelen* and *Heptapterus* sp. The expressive growth these species present during development allows a larger mouth gape can allowing the use of larger items like *Aegla* sp., fish, Megaloptera, Gastropoda, terrestrial insect remains and Oligochaeta by the larger sizes. In this study the difference between the largest and smallest individuals was 26.84 cm and 26.08 cm for *Heptapterus* sp. and *R. quelen* respectively. With the increase in fish size the individuals need to maximize their energetic gain by ingesting larger prey with a higher caloric [71]. Sometimes the ontogenetic diet shifts may be seen as a

consequence of the absolute size increment of their mouth gape [72], the larger fish size enables ingestion of larger preys items due to an increase in searching ability and capture efficiency [73,74].

Trichomycterus poikilos does not achieve a largest size than other species and by having a relatively thin, elongated and depressed body they can explore places not explore by the other species [35] as a explore the small spaces among rocks very well, what seems to have occurred in the present study. So, the items used by *T. poikilos* were smaller size however the ingestion of fish, *Aegla* sp. and terrestrial plant remains occurred only in the larger length classes. Ontogenetic differences in the diet of two *Trichomycterus* species (*T. crassicaudatus* and *T. stawiarski*) were also detected by [67]. *Trichomycterus chiltoni* showed intraspecific differences in its diet which were related to ontogeny because the species reached a larger size which allowed for the consumption of larger prey (therefore broadening the trophic spectrum because of morphological characteristics such as the mouth and body size) [75]. This differentiation of diet by influence of ontogeny was also found by many others studies [70,76,77,78,79].

Dietary differences cited above are also supported by the niche breadth data. All species showed high niche breadth. Most of items were ingested occasionally, expanding the trophic spectrum of

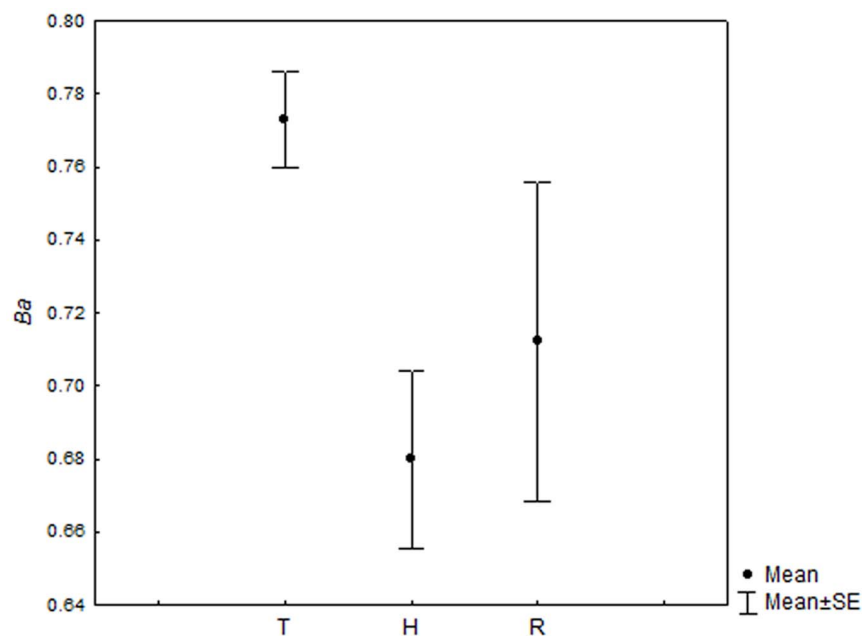


Figure 3. Values of trophic niche breadth for fish species analyzed in Alto Jacuí sub-basin. Values of trophic with mean \pm standart error for each specie analyzed in sampling units. For specie code see Table 1.
doi:10.1371/journal.pone.0110999.g003

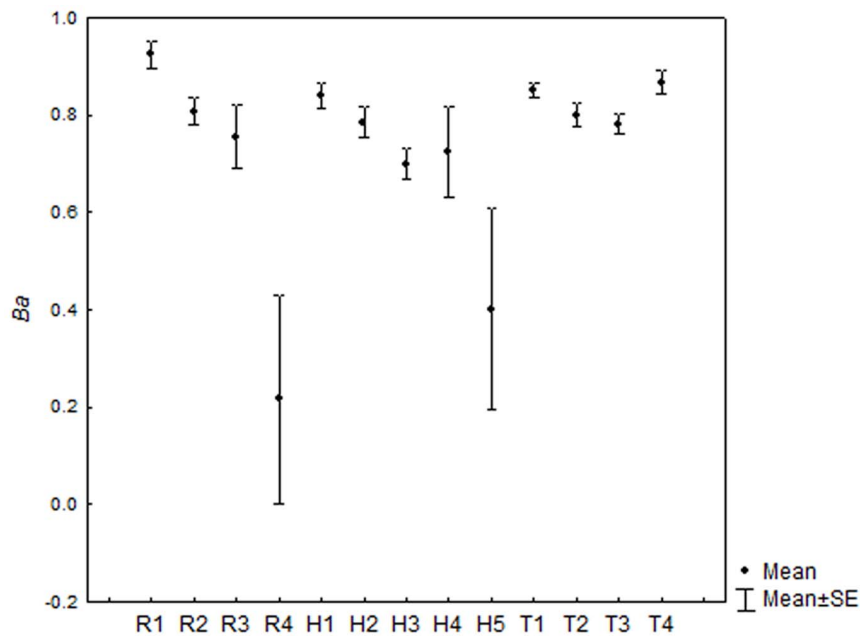


Figure 4. Values of trophic niche breadth for size class of each fish specie analyzed in Alto Jacuí sub-basin. Values of trophic with mean \pm standart error for each specie/size class analyzed in sampling units. For specie/size class code see Table 1. doi:10.1371/journal.pone.0110999.g004

these species that have a diverse diet and wide niche breadth. This broad food spectrum is already expected in Neotropical stream fish, because such streams have a wide range of available resources [27,61,80,81,82]. Neotropical stream fish have a tendency towards generalism thus having the ability of trophic plasticity [83].

The mean niche breadth values were low only in the high size class of *R. quelen* and *Heptapterus* sp. In the early stages of life the species tend to exhibit more generalist behavior and with increasing body size they begin to exhibit more specialist behavior,

as was found by [19] when they studied ontogenetic diet shifts among five species of *Crenicichla*.

The low overlap among the three species also indicates the resource partitioning. There are studies that show *R. quelen* and *Heptapterus* sp. not overlapping [61] and *R. quelen* overlapped with *Trichomycterus* sp. [27]. However, we believe that much of this low overlap was perceived because we did not group food items into broad categories- a discussion approached by other authors [27,66,84]. This in our opinion becomes very important in this type of analysis where one can notice the feeding preference of

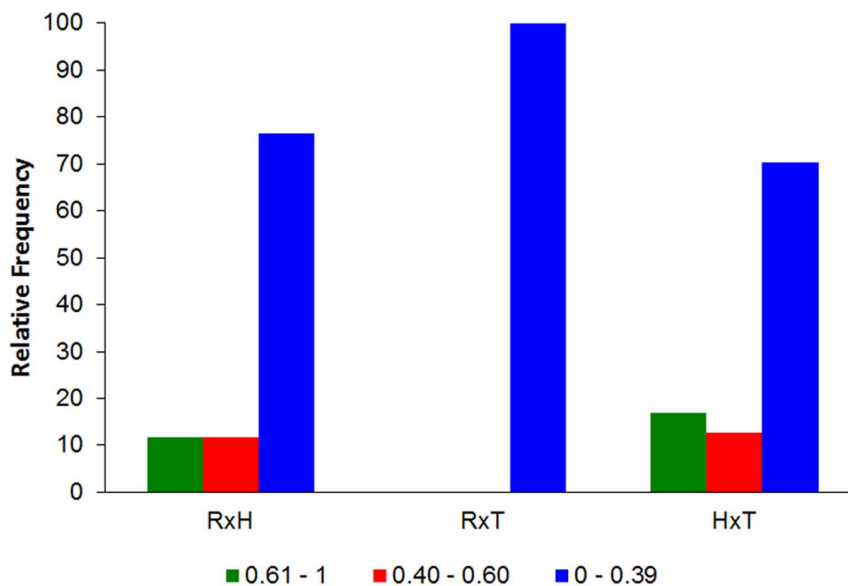


Figure 5. Pianka's index results. Relative frequency of Pianka's index of all pairwise interactions between the species analyzed per sampling unit. For specie code see Table 1. doi:10.1371/journal.pone.0110999.g005

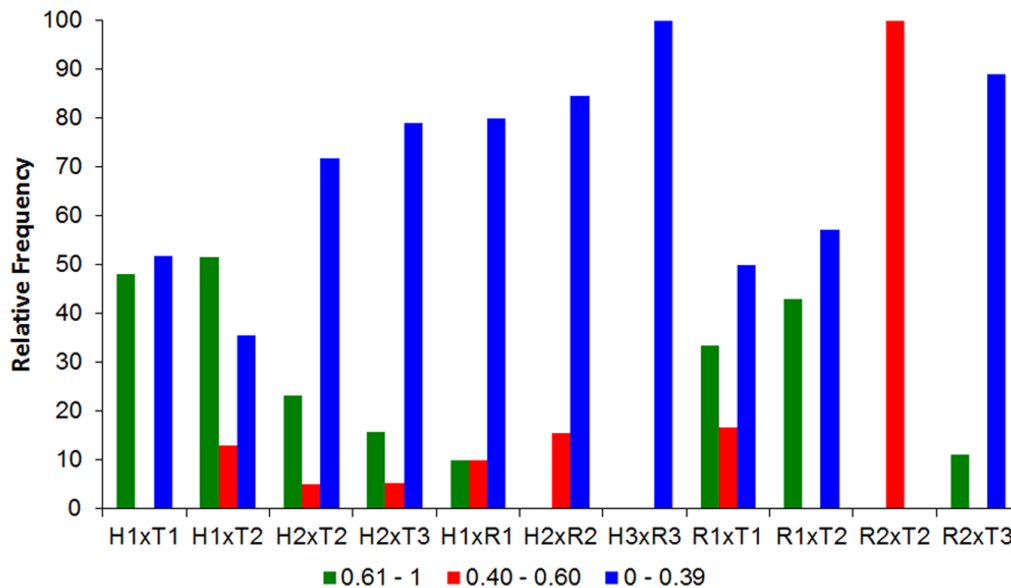


Figure 6. Pianka's index results. Relative frequency of Pianka's index of all pairwise interactions between the species with compatible size classes per sampling unit. For specie/size class code see Table 1. doi:10.1371/journal.pone.0110999.g006

the species for certain aquatic larvae, for example, the strong preference of *T. poikilos* for Simuliidae larvae. If we think in broader trophic categories, we definitely would have an increased dietary overlap of these species. In overall the overlap niche values were low, however only in smaller size classes had high values. The null model showed that there is a resource sharing between the species/size class, this pattern were seen in all spatial-temporal units. The items that are shared by species are those that are most abundant (personal observations) and it is known that the abundance of some items is often responsible for the coexistence of species [77]. Sharing of resources does not mean existence of competition; the high abundance of resources and stochastic processes can promote relaxation of interspecific competition and

facilitating coexistence which was also reported in other study [85]. We note that most items found in this study are shared by species, with overlap avoided both by the abundance of items in the aquatic system [4,27,54] and by the different microhabitats used for feeding, periods of activity, and tactics of capture. This pattern of features that avoid overlap and often explain the coexistence of sympatric species is well reported in Neotropical streams (e.g. [28,86,87,88,89,90]).

It is very difficult and complex we make inferences about species coexistence [85,91]. The three Siluriformes species studied here are coexisting, but the force that allows this coexistence is difficult to prove. The current scenario shows species with morphological similarities, partitioning some resources and sharing abundant

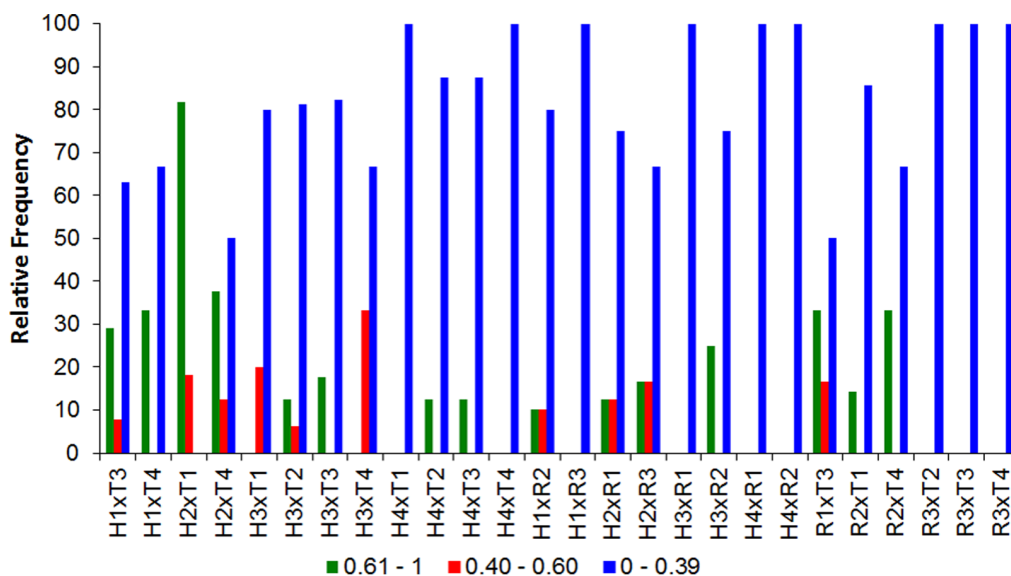


Figure 7. Pianka's index results. Relative frequency of Pianka's index of all pairwise interactions between the species with not compatible size classes per sampling unit. For specie/size class code see Table 1. doi:10.1371/journal.pone.0110999.g007

resources resulting no competition among them (4). But we cannot say with absolute certainty that the current scenario has not been structured over time through large negative pressures of the past between these species [10,92]. Differences in the species population numbers in different sample replicates (Table S1) was seen, and this may be related to stochastic events and other precepts of the neutral theory or which competition became more abundant a specie at a stream than other [93]. However there is no evidence that the closest similarities in these streams had higher population numbers. To prove this theory we must increase our knowledge of dispersal of fish in continental basins, because we know of dispersal limitation in aquatic environments [94] and their phylogenetic relationships [95].

In conclusion, our hypothesis was accepted, there is food segregation of three species of Siluriformes studied and it is related to the differential use of items in different stages of life. This because the differences in diet among species are related to morphological differences and life habits. There are more pronounced ontogenetic changes in *Heptapterus* sp. and *R. quelen* than in *T. poikilos*, given mainly by shifts in the morphology of these species. This process of diet shift throughout species growth is undoubtedly a way to expand or shift the trophic niche of the species in order to avoid inter- and intraspecific competition and maintain species coexistence that also maintain for the sharing resource of abundant items.

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Supporting Information

Table S1 Number of analyzed stomachs per sample unit (stream and sampled month) for each size class for the species studied. For stream and specie/size class code see Material and Methods and Table 1. (DOCX)

Table S2 Stomach content analyzed (% by volume) for the species of Siluriformes sampling in ten streams in Alto Jacuí sub-basin. For species and streams code see Material and Methods and Table 1. Asterisk indicates values less than 0.1%. (DOCX)

Acknowledgments

We thank the colleagues of the Ichthyology Lab at the Universidade Federal do Rio Grande do Sul for Field work; the MSc. Juliano Ferrer for help in identifying the species studied; and the Orlandi and Bonato family for help and support in the field work.

Author Contributions

Conceived and designed the experiments: KOB CBF. Performed the experiments: KOB. Analyzed the data: KOB CBF. Contributed reagents/materials/analysis tools: KOB CBF. Contributed to the writing of the manuscript: KOB CBF.

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CAPÍTULO 4

Dietary differentiation and its relationship with mouth and tooth morphology of Characidae fishes (Teleostei: Ostariophysi: Characiformes) in a Neotropical sub-basin, Brazil

Artigo submetido à revista científica Journal of Fish Biology

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Karine O. Bonato, Edward D. Burress and Clarcie B. Fialho

1 **Dietary differentiation and its relationship with mouth and tooth morphology of**
2 **Characidae fishes (Teleostei: Ostariophysi: Characiformes) in a Neotropical sub-**
3 **basin, Brazil**

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5
6 **DIETARY DIFFERENTIATION OF CHARACIDAE FISHES**

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8
9 K. O. BONATO^{1*}, E. D. BURRESS² AND C. B. FIALHO¹

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11
12 *¹Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento*
13 *de Zoologia, Programa de Pós-Graduação em Biologia Animal, CEP 91501–970, Porto*
14 *Alegre, Rio Grande do Sul, Brazil*

15 *²Department of Biological Sciences and Auburn University Museum of Natural History,*
16 *Auburn University, Auburn, AL, U.S.A.*

17
18
19 *Author to whom correspondence should be addressed at present address: Avenida
20 Bento Gonçalves, 9500, Agronomia, Prédio 43435, Sala 104, Ictiologia/Zoologia –
21 Porto Alegre – Rio Grande do Sul – Brazil – CEP: 91501–970. Tel.: 55-51-3308-7727;
22 email: kakabio2005@yahoo.com.br

23 **ABSTRACT**

24

25 This study aims to answer: 1) is there a significant difference among the diets of 11
26 Characidae fishes? and 2) do morphological features of the mouth and teeth correlate
27 with the diet of species? To answer these questions, fishes were collected bimonthly
28 during 2012 and 2013 using electrofishing techniques in the Alto Jacuí sub-basin. A
29 total of 1,525 stomachs were analyzed representing 11 species of characid fishes. The
30 PERMANOVA showed the existence of a significant differences among the diet of
31 species, and SIMPER analysis indicated that the main food items that contributed to this
32 differentiation were terrestrial and aquatic plants, terrestrial and aquatic insect remains,
33 and adult Hymenoptera. Lastly, CAP analyses showed that several morphological
34 features were associated with dietary patterns. For example, sub-terminal mouth
35 positions were correlated with the ingestion of benthic items (i.e., aquatic insects and
36 organic matter). Large mouths were associated with species that consume large items
37 such as macrocrustaceans (e.g., *Aegla*) and fish. Species that have small teeth variation
38 along the outer row of the premaxilla, as well as those that have fewer teeth on the
39 maxilla, consumed high proportions of plants and terrestrial insects. Species with
40 intermediate and high numbers of cusps in the maxilla teeth tended to consume aquatic
41 insects. Here, we demonstrate that 11 species of characid fishes have different diets and
42 that those diets were correlated with variation in mouth and tooth morphology.
43 Therefore, diet and morphology are likely co-evolved such that divergence in trophic-
44 related morphology likely facilitates dietary differentiation among characid fishes.

45

46 Key words: stream; feeding; oral features.

INTRODUCTION

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48

49

50 Selective pressures in certain environments may generate much of the observed
51 morphological diversity among species (Beaumord & Petrere, 1994). These pressures
52 may result in phylogenetically similar species to adaptively diverge (Reilly &
53 Wainwright, 1994) to avoid competition and thereby facilitate coexistence (Ross, 1986).
54 However, Wootton (1990) emphasized that there may be evolutionary convergence in
55 morphology of phylogenetically unrelated species that use similar food resources. More
56 complex evolutionary changes to morphological characters that result in large changes
57 in dietary patterns is often attributed to increased specialization (Kotrchal, 1989). Some
58 authors emphasize the power of morphology to delimit trophic groups and understand,
59 for example, the community trophic structure (Wiens & Rotenbery, 1980; Pouilly *et al.*,
60 2003; Mise *et al.*, 2013). Therefore, over the years many studies used this approach,
61 linking ecology and morphology (i.e. Chao & Musick, 1977; Yamaoka, 1982; Lauder &
62 Clark, 1984; Balon *et al.*, 1986; Motta, 1989; Winemiller *et al.*, 1995; Santos *et al.*,
63 2011; Dourado *et al.*, 2015). Thus, we believe that using phylogenetically similar
64 species, such as characid fishes, which have high diversity in their mouth and tooth
65 morphologies and sometimes have a similar diets, provides a good system for
66 investigating the ecomorphological influences on feeding habits.

67

68

69 The Characiformes order belong to the Otophysa group, which represent about 75% of
70 the Neotropical freshwater fishes (Malabarba & Malabarba 2014). Characiformes
71 includes about 234 genera (in the Neotropical region) and 2,000 described species

72 (Malabarba & Malabarba, 2010). Characidae is the family with the most species
73 richness within the Characiformes, with 146 genera, representing about 1,096 valid
74 species, 223 of which have been described species in the last 10 years (Oliveira *et al.*,
75 2011; Eschmeyer & Fricke, 2015).

76

77

78 Characid fishes have highly heterogeneous ecologies due their enormous taxonomic
79 diversity (Lima *et al.*, 2003). This family exhibits a wide range of body shape, body
80 size, which varies from small fishes such as *Amazonspinther dalmata* to large species
81 such as the *Salminus*, which are the largest Neotropical Characiformes. Thus, the
82 Characiformes can occupy many trophic niches including species that are piscivorous
83 (e.g., *Salminus*), lepidophagus (e.g., *Roebioxodon geryi* and *Roeboides bonariensis*),
84 omnivorous (e.g., *Astyanax*) (Lima *et al.*, 2003; Javonillo *et al.*, 2010; Neves *et al.*,
85 2015). Additionally, these species ingest a wide variety of food items and range from
86 generalists to specialists.

87

88

89 Other morphological features, beyond body size, that permit characid fishes to explore
90 many feeding habits is their tooth morphology. The Characiformes have a wide
91 diversity of teeth arrangements on the premaxilla, maxilla and dentary. Additionally,
92 tooth morphology also varies such that there are cusps on all or most teeth (Fink &
93 Fink, 1981).

94

95

96 Because their species richness, ecological diversity, and wide distribution, characid
97 fishes have played an important role in the evolution of stream communities throughout
98 Neotropical South America. Thus, in this study, we tested the hypothesis that species
99 show different food preferences that corresponds to differences in their mouth and tooth
100 morphologies such that the co-evolution of these traits has facilitated their
101 diversification into different trophic guilds. Therefore, the objectives of this study were
102 to (i) describe the feeding habits of the 11 Characidae species of Alto Jacuí sub-basin;
103 (ii) test if their diets are different as well as identify the items responsible for this
104 differentiation; and (iii) test the relationship between mouth and tooth morphology and
105 diet.

106

107

108 **MATERIALS AND METHODS**

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110

111 **SAMPLING**

112

113

114 The study was conducted in ten streams (Fig. 1) in northwest Rio Grande do Sul, which
115 correspond to the Alto Jacuí sub-basin – Caixões River (RC) (S29° 01' 54"/W 52° 49'
116 25.1"); Jacuizinho River (RJ) (S 28° 58' 02.9"/W 52° 47' 20.3"); Morcego River (RM) (S
117 28° 53' 55.0"/W 52° 49' 05.6"); Turvo River (RT) (S 28° 43' 47.0"/W 52° 47' 40.4"),
118 Valoroso Stream (RV) (S 28° 41' 32.0"/W 52° 51' 41.5"); Divinéia Stream (RD) (S 28°
119 42' 16.7"/W 52° 52' 25.9"); Arroio Angico (AA) (S 28° 39' 17.9"/W 52° 54' 31.1"); Paz
120 Stream (RP) (S 28° 42' 57.3"/W 52° 50' 41.7"); Santa Clara River (RSC) (S 28° 44'

121 30.1"/W 53° 13' 03.0"); and Quati River (RQ) (S 28° 38' 31.8"/W 52° 37' 07.9"). All
122 streams flow into the Jacuí River, which is one of the main tributaries to the Laguna dos
123 Patos system and the streams are considered headwater streams.

124

125

126 Fish were collected in 2012 and 2013 bimonthly. Each sampling event lasted four days.
127 For the sampling, we used electrofishing with three stages of 30 min each, in stretches
128 of 50 m per sampling stream. After sampling, fish were euthanized with 10% eugenol
129 (Vidal *et al.*, 2008; Lucena *et al.*, 2013a), fixed in 10% formalin and then transferred to
130 70% alcohol for preservation. Fish were identified in the laboratory with identification
131 keys and voucher specimens were deposited in the fish collection of the Departamento
132 de Zoologia at the Universidade Federal do Rio Grande do Sul (*Astyanax jacuhiensis* -
133 UFRGS 19977, *Astyanax laticeps* - UFRGS 19328, *Astyanax obscurus* - UFRGS 19329,
134 *Astyanax procerus* - UFRGS 19323, *Astyanax xiru* - UFRGS 19326, *Bryconamericus*
135 *iheringii* - UFRGS 19974, *Bryconamericus* sp. - UFRGS 17931, *Diapoma alburnus* -
136 UFRGS 19976, *Diapoma dicropotamicus* - UFRGS 19980, *Oligosarcus jacuiensis* -
137 UFRGS 19978, *Oligosarcus jenynsii* - UFRGS 19979). Only adult individuals were
138 analyzed to avoid the ontogenetic influence in the analyses. Individuals were dissected
139 for stomach removal, which were then conserved in 70% alcohol.

140

141

142 **DIET COMPOSITION AND DIETARY DIFFERENTIATION**

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144

145 Stomach contents were identified under optical and stereoscopic microscopes set to the
146 lowest taxonomic level possible. Food items were identified using the identification
147 keys (Merritt & Cummins, 1996; Costa *et al.*, 2006; Mugnai *et al.*, 2010) for
148 invertebrates. Food items were then quantified according to the volumetric method (i.e.,
149 the total volume of a food item consumed by the fish population given as a percentage
150 of the total volume of all stomach contents (Hyslop, 1980) using graduated test tubes
151 and a glass counting plate (Hellawell & Abel, 1971).

152

153

154 We used Permutational Multivariate Analysis of Variance (PERMANOVA) with 9999
155 permutations (Anderson, 2001; Anderson *et al.*, 2008) based on a dissimilarity Bray-
156 Curtis (Legendre & Legendre, 1998) matrix to statistically test the existence of
157 differences among the species' diets in the Alto Jacuí sub-basin. This analysis was
158 based on data volume. If a difference was found, SIMPER (Clarke & Gorley, 2006) was
159 applied to get the food item indicators for each species. These analyses were performed
160 in PRIMER-E v6.1.16 with PERMANOVA+ v1.0.6 (Clarke & Gorley, 2006).

161

162

163 **MOUTH AND TOOTH MORPHOLOGY**

164

165

166 The measurements for mouth and tooth were base in the description of 11 characid
167 species (see Table I). The mouth and teeth features are done through counts and
168 description of the form. The choice of measurements were based in the characteristics
169 that are relevant to the diet. These features determine what items can be ingested and

170 how they are captured by species. The measurements choice were: mouth position
171 (descriptive), mouth size (measured and descriptive), number of rows in premaxilla
172 (counted), number of teeth in outer row (counted), number of cusps in outer row teeth
173 (counted), number of teeth in inner row teeth (counted and descriptive), number of
174 cusps in the inner row teeth (counted), number of teeth in maxilla (counted and
175 descriptive), number of cusps in maxilla teeth (counted and descriptive), number of
176 teeth in dentary (counted and descriptive), central cusp shape (descriptive), teeth shape
177 (descriptive).

178

179

180 **CORRELATION BETWEEN DIET AND MPOUTH/TOOTH MORPHOLOGY**

181

182

183 We performed a Constrained Principal Analysis on Coordinates (CAP) to determine the
184 ratio of mouth/teeth morphological factors and diet of species. CAP is the canonical
185 form of PCoA and can be applied to a matrix of distances (used Bray-Curtis distance)
186 between objects using any resemblance matrix. The CAP analysis permits comparison
187 of two sets of matrices measured in the same sampling units (Legendre & Legendre,
188 1998; Legendre & Anderson, 1999). To facilitate the visualization of the CAP analysis
189 subjects were grouped into their respective sampling units (i.e. by stream and date of
190 sample). We used ANOVA analysis with 9999 permutations to test the significance of
191 the CAP and the axes generated by this analysis and the correlation between variables,
192 and deleting the possible correlated variables. All analyses were performed using R
193 software (R Core Team 2015) with the Vegan package (Oksanen *et al.*, 2009).

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195

196

RESULTS

197

198

199 **DIET COMPOSITION AND DIETARY DIFFERENTIATION**

200

201

202 The contents of 1,525 stomachs (Table SI, Supporting Information) belonging to 11

203 Characidae species (Table II) were analyzed. The Stevardiinae classification follows

204 Thomaz *et al.* 2015. We recorded 58 food items and the most consumed for each

205 species is show in Table III. In order to classify the species into trophic guilds we

206 summed the percentage of consumed items and the groups that totaled 60% or more of

207 contribution was the responsible for the denomination. So, *Astyanax jacuhiensis* (Cope,

208 1894) and *Astyanax procerus* (Lucena, Castro & Bertaco, 2013) were classified as

209 omnivores and the most of the ingested items were of terrestrial origin; however,

210 *Bryconamericus iheringii* (Boulenger, 1887) was also omnivorous, but its diet was

211 based in items of aquatic origin. *Astyanax laticeps* (Cope, 1894) was classified as

212 omnivorous too, but the items ingested were both aquatic and terrestrial origin.

213 *Astyanax obscurus* (Hensel, 1870) showed an invertivorous diet. The herbivore diet was

214 showed by *Astyanax xiru* (Lucena, Castro & Bertaco, 2013), which ingested both

215 aquatic and terrestrial plants. Nevertheless, *Diapoma alburnus* (Hensel, 1870), *Diapoma*

216 *dicropotamicus* (Malabarba & Weitzman, 2003) and *Bryconamericus* sp. exhibited an

217 aquatic insectivorous feeding. *Oligosarcus jacuiensis* Menezes & Ribeiro, 2010 had a

218 piscivorous diet with tendency to invertivory, but *Oligosarcus jenynsii* (Günther, 1864)

219 was piscivorous (Table III).

220

221

222 The existence of diet differentiation among the species ($F= 13.5$, $P= 0.0001$) was
223 comproved by PERMANOVA. The most significant food items that contributed to
224 species' diet differentiation were indicated by SIMPER. The items indicated for
225 SIMPER for the pairs of species were basically the same (Table IV).

226

227

228 MOUTH AND TOOTH MORPHOLOGY

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230

231 The 12 morphological mouth/tooth features chosen were categorized for CAP analysis
232 (Table SII, Supporting Information).

233 Mouth position: only *B. iheringii* and *Bryconamericus* sp. have sub-terminal mouth,
234 other species have terminal mouth;

235 Mouth size: only *O. jacuiensis* and *O. jenynsii* have large mouth, the other species have
236 short mouth;

237 Number of rows in premaxilla: only *O. jacuiensis* and *O. jenynsii* have one row, the
238 other species have two rows;

239 Number of teeth in outer row: in *O. jacuiensis* and *O. jenynsii* the outer row is absent,
240 the broad variation (3-6) is showed by *B. iheringii* and *D. alburnus*;

241 Number of cusps in outer row teeth: the broad variation is found in *B. iheringii* and *D.*
242 *alburnus*;

243 Number of teeth in inner row teeth: the broad variation (3-6) in *B. iheringii*, the most of
244 species have to 5 teeth;

245 Teeth number of cusps in the inner row teeth: the most of species have 3-5 cusps, *A.*
246 *procerus* and *A. xiru* have to 7 cusps;
247 Number of teeth in maxilla: in *A. jacuhiensis* is absent, only one teeth in *A. procerus*
248 and *A. xiru*, and the broad variation (22-34) in *O. jacuiensis*;
249 Number of cusps in maxilla teeth: vestigial cusps in *O. jacuiensis* and *O. jenynsii*, *A.*
250 *procerus*, *A. xiru* and *B. iheringii* have the five cusps;
251 Number of teeth in dentary: *O. jacuiensis* has one canine anterior, three conical and
252 more 12-25, *O. jenynsii* have 12-21, *A. jacuhiensis* with 12-13 teeth, and
253 *Bryconamericus* sp. have only six;
254 Central cusp shape: all species have the central cusps more longer than other cusps, only
255 *A. jacuhiensis*, *A. obscurus*, *A. proceros* and *A. xiru* have cusps slightly curved
256 posteriorly towards inside mouth;
257 Teeth shape: teeth are anteroposteriorly compressed in *B. iheringii*, *Bryconamericus* sp.,
258 *O. jacuiensis* and *O. jenynsii*.

259

260

261 **CORRELATION BETWEEN DIET AND MOUTH/TOOTH MORPHOLOGY**

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263

264 This analysis was based in 196 sampling units. Some morphological tooth features were
265 correlated, in which case the less ecologically informative were removed from the
266 analysis (TSPM, NCOR, NCIR, NTD, CCS, TS). Of the six remaining, only NTIR was
267 not significant (Table V). The CAP showed an explanation of 15.1% of variation
268 constrained of the food items. The CAP was significant ($F = 5.6$, $p = 0.0001$) as well as
269 its first two axis (CAP1 - $F = 14.5$, $P = 0.0001$; CAP2 - $F = 11.1$, $P = 0.0001$). The

270 explanations of the first two axes were 43.3% and 33.1%, respectively. The CAP
271 confirms the significant differences found by PERMANOVA in the species' diet. The
272 items indicated by SIMPER are the same that appear more isolated and are influencing
273 the species in both axes of the CAP analysis (Fig. 2).

274
275

276 Mouth position (Pmouth) separated the species with terminal mouths represented by
277 negative scores (i.e. AL, AO, AP, AS, AX, OA and OI) from those with sub-terminal
278 mouths represented by positive scores (i.e. BI and BY). The species with sub-terminal
279 mouths (i.e. BI, BY) tended to eat more aquatic insects, which are benthic or eat bottom
280 items like detritus and organic matter, and those with terminal mouth ingested more
281 quantities of terrestrial insects and plants because of being able to capture these types of
282 items on the surface of the water column. Mouth size (Tmouth) separated the species
283 with large mouths (OA and OI) from those having a small mouth (i.e. BI, BY, DA,
284 DD). This situation was influenced by items of large size that can only be ingested by
285 the species with large mouths. Species like OA and OE were influenced by
286 consumption of large items such as fish and terrestrial insects (i.e. Hymenoptera).

287
288

289 Number of teeth in the outer row (NTOR) approached those species that have a similar
290 minimum number of teeth in this row and had similar variation. The species (all
291 *Astyanax* species) with small variation in the number of teeth showed the highest
292 ingestion of plants, mainly terrestrial plants. The largest number of teeth on the maxilla
293 (NTM) seems permit the species consumed more aquatic insects; however, species with
294 the fewest teeth fed more on terrestrial items. Species with intermediate and more

295 numerous cusps on the maxilla (NCM) ingested more aquatic insects. Nevertheless, the
296 smallest number of cusps on the maxilla seems to permit the consumption of large sized
297 and terrestrial items.

298

299

300

DISCUSSION

301

302 The characid fishes from Neotropical Brazil ingested about 58 food items revealing a
303 diverse supply of food resources, which supports the idea that most Neotropical fish
304 have highly variable diets (Lowe-McConnell, 1999). The five guilds presented here
305 indicate the great importance of plants and insects for the maintenance of the species in
306 the Alto Jacuí sub-basin.

307

308

309 The five trophic guilds were: Piscivorous with secondary degrees of invertivory -
310 *Oligosarcus jenynsii* and *O. jacuiensis* - there is not consistent information about the
311 diet of *O. jacuiensis*, but *O. jenynsii* is mostly documented in the literature as
312 carnivorous and piscivorous (Hartz *et al.*, 1996; Nunes & Hartz, 2006; Silveira *et al.*,
313 2011); Invertivorous - *A. obscurus* – information about dietary habits in the literature is
314 absent; Aquatic insectivorous - *Diapoma alburnus*, *D. dicropotamicus*, *Bryconamericus*
315 sp. - information about the diet of *D. alburnus* is based on its previous name and for *D.*
316 *dicropotamicus* there is not information about feed biology. In contrast with our results
317 it was considered a terrestrial insectivore by Delariva *et al.* (2013), omnivore-
318 bentophagous by Hartz *et al.* (1996), and insectivore-zooplanktivore by Vilella *et al.*
319 (2002); Omnivorous – *A. jacuhiensis*, *A. procerus*, *B. iheringii* - in general the *Astyanax*

320 species were classified as omnivores, herbivores or insectivores (Cassemiro *et al.*, 2002;
321 Vilella *et al.*, 2002; Bennemann *et al.*, 2005); the *Bryconamericus* genus is known to
322 have high trophic plasticity. For example, it is classified in many trophic groups because
323 they ingest many prey items including aquatic insects, algae and can also consume high
324 proportions of detritus in degraded environments (Orcioli & Bennemann, 2006;
325 Brandão-Gonçalves *et al.*, 2009; Mazzoni & Rezende, 2009; Bonato *et al.*, 2012);
326 Herbivorous - *A. xiru* is an herbivore but seems to ingest animal resources too, but
327 information in literature about this species is absent.

328

329

330 According to Gerking (1994), there has been resistance to classify fish as herbivorous,
331 because it was believed that a totally herbivorous diet could not allow for the proper
332 growth and reproduction of fish species. Over time there have been an increasing
333 number of studies about the nutrition of herbivorous diets (see Clements *et al.*, 2009)
334 and these studies indicate that plants are assimilated and the nutritional value supports
335 proper growth of animals (Hemmi & Jormalainen, 2002; Rubenstein & Wikelski, 2003;
336 Smit *et al.*, 2006).

337

338

339 We demonstrated with our data that these variations in feeding patterns among these
340 species are strongly related with the differentiation of morphological features. Thus, the
341 theory of ecomorphology says that morphological features of species should reflect their
342 ecology and therefore predict the feeding habits of species (Gatz, 1979). Additionally,
343 this pattern suggests that fishes with comparable morphological traits should use similar
344 resources (Winemiller, 1990). According to Wetzmain & Malabarba (1998), the

345 numbers of teeth in a given row on the premaxilla has been one of the prominent
346 sources supporting the recognition that this family is polyphyletic. The same authors
347 emphasize that tooth shape and certain types of tooth arrangements, especially on the
348 premaxilla, have been more successful in predicting relationships among characids. We
349 observed that the morphological features of mouth and teeth may explain the dietary
350 patterns. Although some studies have obtained the same result indicating a link between
351 morphology and diet (Motta, 1995; Winemiller *et al.*, 1995; Piet, 1998; Fugi *et al.*,
352 2001), others have not found this relation (Barnett *et al.*, 2006).

353

354

355 The characteristic that best separated the *Oligosarcus* species from the other species was
356 the mouth size. Carnivorous species must have sufficient gape that permits the ingestion
357 of large items as fishes and *Aegla*. This easy differentiation of carnivorous and
358 piscivorous species from other dietary patterns is seen in many studies (Motta, 1995;
359 Winemiller *et al.*, 1995; Barnett *et al.*, 2006; Dourado *et al.*, 2015), which also indicated
360 others features such as longer lower jaws, longer teeth size, an upturned mouth, and
361 greater snout length that also explain their dietary differentiation. In Santos *et al.* (2011)
362 study, they verified the feeding habit differentiation of *Oligosarcus hepsetus* from other
363 species such that they explored lotic environments and had carnivorous feeding habits,
364 which they linked to the morphological features as higher relative height of the mouth
365 and mouth aspect.

366

367

368 The *Bryconamerycus* species separate from the others mainly by having a sub-terminal
369 mouth allowing greater intake of benthic items such as detritus, organic matter and

370 benthic aquatic insects. The *Diapoma* species, despite having a terminal mouth, eat
371 more benthic items too. This is probably related to the shape of the body, which is
372 elongate and allows them to more efficiently exploit the bottom of streams and among
373 rocks. Although tooth number in the outer row of the premaxilla has been identified as
374 crucial in dietary differentiation, it does not seem to distinguish the species analyzed
375 here. Maybe it is not the number that defines this separation. Although *Bryconamericus*
376 and *Diapoma* both have a large number of teeth range (3-6) on the premaxilla, they have
377 another feature in common, which is the angle of these teeth. This angle is greater than
378 in *Astyanax*, in which the teeth are projected more externally (see Malabarba &
379 Malabarba, 1994) and it seems to allow the species to forage along the substrate and the
380 spaces between rocks whereas *Bryconamericus* scrapes algae from surfaces. The high
381 number of teeth on the maxilla may also be responsible for this foraging behavior by
382 further facilitating eating algae.

383

384

385 *Astyanax* species, in general, has a similar diet as the other species, the origin of items
386 (animal and plant) appears to be of more terrestrial origin. The more superior position of
387 the mouth in *Astyanax* permits the individuals to forage from the surface water and eat
388 floating terrestrial insects as well as drift feeding. According to Peretti & Andrian
389 (2008) the use of Hymenoptera and Coleoptera by *Astyanax* is explained by the type of
390 teeth. The insertion almost forming a right angle (90°) to its premaxilla and dentary does
391 not allow scraping substrates, but makes it easier to bite and tear plants and ingest
392 terrestrial insects. Therefore, in this study *Astyanax* may be considered browsers that
393 bite off pieces and tear plants above the substrate, usually by pointing the head upward
394 (Jones, 1968). Unlike *Bryconamericus* are grazers which ingest algae from close to the

395 substrate and may ingest some of the substrate; this type of species feeds by pointing the
396 head down and applying their lips and teeth to the substrate, so they can feed by rasping
397 and suction (Jones, 1968). Sazima (1986) observed that *Kyphosus sectatrix* and
398 *Metynnus maculatus* have specialized, shearing teeth of plant-eating fish species. The
399 smaller teeth on the maxilla seem to be offset by the higher number of cusps on the
400 teeth of these species.

401

402

403 The related species *A. xiru* and *A. procerus* showed a differentiation in their feeding
404 habits even though they share very similar morphology often being difficult to identify.
405 The small differences between these species are restricted to body height and the size of
406 the caudal peduncle. Mise *et al.* (2013) compared the diet and ecomorphology of three
407 species of *Astyanax* in Segredo reservoir (PR-Brazil) and found a similar pattern.
408 Species of *Astyanax* are highlighted in the literature as having few morphological,
409 ecological, and behavior differences (Santos *et al.*, 2011). The omnivores and
410 invertivores that are frequently noted in this group indicate they have broad
411 morphological variations, probably related to the lack of specialization that
412 characterizes these trophic groups (Horn, 1998; Pouilly *et al.*, 2003).

413

414

415 In conclusion, with this work we contributed with basic information about the trophic
416 biology of several recently described species, we reinforced the hypothesis of the
417 Characidae as a diverse family that exploits distinct habitats and associated broad
418 trophic spectrum. The species varied between specialists such as herbivorous and
419 aquatic insectivorous to generalists such as omnivores and invertivores. This ecological

420 variability is only possible due to the different morphological features exhibited and
421 maintained in the populations. Thereby, with this study we demonstrate that mouth and
422 teeth morphology that differed among the species likely permitted the differentiation of
423 food habits in the diet.

424

425

426 We hope that with this effort in adding biological and ecological information, in the
427 near future, we can have more clear and objective answers about the evolutionary
428 patterns within Characidae group. Because many questions still remain unresolved in
429 many taxonomic levels of the Characiformes mainly in the named “Clade C” (Vari &
430 Malabarba, 1996). Recently, Thomaz *et al.* (2015) established the Stevardiinae as
431 monophyletic, placed *Bryconamericus iheringii* with *Hypobrycon*, and designated
432 *Cyanocharax* as *Diapoma*. This evolutionary similarity is consistent with our dietary
433 data such that we observed similar insectivorous diets among this sub-group including
434 *D. alburnus*, *D. dicropotamicus* and *Bryconamericus* sp. So, with greater understanding
435 of the relationships within these groups we can infer with more certainty, for example,
436 why species of *Astyanax* showed similar diets. If this situation may be due to ancient
437 diversification in the presence of ecological opportunity as has been shown with other
438 groups of Neotropical fishes (López-Fernández *et al.*, 2012) or a recent diversification
439 due a high competition among *Astyanax* species with one specie began to exploit a little
440 explored niche.

441

442

443 We thank the colleagues of the Ichthyology Lab at the Universidade Federal do Rio
444 Grande do Sul for Field work; the MSc. J. Ferrer, MSc. P.C. Silva and Dr. L.R.

445 Malabarba for help in identifying the species studied; Orlandi and Bonato family for
446 help and support in the field work; MSc. R.B. Dala Corte for help with figure
447 construction; and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
448 (CAPES, Proc. 1104786, to KOB) for financial support.

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685 TABLE I. The twelve morphological teeth features for 11 characid fishes sampled in the Alto Jacuí sub-basin, their respective codes and the references used to
 686 obtained the information for each species.

687

Morphological teeth features	Species										
	AJ	AL	AO	AP	AX	BI	BY	DA	DD	OA	OE
Mouth (Pmouth)	Terminal	Terminal	Terminal	Terminal	Terminal	Sub-terminal	Sub-terminal	Terminal	Terminal	Terminal	Terminal
Mouth Size (Tmouth)	Short	Short	Short	Short	Short	Short	Short	Short	Short	Large, that extends beyond the eye	Large, that extends beyond the eye
Number of rows in premaxilla (TSPM)	2	2	2	2	2	2	2	2	2	1	1
Number of teeth in outer row (NTOR)	4 teeth (rarely 3-5 on one	4-5	3-5	4-6	4-6	3-6	3-5	3-6	3-5	0	0

	side)										
Number of cusps in outer row teeth (NCOR)	3 teeth (rarely 4)	3	5	5	5	3-5	3	3-5	3	0	0
		5 teeth gradually decreasing in length from the first to fourth, last distinctly smaller	5 teeth gradually decreasing in length from the first to fourth, last distinctly smaller	5 teeth gradually decreasing in length from the first to fourth, last distinctly smaller	5 teeth gradually decreasing in length from the first to fourth, last distinctly smaller						
Number of teeth in inner row (NTIR)	5 teeth (rarely 4)					3-6	4	4-5	4 or 5 teeth	3-5	3-5
Number of cusps in inner row teeth (NCIR)	5, 6 or 7 cusps (rarely 4 in the most			6-7 cusps (anterior teeth with 5 cusps and	7 cusps (the first teeth with 5 cusps and	3-5	3	3-5	3-5 cusps where 4° and 5° are very small almost	Conical teeth with 3 cusps where 2 are vestigial	Conical teeth with 3 cusps where 2 are vestigial

	posterior)			the last 5 or 6 cusps)	the last 5 or 6 cusps)				imperceptible		
Number of teeth in maxilla (NTM)	0	1-3	1 (rarely 2)	1	1	1-6 teeth gradually decreasing of size	3 -6	3-8	4-6	22-34	19-31
Number of cusps in maxilla teeth (NCM)	0	1-3	1-3	4-5	3-5	3-5	3	Conical teeth to 3 cusps	Conical teeth to 3 cusps	Nearly conical teeth with vestigial lateral cusps	Nearly conical teeth with vestigial lateral cusps
Number of teeth in dentary (NTD)	12-13	Longer 4 anterior teeth and more 5-7 teeth	11	9-10	9-10	Longer 4 or 5 teeth and more 1- 6 smaller teeth	6	Longer 4 teeth and 7- 9 rarely 10 smaller teeth	Large 4 teeth and a serie of 6 - 10 smaller teeth	1 anterior canine, 3 conical teeth and more posterior row of 12 - 25	12-21
Central cusp shape (CCS)	Central cusp in all teeth	Central cusp in all teeth is	Central cusp in all teeth	Central cusp in all teeth	Central cusp in all teeth	Central cusp in all teeth is more long as other	Central cusp in all teeth is more long as other	Central cusp in all teeth is	Central cusp in all teeth is more long as	Central cusp in all teeth is more long as other	Central cusp in all teeth is more long as other

	is more long as other cusps; cusp tips slightly curved posteriorly towards inside mouth	more long as other cusps	is more long as other cusps; cusp tips slightly curved posteriorly towards inside mouth	is more long as other cusps; cusp tips slightly curved posteriorly towards inside mouth	is more long as other cusps; cusp tips slightly curved posteriorly towards inside mouth	cusps	cusps	more long as other cusps	other cusps	cusps	cusps
Teeth shape	Not	Not	Not	Not	Not	Anteroposteriorly	Anteroposteriorly	Not	Not	Anteroposteriorly	Anteroposteriorly
(TS)	compressed	compressed	compressed	compressed	compressed	compressed	compressed	compressed	compressed	compressed	compressed
References used	C.A.S. Lucena, comunic. pess.	Bertaco & Lucena, 2010	Bertaco & Lucena, 2010	Lucena <i>et</i> <i>al.</i> , 2013b	Lucena <i>et</i> <i>al.</i> , 2013b	Tatsumi, 2006; A. Hirschmann, comunic. pess.	Silva, 2008; A. Hirschmann, comunic. pess.	Malabarba, 1983; Malabarba, 1987; Malabarba	Malabarba, 1983; Malabarba, 1987; Malabarba &	Menezes & Ribeiro, 2010; L.R. Malabarba, comunic. pess.	Menezes & Ribeiro, 2010; L.R. Malabarba, comunic. pess.

& Weitzman,
Weitzman, 2003
2003

688 TABLE II. Taxonomic position of 11 characid fishes in the Alto Jacuí sub-basin with their
 689 respective species code and the number of stomachs analyzed for each species (these total
 690 number is based in the seven sampling realized).

Ordem/Família/Espécie	Species Code	Number of Stomachs
CHARACIFORMES		
Characidae		
Clade C *		
<i>Astyanax jacuhiensis</i> (Cope, 1894)	AJ	39
<i>Astyanax laticeps</i> (Cope, 1894)	AL	5
<i>Astyanax obscurus</i> (Hensel, 1870)	AO	23
<i>Astyanax procerus</i> Lucena, Castro & Bertaco, 2013	AP	277
<i>Astyanax xiru</i> Lucena, Castro & Bertaco, 2013	AX	64
<i>Oligosarcus jacuiensis</i> Menezes & Ribeiro, 2010	OA	10
<i>Oligosarcus jenynsii</i> (Günther, 1864)	OE	5
Stevardiinae **		
<i>Bryconamericus iheringii</i> (Boulenger, 1887)	BI	885
<i>Bryconamericus</i> sp.	BY	156
<i>Diapoma alburnus</i> (Hensel, 1870)	DA	58
<i>Diapoma dicropotamicus</i> (Malabarba & Weitzman, 2003)	DD	3

691 *(Sense Jonovillo *et al.*, 2010; Oliveira *et al.*, 2011); **(Sense Malabarba & Weitzman,

692 2003)

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697 TABLE III. Stomach content analysis (% by volume) for the species of Characidae in the
 698 Alto Jacuí sub-basin. For species' code see Table I.

Items	AJ	AL	AO	AP	AX	BI	DA	DD	BY	AO	OE
Chironomidae	0.0	0.1	0.0	0.2	*	0.6	2.1	2.0	3.2	0.0	0.0
Simuliidae	0.6	0.0	0.8	1.1	0.8	1.3	4.1	3.9	5.3	*	0.0
Ceratopogonidae	0.0	0.0	0.0	0.0	0.0	*	0.3	0.3	0.0	0.0	0.0
Psychodidae	*	0.0	0.0	*	0.2	*	0.0	0.0	*	0.0	0.0
Empididae	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0
Stratiomyidae	0.0	0.0	0.0	0.0	0.0	*	0.0	0.0	0.0	0.0	0.0
Sarcophagidae	0.0	0.0	0.0	1.0	8.5	0.0	0.0	0.0	0.0	0.0	0.0
Tabanidae	0.0	0.0	0.0	*	0.0	*	0.8	0.8	0.0	0.0	0.0
Tipulidae	0.0	0.0	0.0	0.0	*	*	0.0	0.0	0.0	0.0	0.0
Diptera pupae	0.5	0.0	0.5	0.2	0.1	0.3	2.3	3.2	0.2	0.0	0.0
Trichoptera	0.1	0.0	1.2	1.3	0.2	5.5	8.0	7.7	10.6	0.0	0.0
Ephemeroptera	1.3	28.6	0.0	1.9	1.3	4.0	39.7	37.9	48.5	1.3	4.1
Plecoptera	0.0	0.0	0.0	0.1	0.7	1.1	1.2	1.2	3.3	0.0	0.0
Simuliidae pupae	0.0	0.0	0.0	*	*	0.1	*	*	0.1	0.0	0.0
Trchoptera pupae	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.2	0.3	0.0	0.0
Odonata nymph	0.0	0.0	0.0	0.6	0.0	2.2	2.0	1.9	1.2	0.0	0.0
Aquatic Coleoptera larvae	2.3	0.0	2.1	0.5	0.5	1.2	1.3	1.2	0.7	0.0	0.0
Terrestrial Lepidoptera larvae	1.7	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aquatic Lepidoptera larvae	0.0	0.0	0.0	0.6	0.2	1.7	0.0	0.0	12.7	0.0	0.0
Lepidoptera pupae	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Aquatic insects remains	1.2	14.3	2.5	4.7	1.1	12.0	11.3	10.8	5.7	*	0.0
Adult Diptera	0.0	0.0	0.0	0.4	0.0	0.6	1.2	1.1	0.0	0.0	0.0
Adult Orthoptera	0.0	0.0	0.8	0.3	0.1	*	0.0	0.0	0.0	0.0	0.0
Aquatic adult Hemiptera	0.0	0.0	0.1	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0

Terrestrial adult Hemiptera	0.1	0.0	0.4	*	0.7	0.2	0.0	0.0	0.0	0.2	0.0
Adult Hymenoptera	8.0	0.0	26.0	15.0	4.1	1.7	7.5	9.7	0.4	3.0	0.6
Adult Coleoptera	9.6	0.0	4.1	4.5	0.6	1.7	2.9	2.8	0.5	11.7	0.0
Adult Lepdoptera	3.5	0.0	19.8	0.8	0.0	0.3	0.4	0.4	0.0	0.0	0.0
Adult Odonata	0.0	0.0	0.5	0.0	0.0	*	0.0	0.0	0.0	0.5	0.0
Adult Plecoptera	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.6	0.0
Terrestrial insects remains	2.9	14.3	5.3	6.5	2.5	1.1	6.2	6.8	1.8	0.0	2.9
Aranae	3.4	0.0	0.6	0.9	0.0	0.4	0.2	0.2	0.0	0.0	0.0
Megaloptera	0.0	0.0	0.0	0.0	0.0	0.1	0.4	0.4	0.0	0.0	0.0
Diplopoda	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Aegla</i> sp.	9.7	0.0	22.4	*	0.0	0.2	0.0	0.0	0.0	16.9	0.0
Bivalvia	0.0	0.0	0.0	0.0	0.0	*	0.0	0.0	0.0	0.0	0.0
Gastropoda	0.0	0.0	0.0	0.5	0.0	0.6	0.0	0.0	0.1	0.0	0.0
Oligochaeta	2.2	0.0	0.0	6.3	0.0	2.9	0.8	0.8	0.4	0.0	0.0
Microcrustacea	0.0	0.0	0.0	*	0.2	0.2	0.0	0.0	0.0	0.1	0.0
Acari	0.0	0.0	0.0	*	*	*	*	*	0.0	0.0	1.0
Terrestrial plants	36.3	21.4	12.0	21.9	39.3	13.7	1.7	1.6	2.6	0.0	12.0
Aquatic plants	0.0	21.4	0.1	19.7	34.2	8.7	5.2	4.9	0.0	0.0	0.0
Diatomacea algae	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	*	0.0	0.0
Filamentous algae	0.0	0.0	0.1	2.3	0.8	7.1	0.0	0.0	0.4	0.0	0.0
Sediment	0.0	0.0	0.0	0.1	0.0	0.8	0.0	0.0	0.4	0.0	0.0
Undetermined organic matter	0.0	0.0	0.0	3.9	3.8	7.3	0.0	0.0	0.3	0.0	0.0
Vegetal organic matter	0.0	0.0	0.5	2.4	0.0	1.7	0.0	0.0	0.7	0.0	0.0
Animal organic matter	0.0	0.0	0.0	0.2	0.0	0.9	0.0	0.0	0.4	0.0	0.0
Testae Amoebae	0.0	0.0	0.0	0.0	0.0	*	0.0	0.0	0.0	0.0	0.0
Detritus	0.0	0.0	0.0	0.2	0.0	16.4	0.0	0.0	0.0	0.0	0.0
Scale	0.0	0.0	0.0	*	0.0	0.1	0.0	0.0	0.0	0.0	0.0

Nematoda	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Fur	0.0	0.0	0.0	0.0	*	*	0.0	0.0	0.0	0.0	0.0
Fish	15.9	0.0	0.0	0.0	0.1	1.0	0.0	0.0	0.0	60.1	79.4
Aquatic Isopoda	0.0	0.0	0.0	0.3	0.0	0.7	0.0	0.0	0.0	0.5	0.0
Homoptera	0.0	0.0	0.0	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Neuroptera	0.0	0.0	0.0	0.0	0.0	*	0.0	0.0	0.0	0.0	0.0
Pollen	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0

699 † The asteristicks (*) indicates values less than 0.1%; bold values indicate the items

700 most consumed

TABLE IV. Dissimilarity of pairwise comparison and the contribution (%) of the food items obtained by SIMPER analyses for the 11 Characidae species sampling in Alto Jacuí sub-basin. Diss= dissimilarity; AP= aquatic plants; AIR= aquatic insects remains; TIR= terrestrial insects remains; TP= terrestrial plants; AH= adult Hymenoptera; AC= adult Coleoptera; EPH= Ephemeroptera; DET= detritus; TRI= Trichoptera, DP= Diptera pupae, SIM= Simuliidae; AC=Acari. For species' code

Table I.

Species	Diss (%)	AP	AIR	TIR	TP	AH	AC	EPH	DET	TRI	DP	SIM	FISH	Species	Diss (%)	AP	AIR	TIR	TP	AH	AC	EPH	DET	TRI	DP	SIM	FISH	AC	
AJxAL	84.5	25.4	11.2	10.1										AJxBY	94.3			17.4		6.2	17.7		7.7						
AJxAO	84.9				22.1	13.6	10.8							ALxBY	89.1		9.5		13.1			24.0		10.4					
ALxAO	91.0		12.6	10.0	18.8	12.9								AOxBY	95.7				8.5	9.3		20.2		9.0					
AJxAP	87.5				19.6	11.1								APxBY	90.2				8.3	8.0		17.5		8.5					
ALxAP	88.1	11.9	11.7	10.4	16.8	10.4								AXxBY	92.1	8.7			13.5	9.5		16.9							
AOxAP	89.3		10.0		12.7	14.6								BIxBY	86.1		10.3		9.7			14.3	6.1	8.6					
AJxAX	83.4	10.0			21.2	12.6								DAxBY	76.8		12.7					18.5		10.9		9.1			
ALxAX	86.0	15.0	10.2	10.0	20.9	13.3								DDxBY	94.6					14.4		20.5			17.0				
AOxAX	87.2	10.7			17.7	15.5								AJxOA	93.7				18.0	10.3	10.5							16.6	
APxAX	85.9	13.3			16.5	13.4								ALxOA	97.6				11.8	9.0		10.6						19.0	
AJxBI	87.2		10.2		15.4	6.1	6.1							AOxOA	93.9				7.5	13.9	10.4							17.1	
ALxBI	87.5		13.3		14.2			8.6	7.5					APxOA	94.6				6.8	11.6	8.2	7.6						15.3	
AOxBI	90.6		11.1		11.8	8.7			6.4					AXxOA	94.3	8.6			12.9	12.2	7.8							14.8	
APxBI	88.3	7.4	10.5		11.5	7.5		6.4						BIxOA	96.0		8.9		8.4	6.5	6.0	6.5						12.4	
AXxBI	87.5	9.4	10.0		13.9	8.6								DAxOA	92.2		10.3			9.2		14.0						15.6	

AJxDA	91.9		10.6		18.2	7.8		12.4		DDxOA	94.0			17.4			17.7	17.5			
ALxDA	87.7		15.8	8.7	13.0			17.4		BIxOA	93.7			7.2	18.1		7.6		15.3		
AOxDA	91.5		12.7		8.0	11.5		13.8		AJxOE	82.7			19.1					18.7		
APxDA	87.5	8.1	11.8			9.9		13.3		AlxOE	84.1		11.1	19.7					21.9	12.9	
AXxDA	89.4	10.3	10.5		13.6	10.9		12.5		AOxOE	91.7			16.3	10.8					17.6	
BlxDA	85.3		11.6		9.5			11.2	6.8	APxOE	89.5			15.6	9.0					16.5	
AJxDD	92.6			10.2	20.7	16.7			17.4	AXxOE	86.5			17.5	10.5					16.5	
ALxDD	94.6			14.8	13.8	20.1			24.0	BIxOE	89.0	9.0		12.7					13.8	6.7	
AOxDD	90.0			11.2		21.6			20.5	DAxOE	89.6	9.3		14.3						16.3	
APxDD	90.9			11.2	8.0	17.9			17.8	DDxOE	94.9			15.4	14.3				16.2	17.5	
AXxDD	90.0	10.2	10.5	15.3	17.6				16.9	BYxOE	85.7			14.8						17.1	9.0
BlxDD	95.3		9.8	7.2	9.5	11.5			13.1	OAxOE	88.8			14.5							24.0
DAxDD	90.5		11.6	9.8		16.2		14.2	17.6												

705 TABLE V. ANOVA results (F and *p*) of six non-
 706 correlated morphological tooth features of 11
 707 species sampling in the Alto Jacuí sub-basin. For
 708 morphological tooth features see Table III.

Morphological teeth features	F	P
Pmouth	12.8	0.0001
Tmouth	4.3	0.0001
NTOR	8.4	0.0001
NTIR	1.5	0.0760
NTM	3.9	0.0001
NCM	2.7	0.0001

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Figure Captions

713 FIG. 1. Sampling streams in the Alto Jacuí sub-basin, state of Rio Grande do Sul, Brazil.

714 For stream codes see Material and Methods.

715 FIG. 2. Ordination of the Characidae species in the Alto Jacuí sub-basin, state of Rio

716 Grande do Sul, Brazil, produced by the first two axes of the Constrained Principal

717 Analysis on Coordinates (CAP1 and CAP2) applied to the correlation of six

718 morphological tooth features and items ingested by the species. For morphological tooth

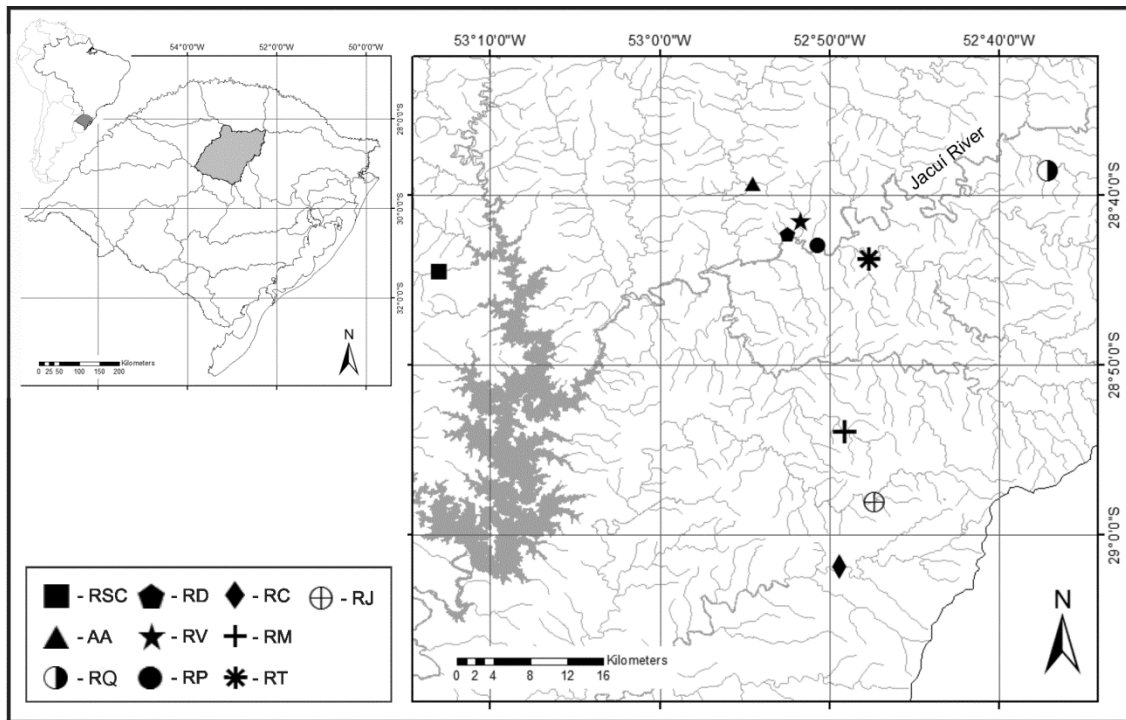
719 features variables see Table IV. TV= terrestrial plants; AV= aquatic plants; Hyme=

720 adult Hymenoptera; TIR= terrestrial insects remains; Olig= Oligochaeta; Filamen=

721 Filamentous algae; Ephemer= Ephemeroptera; Trichop= Trichoptera; Simul=

722 Simuliidae; DP= Diptera pupae; Det= detritus; AIR= aquatic insects remains; IOM=

723 undetermined organic matter.

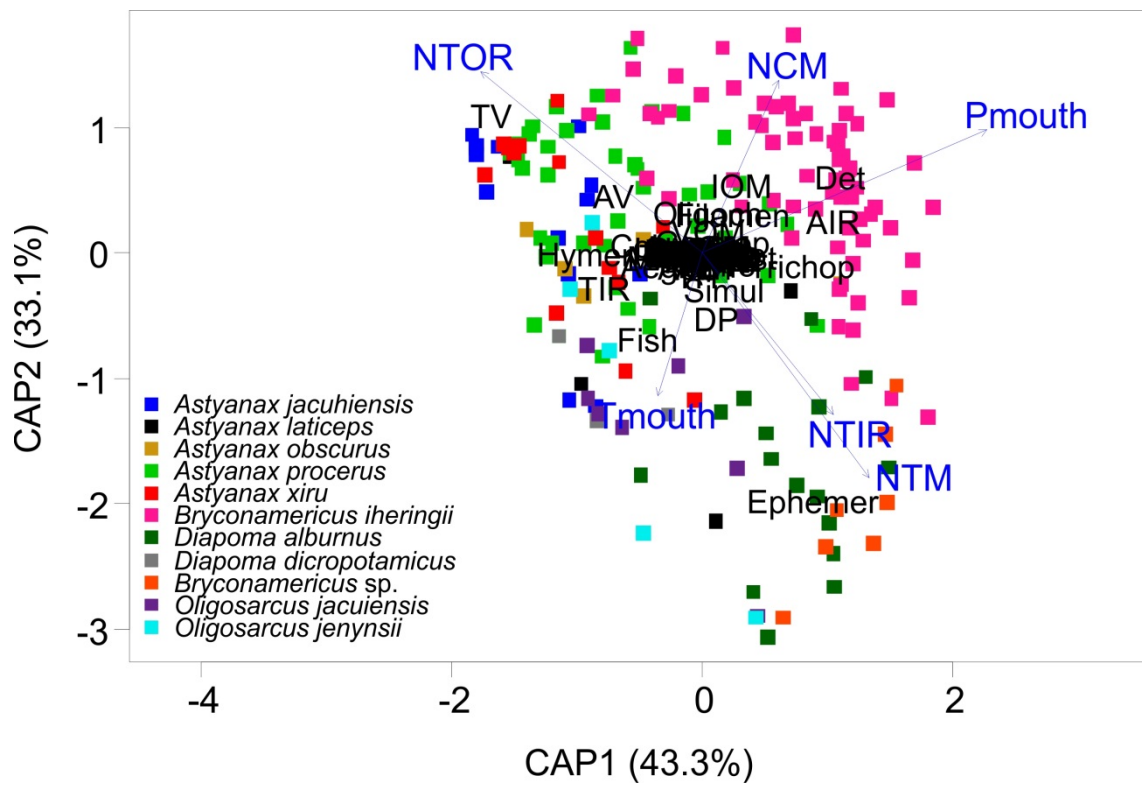


724

725 FIG. 1.

726

727



728

729 FIG. 2.

730

Supporting Information

731 Supporting Information may be found in the online version of this paper:

732 **TABLE SI.** Stomach contents analysis (by volume) for the species of Characidae

733 sampling in the Alto Jacuí sub-basin, state of Rio Grande do Sul, Brazil.

734 **TABLE SII.** Categorized morphological tooth features for the Characidae species to the

735 Constrained Principal Analysis on Coordinates (CAP) analyses.

CAPÍTULO 5

Resource partitioning among syntopic Characidae corroborated by gut content and stable isotope analyses

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Karine O. Bonato, Edward D. Bures, Clarice B. Fialho & Jonathan W.
Armbruster

1 **Resource partitioning among syntopic Characidae corroborated by gut content and stable isotope**
2 **analyses**

3

4 **Gut content and stable isotopes analyses for Characidae**

5

6 **Karine O. Bonato^{1*}, Edward D. Burress², Clarice B. Fialho¹ and Jonathan W. Armbruster²**

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8 ¹Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Zoologia,
9 Programa de Pós-Graduação em Biologia Animal, CEP 91501-970, Porto Alegre, Rio Grande do Sul,
10 Brazil

11 ²Department of Biological Sciences and Auburn University Museum of Natural History, Auburn
12 University, Auburn, AL, U.S.A.

13

14 *Correspondence:* Karine Bonato, Ichthyology laboratory – Instituto de Biociências - Avenida Bento
15 Gonçalves, 9500, Agronomia, Prédio 43435, Sala 104, Ictiologia/Zoologia – Porto Alegre – Rio Grande
16 do Sul – Brazil – CEP: 91501-970. Telephone number: 55-51-3308-7727. Email:
17 kakabio2005@yahoo.com.br

18

19 Abstract - The degree of niche overlap among syntopic organisms and the correspondence between
20 consumption and assimilation are major topics in trophic ecology. Here, we calculated niche overlap of
21 four syntopic Characidae species using the proportions of food items present based on gut content
22 analysis. Secondly, to evaluate the importance of these food items as nutrient sources, we used carbon
23 (¹³C) and nitrogen (¹⁴N) isotopes analyses to estimate relative assimilation. We measured diet overlap by
24 calculating Pianka's index for each sampling period, which spanned seven months. To estimate the
25 relative importance of food items to characids, we used a dual isotope Bayesian mixing model. All

26 characids consumed large proportions of plant material and aquatic insects. Pianka's index indicated low
27 dietary overlap throughout most of the sampling period. In December 2012, there was intermediate
28 dietary overlap and in June 2013 there was high dietary overlap. The low degrees of dietary overlap were
29 due to discrepancies in the relative consumption of Ephemeroptera and aquatic insect remains, which are
30 abundant in the aquatic systems. Secondly, there was high correspondence between the gut content
31 analyses and isotope mixing model estimates. *Astyanax xiru* and *Astyanax procerus* assimilated large
32 fractions of terrestrial invertebrates and aquatic plants. *Bryconamericus iheringi* assimilated primarily
33 aquatic invertebrates and secondarily algae, whereas *Bryconamericus* sp. assimilated primarily aquatic
34 and terrestrial invertebrates. Therefore, we demonstrate that the congeneric species have more similar
35 diets, but that these four Characidae species coexist by resource partitioning, and that they assimilate
36 nutrients consistent with the relative consumption of food items.

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38 Key words: stream fish; diet; prey assimilation; niche overlap

39 **Introduction**

40

41 Characidae have highly heterogeneous ecologies due its enormous taxonomic diversity (Lima et
42 al. 2003). This family can occupy many trophic niches including species that are piscivorous (e.g.,
43 *Salminus*), lepidophagus (e.g. *Roebioxodon geryi* and *Roeboides bonariensis*), and omnivorous (e.g.
44 *Astyanax*) (Lima et al. 2003; Javonillo et al. 2010; Neves et al. 2015). Therefore, characids ingest a wide
45 variety of food items, occupy different parts of the food web, and range from generalists to specialists.
46 The characid genera *Astyanax* and *Bryconamericus* are the most speciose and normally occur in
47 abundance in rivers and streams. These species, particularly the Neotropical ones, exhibit varied diets and
48 may function as herbivores, omnivores, or mesopredators in the ecosystems they inhabit (Winemiller et
49 al. 2008; Burrell et al. 2013). Moreover they have the ability to change their eating habits (mainly
50 *Bryconamericus*) in response to variations in the availability of certain items (Abelha et al. 2001; Orcioli
51 & Bennemann 2006; Brandão-Gonçalves et al. 2009; Bonato et al. 2012).

52 For a certain population of 11 sympatric Characidae species, Bonato & Fialho (in preparation)
53 observed that there were differences among the species' diets, which was closely related to the species'
54 mouth and tooth morphology. These authors found two *Astyanax* (*Astyanax procerus* Lucena, Castro &
55 Bertaco, 2013 and *Astyanax xiru* Lucena, Castro & Bertaco, 2013) and two *Bryconamericus* species
56 (*Bryconamericus iheringii* (Boulenger, 1887) and *Bryconamericus* sp.) occurring syntopically in a studied
57 stream. The gut content analysis revealed that *Astyanax* species have pelagic diets such that they ingest
58 high proportions of aquatic plants (i.e. *A. xiru* was considered herbivorous) and terrestrial insects. In
59 contrast, the *Bryconamericus* species were more benthivorous because they ingested large proportions of
60 aquatic insects, aquatic plants, and algae. Stomach content analysis usually indicates food consumed
61 recently, but not the dietary patterns over time or differential assimilation of food items because of
62 differences among food items associated with detectability, measurability, and digestibility (Beneditto et
63 al. 2013; Park et al. 2015). According to Gerking (1994), there was resistance to classify fish as

64 herbivorous, because it was believed that a totally herbivorous diet could not allow for the proper growth
65 and reproduction of fish species. This is due to that fish intestines do not produce enzymes capable of
66 breaking down plant cell (e.g. cellulose); therefore, fishes need other tools to assist with breakdown of
67 plant materials such as microorganisms that produce necessary enzymes, mechanical processing to
68 physically rupture cell walls, and/or longer guts to allow for higher intake of food and maintain these food
69 for longer time in the gut to maximize nutrient assimilation (German et al. 2009; Wagner et al. 2009;
70 Pelster et al. 2015). Accordingly, many studies suggest that fishes can assimilate ingested plants and that
71 plants can provide sufficient nutritional value to support proper growth (Hemmi & Jormalainen 2002;
72 Clements et al. 2009; Smit et al. 2006).

73 Gerking (1994) stable isotope studies may provide evidence about the relative amounts of
74 nutrients derived from plant and animal origins. The stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$)
75 provide powerful tools for estimating trophic positions that integrates the assimilation of energy and
76 carbon flow to consumers in food webs (De Niro & Epstein 1981; Post 2002). This technique is based on
77 the fact that the isotopic ratios of a consumer are dependent upon the ratios of its diet with a trophic
78 enrichment factor between consumer and diet (Anderson & Cabana 2007; Compson et al. 2014; Di
79 Benedetto et al. 2011; Di Benedetto et al. 2012; Cresson et al. 2016). Stable isotopes of nitrogen ($\delta^{15}\text{N}$) can
80 be used to estimate trophic levels and food assimilation over time, and carbon ($\delta^{13}\text{C}$) indicates different
81 sources of carbon, indicating the origin of food (e.g. aquatic or terrestrial) (DeNiro & Epstein 1978;
82 Peterson & Fry 1987; Fry 2008). Additionally, gut content and isotope analyses may give complementary
83 answers about niche overlap index results (Genner et al. 1999; Bearhop et al. 2004; Quevedo et al. 2009)
84 and reveal if rates of assimilation is consistent with rates of consumption. Using multiple approaches
85 allows for more robust assertions about the trophic patterns and trophic interactions among species such
86 as coexistence by food niche partitioning.

87 Thus, many questions remain pertaining to the trophic ecology of even well-studied fishes such as
88 *Astyanax* and *Bryconamericus*. Do *Astyanax* assimilate the large amount of ingested plant material or do

89 the smaller proportions of invertebrates supply most of their nutrients? Therefore, to understand the
90 complex trophic relationships among four syntopic characid species, the objectives of this study were to
91 (i) evaluate if the relative assimilation of food items corresponds to their relative consumption as
92 described by conventional gut contents analyses and (ii) assess the existence of resource partitioning
93 among the species.

94

95 **Material and methods**

96

97 **Sampling**

98

99 We studied four syntopic species of Characidae in the Caixões River (S29° 01' 54"/W 52° 49'
100 25.1"). This stream is located in the Alto Jacuí sub-basin that bellows to Laguna dos Patos system in Rio
101 Grande do Sul, Brazil. For diet analysis, fish were collected in 2012 and 2013 bimonthly. We sampled
102 fish using electrofishing with three stages of 30 min each, in stretches of 50 m of the stream. After
103 sampling, fish were euthanized with 10% eugenol (Vidal et al. 2008; Lucena et al. 2013a), fixed in 10%
104 formalin and then transferred to 70% alcohol for preservation. Fish were identified in the laboratory with
105 identification keys and voucher specimens were deposited in the fish collection of the Departamento de
106 Zoologia at the Universidade Federal do Rio Grande do Sul (*Astyanax procerus* - UFRGS 19323,
107 *Astyanax xiru* - UFRGS 19326, *Bryconamericus iheringii* - UFRGS 19974, *Bryconamericus* sp. - UFRGS
108 17931). Individuals were dissected for stomach removal, which were then conserved in 70% alcohol.

109 For isotope analysis, sampling occurred in February (2015) using the same method cited above.
110 Specimens were temporarily stored on ice in the field and later stored in a -80°C freezer in the laboratory.
111 The individuals used for all analyses were representative of adult size classes. Additionally, we manually
112 sampled invertebrates that represent potential prey items, including (i) aquatic insects (Ephemeroptera,
113 Trichoptera, Plecoptera, Lepidoptera larvae, Megaloptera, Coleoptera larvae and adult), (ii) terrestrial

114 insects (Lepidoptera larvae and adult, adult Hemiptera, adult Coleoptera, adult Orthoptera, adult
115 Hymenoptera (i.e. ants and bees) and adult Odonata), (iii) other invertebrates (*Aegla* sp., Oligochaeta,
116 Bivalvia, snail and Aranae) and (iv) plants (terrestrial leaves, filamentous algae, diatoms, Cyanophyceae,
117 Bryophytae and aquatic plants (Podostemaceae)).

118

119 Feeding overlap

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121 We used Pianka's index (Pianka 1973) to estimate the species' feeding overlap within each
122 sampling month to test the assumptions of niche overlap theory (Pianka 1974; Ross 1986). Overlap values
123 range from zero to one, where zero indicates the absence of overlap and one indicates complete overlap.
124 We established three categories to improve understanding of the overlap results, high overlap is given by
125 overlap values >0.6 , intermediate values overlap between 0.4 - 0.6 and low overlap values <0.4
126 (Grossman 1986).

127 To evaluate the significance of Pianka's index we used the null model with RA3 algorithm
128 (Winemiller & Pianka 1990). We performed 9,999 Monte Carlo randomizations that represent a null
129 model that we can compare (mean niche overlap values for all group pairs) with the observed data. In this
130 model, mean overlap values that are significantly lower than those expected by chance might indicate
131 food partitioning; however, values higher than those expected by chance might indicate food sharing
132 (Albrecht & Goteli 2001). The observed results were compared with the null model at the significance
133 level $p < 0.05$ to evaluate if the observed pattern differs of the overlap values simulated at random. Both
134 feeding overlap and null model were computed using R Project for Statistical Computing program
135 (version 3.2.3) in the EcoSimR package (version 0.1.0) (R Core Team 2016). The methodology used gut
136 analyses data in Bonato and Fialho (in preparation) and the data used to calculate the Pianka's index in
137 the Supporting Information (SI Table1).

138

139 Stable isotope methods

140

141 Dissected caudal muscle tissues were lyophilized until dry (i.e., asymptotic mass), ground into a
142 homogenous powder and weighed into tin capsules. Invertebrates and basal resources were lyophilized
143 whole and homogenized. Samples were analyzed for ^{15}N and ^{13}C at the University of Georgia stable
144 isotope laboratory (Athens, Georgia, USA). All values are written in delta notation, which represents the
145 deviation of stable isotope ratios ($^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$) from universal standards: PDB limestone (C),
146 atmospheric nitrogen (N) and standard mean ocean water (H). Lipid content can bias ^{13}C analysis (Post et
147 al. 2007) because lipids are depleted relative to proteins and carbohydrates. However, consumer C:N
148 ratios were less than 3.5 indicating that lipid content was sufficiently low to prevent bias (Post et al.
149 2007). Furthermore, lipid content (i.e., estimated from C:N) was similar among consumers suggesting
150 uniform effects (if any) from lipids, thus limiting potential bias in our analyses.

151 To estimate the relative assimilation of prey items, we employed a dual-isotope Bayesian mixing
152 model in R Project for Statistical Computing program (R Core Team 2011) with the IsotopeR package,
153 ver. 0.4.7 (Hopkins & Ferguson 2012). Potential prey items were partitioned into four functional groups;
154 however, to account for all items individually, they were coded as sub-sources in the mixing model (Table
155 2). We incorporated error associated with 1) fractionation estimates, 2) variation in consumer and source
156 isotope ratios, 3) source concentration (of C and N) and 4) digestibility of sources (see below for details).
157 We corrected consumer isotopic signatures using generic fractionation rates supported by meta-analyses
158 (Post 2002): 3.5‰ and 0.5‰ for ^{15}N and ^{13}C , respectively. We incorporated error (e.g., SD) associated
159 with these rates as they were reported in Post (2002): 0.98‰ (^{15}N) and 1.3‰ (^{13}C). Source digestibility
160 was incorporated using rates reported in prior literature (Whitledge & Rabeni 1997; Roth et al. 2006):
161 plants (41%), detritus (14%), algae (39%), and invertebrates (92%). To prevent erroneously attributing
162 prey items that are not consumed, we utilized a threshold to determine what prey items were included in
163 the models. Any prey items (or functional groups; Table 3) that represented $\geq 5\%$ of the gut contents by

164 volume were included for that consumers model. Therefore, our estimates are not independent
165 assessments of these consumers' diets, but rather estimates of the relative assimilation of known food
166 resources. The model was run using three MCMC chains and 10,000 MCMC runs with 10% burnin.

167

168 **Results**

169

170 Feeding overlap

171

172 The Pianka's index results indicated a low feeding overlap among the majority of sampling
173 months. In December 2012, there was an intermediate overlap and June 2013 was the only month that had
174 a high overlap (Table 1). The higher overlap during these months resulted from sharing of few resources.
175 In December (Fig. 1), all four species consumed large proportions of Ephemeroptera. The overlap among
176 *A. procerus*, *A. xiru* and *B. iheringii* was also due to higher intake of terrestrial plants, and also between
177 *A. procerus*, *B. iheringii* and *Bryconamericus* sp., which consumed more aquatic insect remains. In June
178 2013 (Fig. 1), *A. xiru* was not sampled. However, the high overlap among *B. iheringii*, *Bryconamericus*
179 sp. and *A. procerus* resulted from the consumption of large proportions of Ephemeroptera and secondary
180 of aquatic insects remains. Additionally, aquatic Lepidoptera larvae were used by both *Bryconamericus*
181 species.

182

183 Table 1.

184

185 *Fig. 1.*

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188

189 Stable isotope Results

190

191 The four focal characid species had higher ^{15}N ratios than all surveyed prey items (Fig. 2).
192 *Astyanax xiru* and *A. procerus* had similar ^{15}N and ^{13}C ratios, whereas *B. iheringii* had less negative ^{13}C
193 ratios, and *Bryconamericus* sp. had higher ^{15}N ratios (Fig. 2). The prey items exhibited diverse isotope
194 ratios and functional groups of these items were generally isotopically distinct (Table 2; Fig. 2). Based on
195 estimates using a dual isotope mixing model, *Astyanax xiru* and *A. procerus* assimilated large fractions of
196 terrestrial invertebrates and aquatic plants (Table 3). *Bryconamericus iheringii* assimilated primarily
197 aquatic invertebrates and secondarily algae, whereas *Bryconamericus* sp. assimilated primarily aquatic
198 and terrestrial invertebrates (Table 3).

199

200 *Fig. 2.*

201

202 *Table 2.*

203

204 *Table 3.*

205

206 **Discussion**

207

208 Initial studies of stomach contents classified *Astyanax procerus* as an omnivore that primarily
209 ingests items of terrestrial origin, whereas *Astyanax xiru* were classified as an herbivore that ingested both
210 aquatic and terrestrial plants. In contrast, *Bryconamericus iheringii* was also omnivorous, but ate more
211 items of aquatic origin and *Bryconamericus* sp. consumed primarily aquatic insects. With the Pianka's
212 index and the null model results we demonstrate low feeding overlap among these species. Similar result
213 was found for some Siluriformes species in the same sub-basin (Bonato & Fialho 2014). The high overlap

214 showed by the index occurred when species consumed largest volume of items like Ephemeroptera and
215 aquatic insects remains in two sampling months. Aquatic invertebrates (i.e. larval insects) are important
216 food sources and they are in abundance in Neotropical streams (Boulton et al. 2008; Winemiller et al.
217 2008) and, by personal observations they are abundant in the Caixões River. In that study, Bonato &
218 Fialho (2014) also found some high overlap between *Trycomicterus poikilos* and *Heptapterus* sp. due
219 aquatic insect consumption. Guo et al. (2015) demonstrated with their results that even with a high
220 overlap, two invasive gobies coexisted at similar abundances in Lake Erhai on China, because the species
221 had access to sufficiently many food resources that prevented the development of competitive
222 interactions.

223 According to Hardin (1960) species cannot coexist because competing for resources could lead to
224 the exclusion of one or the other species or a population decrease, so the coexistence of species imply in
225 existence of resource partition (Pianka 1974; Ross 1986; Townsend et al. 2006). However, the feeding
226 specialization showed by the two *Astyanax* species in which they largely consume plants may prevent
227 dietary overlap with *Bryconamericus*. Guo et al. (2015) emphasizes that specialization in food resource is
228 often crucial to enhance coexistence by reducing interspecific competition among closed-related
229 competing fish species. Another mechanism that allows the co-occurrence of different species would be
230 the use of different habitats for feeding. *Astyanax* are species that forage in the water column and from the
231 water surface, whereas *Bryconamericus* are benthic and thus forage from the substrate (Casatti 2002;
232 Orcioli & Benneman 2006; Peretti & Adrian 2008). Also, morphology is a factor that may prevent or
233 reduce niche overlap between species (Skúlason & Smith 1995; Rezende et al. 2013; Song & Kim 2014).
234 Traits such as head shape, mouth angle, and gill morphology are closely associated with the trophic
235 characteristics of fishes (Gerking 1994). Additionally, tooth morphology, number, and diversity are
236 associated with the type of foods that fishes consume (Burress 2016). Features such as mouth position
237 may be important for dietary patterns among characids. For example, *Astyanax* and *Bryconamericus* sp.

238 have terminal jaws and *B. iheringii* has sub-terminal jaws. Tooth angle, and the distribution of teeth in the
239 jaws may also allow these species to exploit different food items (Bonato & Fialho, in preparation).

240 The four characid species had higher ^{15}N ratios than all surveyed prey items, which is indicative
241 of being consumers. *Bryconamericus iheringii* assimilated primarily aquatic invertebrates and secondarily
242 algae. In fact, the carbon assimilated by this species is likely derived from algae because the species had
243 less negative ^{13}C ratios similar to that of algae. *Bryconamericus* sp. had higher ^{15}N ratios that resulted in
244 estimates that they primarily assimilate aquatic invertebrates followed by terrestrial invertebrates. Based
245 on these results, we can maintain the previous trophic guild classification, derived from gut content
246 analysis, that *B. iheringii* is an omnivore and reclassify *Bryconamericus* sp. as an invertivore.

247 *Bryconamericus* species have been classified in many trophic guilds because they ingest many prey,
248 including algae and detritus in degraded environments (Orcioli & Bennemann 2006; Brandão-Gonçalves
249 et al. 2009; Mazzoni & Rezende 2009; Bonato et al. 2012). *Bryconamericus iheringii* seems to graze
250 algae from rocky surfaces. There are few studies on the isotopic composition of Neotropical stream fishes
251 available. But, in a coastal lake of the Rio Grande do Sul state, Rodrigues et al. (2013) observed that *B.*
252 *iheringii* was the most enriched species in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and classified the species in the second
253 trophic level and isotopically associated them with zooplankton. Brito et al. (2006) also classified *B.*
254 *microcephalus* as secondary consumer based on their isotopic relationship with algae in a stream
255 environment. Additionally, in a community-wide isotopic analysis of a tributary to the Uruguay River,
256 Burress et al. (2013) observed that *B. iheringii* had similar isotopic values as other omnivorous species.
257 *Bryconamericus iheringii* also had different $\delta^{13}\text{C}$, but similar $\delta^{15}\text{N}$ values as *Astyanax* sp. (Burress et al.
258 2013). Therefore, *B. iheringii* are omnivorous, but may be a opportunist such that they have slightly
259 different trophic roles in different environments.

260 We observed that *A. xiru* and *A. procerus* had similar ^{15}N and ^{13}C ratios and assimilated large
261 fractions of terrestrial invertebrates and aquatic plants. Therefore, we corroborate that *A. procerus* is an
262 omnivore, and provide a novel classification of *A. xiru* as an omnivore. There are no published ecological

263 data on either *Astyanax* species, because they were recently described (Lucena et al. 2013b). However, in
264 the upper Uruguay river basin, Neves & Delariva (in preparation) found that *A. xiru* was herbivorous
265 since more than 75% of the ingested material was of terrestrial plants and seeds. According to previous
266 studies, isotopic signatures indicate that macrophytes are an important resource for *Astyanax* species in
267 lagoon (Garcia et al. 2006; Rodrigues et al. 2013) and stream ecosystems (Burrell et al. 2013). In these
268 aquatic environments the *Astyanax* species were classified as omnivore and invertivore respectively.

269 Herbivorous fishes are expected to supplement their diets with animal resources to meet
270 nutritional needs for proper development, especially since these species do not have as long intestines to
271 maximize absorption of nutrient-poor vegetal resources (Horn 1989; Horn & Messer 1992). Thus, with
272 our data it is questionable the classification of *A. xiru* as herbivorous. Based on preliminary gut content
273 analyses, the ingested volume of terrestrial invertebrates by *A. xiru* was low; however, it seems that these
274 items represent a large proportion of assimilated nutrients. Furthermore, Raubenheimer et al. (2005)
275 showed that complementary feeding may occur in a temperate girellid species such that algae is the
276 primary source of energy but that animal matter is the primary source of protein. In a review about
277 nutrition ecology of marine herbivorous, Clements et al. (2009) commented that isotopes from high
278 protein resources such as animals are incorporated; however, carbohydrates from a low protein source
279 such as algae or seagrasses are catabolized directly for energy. Thus, estimates of assimilation are
280 currently limited by large discrepancies in protein content of food items, particularly for herbivores and
281 omnivores that consume a wide variety of food items. However, the low C/N ratios observed suggest that
282 biases introduced from lipid content are low (Post et al. 2007). Therefore, it may be more likely that $\delta^{13}\text{C}$
283 and $\delta^{15}\text{N}$ are not assimilated equally, which is an assumption of dual isotope mixing models (Hopkins &
284 Ferguson 2012). Indeed, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ appear to be assimilated independently by some stream fishes
285 such that they preferentially assimilate large fractions of $\delta^{15}\text{N}$ from invertebrates, even if they represent a
286 small proportion of their diets (Burrell et al. 2016). Nevertheless, because in our results both plant and
287 animal material appear to be assimilated, we assumed that *A. xiru* is an omnivore.

288 *Astyanax procerus* forage upon aquatic and terrestrial plants, while *A. xiru* seems consume only
289 aquatic plants, but complement their diet with terrestrial and aquatic invertebrates (i.e. insects). It remains
290 unclear if *A. xiru* intentionally consumes invertebrates or if they are inadvertently eaten while taking
291 refuge in terrestrial plant material. Some food items consumed by *A. xiru*, such as ants, are often
292 associated with terrestrial vegetation hanging over or into the water and may be consumed
293 opportunistically from the surface or inadvertently from the plants themselves. Regardless, we
294 demonstrate that both plant and invertebrate resources are important to food resources for *Astyanax*
295 populations.

296 Notwithstanding, these very similar diet of *Astyanax* species seems allowed by the utilization of
297 different microhabitats. There is evidence that in heterogeneous environments, ecologically similar
298 species avoid competition by mechanisms such as specialization in microhabitat use (Jones et al. 2001;
299 Amarasekare 2003). Stream environments are very heterogeneous (Esteves & Aranha 1999) and therefore
300 offer many microhabitats for foraging, which were observed in the Caixões River. The differentiated use
301 of microhabitats as an explanation for the coexistence of populations was also found in studies done by
302 Geheber & Frenette (2015).

303 Therefore, we corroborate the previous studies that these four Characidae populations partition
304 dietary resources that are available in the stream. These patterns may be related to differences in
305 morphology, the segregation of microhabitats for feeding and the high abundance of resources. Based on
306 the isotopic data, we demonstrate that vegetal materials are assimilated by the *Astyanax* species and that
307 species complement their plant-based diets with animal matter, which was highly assimilated relative to
308 the proportions consumed. The small changes in the trophic classification of these species is very
309 important for ecology of these species, particularly because two species were described recently (Lucena
310 et al. 2013b) and *Bryconamericus* sp. is a new species, and therefore, have poorly known ecologies.

311

312

313 **Acknowledgments**

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315 We thank the colleagues of the Ichthyology Lab at the Universidade Federal do Rio Grande do Sul for
316 field work; the MSc. J. Ferrer, MSc. P.C. Silva and Dr. L.R. Malabarba for help in identifying the species
317 studied; and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Proc. 1104786, to
318 KOB) for financial support.

319

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488 Table 1. Pianka's index results for observed niche overlap values for four Characidae species and
489 simulated values for the null model. The asterisks indicate the sampling months with intermediate and
490 high niche overlap.

Sampling unit	Observed index	Mean of simulated index	Variance of simulated index	p (observed \geq simulated)
June 2012	0.20	0.11	0.006	0.12
August 2012	0.10	0.09	0.005	0.34
October 2012	0.37	0.15	0.002	0.06
December 2012 *	0.42	0.19	0.006	0.008
February 2013	0.11	0.11	0.005	0.40
April 2013	0.20	0.17	0.01	0.28
June 2013 *	0.63	0.22	0.001	0.002

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497 Table 2. Functional groupings of prey items used in the mixing models to estimate the diets of *Astyanax*
 498 *xiru*, *Astyanax procerus*, *Bryconamericus* sp., and *Bryconamericus iheringii*.

Source	Sub-source	C/N	¹⁵ N	¹³ C
Aquatic plant	Podostemaceae	12.6	6.0±1.4	-20.1±0.9
Aquatic plant	Podostemaceae	10.9	6.3±1.7	-25.7±1.4
Aquatic plant	Bryophyta	14.1	4.8±0.9	-27.8±2.5
Terrestrial plant	Bamboo	23.7	-0.6	-32.1
Terrestrial plant	Leaves	21.3	1.71	-28.7
Algae	Filamentous algae	9.8	4.6±0.2	-19.4±1.1
Algae	Cyanophyceae	11.2	3.1	-16.8
Aquatic invertebrate	Ephemeroptera	5.4	5.3±0.1	-27.9±0.6
Aquatic invertebrate	Trichoptera	5.5	6.4±0.9	-28.0±2.5
Aquatic invertebrate	Plecoptera	5.4	5.6	-27.9
Aquatic invertebrate	Odonata	5.1	7.5	-27.7
Aquatic invertebrate	Megaloptera	5.2	7.3	-25.9
Terrestrial invertebrate	Ant	5.6	3.65±0.7	-25.3±0.7
Terrestrial invertebrate	Bee	5.6	3.7	-24.4
Terrestrial invertebrate	Oligochaeta	5.5	4.01	-25.2

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505 Table 3. Mean (95% C.I.) assimilation of generalized groups of prey items by four characid species:
 506 *Astyanax procerus*, *Astyanax xiru*, *Bryconamericus iheringi*, and *Bryconamericus* sp.

Prey item	Consumer			
	<i>A. procerus</i>	<i>A. xiru</i>	<i>B. iheringi</i>	<i>Bryconamericus</i> sp.
Aquatic invertebrates	1 (0-90)	12 (2-32)	75 (63-86)	73 (60-88)
Terrestrial invertebrates	37 (0-100)	57 (42-91)		21 (12-32)
Aquatic plants	47 (0-100)	31 (38-79)		
Terrestrial plants	14 (0-77)		2 (1-5)	5 (2-12)
Algae			23 (14-33)	

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509 **Supporting Information**

510 **Table S1.** Food items consumed by four Characidae species in the seven sampling units (months) and
 511 utilized to calculate the Piaka's index and null model.

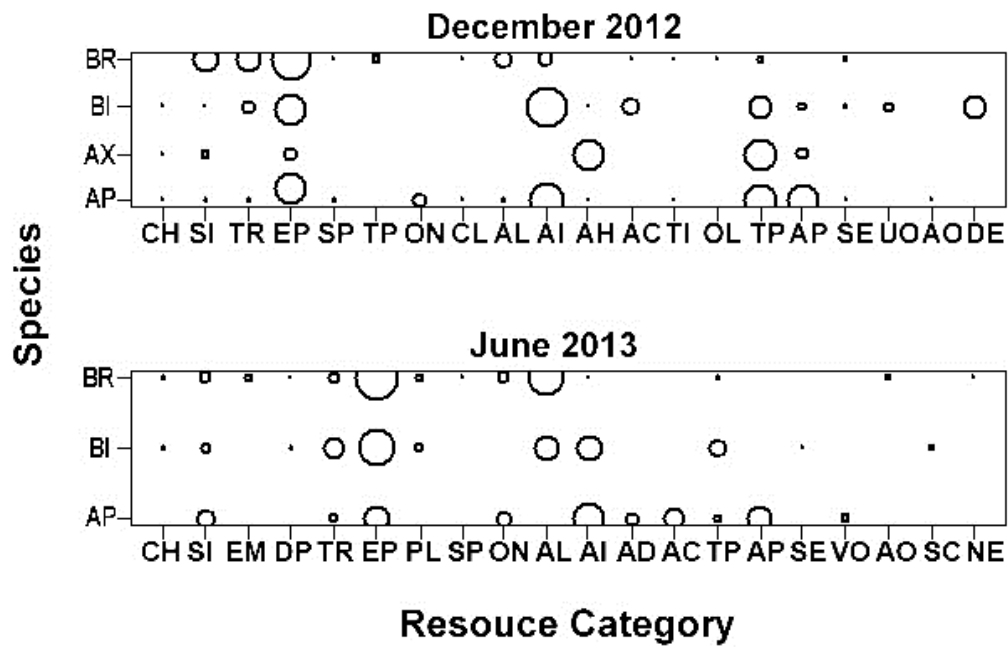
512 **Figure legends**

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514 *Fig. 1.* Observed feeding overlap matrix by consumed resource to four Characidae
515 species sampling. The size of the circles correspond to the proportion of the diet
516 represented by each resource. Species: BR= *Bryconamericus* sp; BI= *Bryconamericus*
517 *iheringii*; AP= *Astyanax procerus*; AX= *Astyanax xiru*. Resources: CH=
518 Chironomidae; SI= Simuliidae; TR= Trichoptera; EP= Ephemeroptera; SP=
519 Simuliidae pupae; TP= Trichoptera pupae; ON= Odonata nymph; CL= aquatic
520 Coleoptera larvae; AL= aquatic Lepidoptera larvae; AI= aquatic insects remains; AH=
521 adult Hymenoptera; AC= adult Coleoptera; TI= terrestrial insects remains; OL=
522 Oligochaeta; TP= terrestrial plants; AP= aquatic plants; SE= sediment; UO=
523 unidentified organic matter; AO= animal organic matter; DE= detritus; EM=
524 Empididae; DP= Diptera pupae; PL= Plecoptera; AD= adult Diptera; VO= vegetal
525 organic matter; SC= scale; NE= Nematoda.

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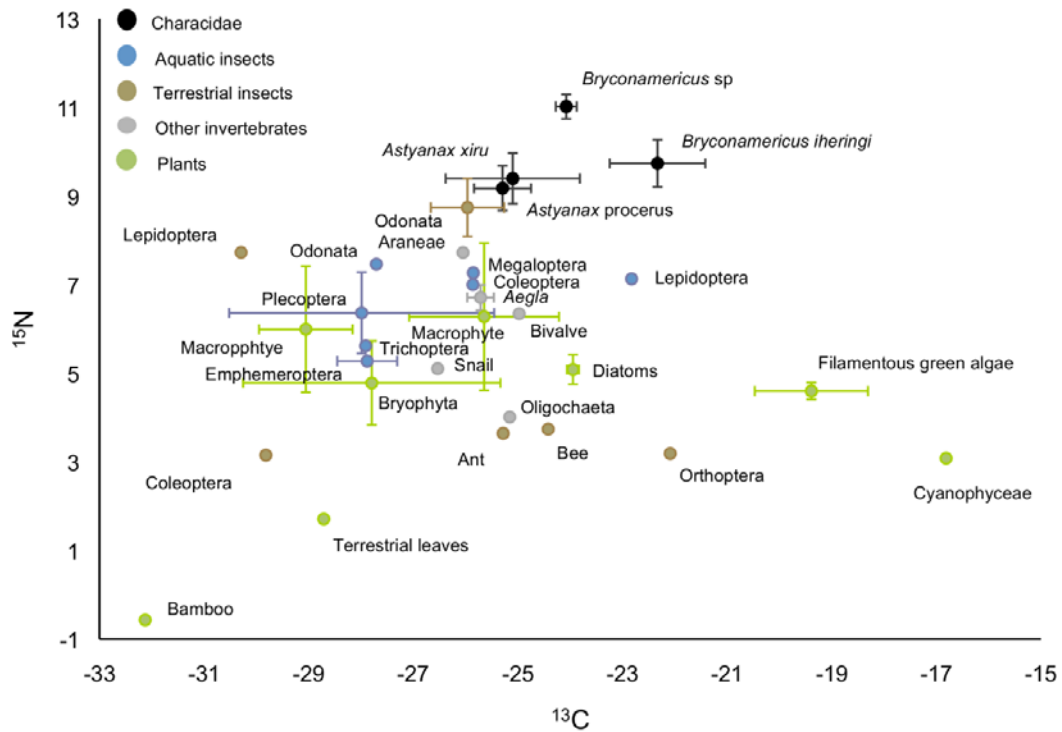
527 *Fig. 2.* Bi-plot (mean±SD) depicting the isotopic relationships among *Astyanax procerus*,
528 *Astyanax xiru*, *Bryconamericus iheringi*, and *Brycomamericus* sp. and their potential prey items.
529 Note that some items are shown that were not included in the mixing models (Table 1) because
530 they are not prey items of the characids.



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532 Fig. 1.

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535 Fig. 2

CONCLUSÕES GERAIS

- As 42 espécies de peixes coletadas para a região estudada perfizeram um total de 26% das espécies encontradas para o sistema da Laguna dos Patos. Este total de espécies foi considerado representativo para a região, principalmente por não se ter estudos de levantamento de ictiofauna para riachos que contemplem uma escala temporal;
- O primeiro registro de ocorrência da espécie *Phalloceros spiloura* para o sistema da Laguna dos Patos, mostra a importância de se coletar em locais pouco explorados cientificamente;
- Nossa hipótese de que existia segregação entre as três espécies de Siluriformes estudadas foi aceita e isto está relacionado ao uso diferencial de itens alimentares nos diferentes estágios de vida. Essas diferenças na dieta refletem as diferenças morfológicas e os diferentes hábitos de vida das espécies;
- Existem mudanças ontogenéticas mais pronunciadas em *Heptapterus* sp. e *Rhamdia quelen* do que em *Trychomicterus poikilos*, por isso os menores valores de sobreposição são encontrados para as duas primeiras espécies, quando analisamos o fator ontogenético;
- O processo de mudança de dieta durante todo o crescimento da espécie é, sem dúvida, uma forma de expandir ou mudar o nicho trófico das espécies, a fim de evitar a concorrência inter e intra-específica e manter a coexistência das mesmas;
- Verificou-se a diferenciação alimentar para 11 espécies simpátricas de Characidae, essas espécies se distribuíram em cinco guildas tróficas. Os itens responsáveis pela diferenciação da dieta foram aqueles usados em maiores proporções por cada espécie, como plantas aquáticas, insetos terrestres e insetos aquáticos;
- Neste estudo, demonstramos, mais uma vez, que a morfologia da boca e dos dentes estão estritamente correlacionados com a dieta das espécies, fazendo, desta forma, com que o fator morfológico contribua para a diferenciação do padrão alimentar das espécies;

- As relações encontradas entre morfologia e padrão alimentar servirão de base para refletirmos e buscarmos maior compreensão sobre os padrões de co-evolução da morfologia e padrões tróficos dentro de um grupo tão diverso como Characidae;
- Não obstante, com este estudo nós corroboramos os estudos prévios de que existe partição de recursos entre espécies de Characidae. Mais uma vez, este padrão está relacionado com as diferenças morfológicas, com a segregação de microhabitats para alimentação e com a alta abundância de alguns recursos.
- Baseado nos dados isotópicos, nós demonstramos que materiais vegetais são assimilados por duas espécies de *Astyanax* e que estas espécies complementam sua dieta herbívora com material animal, o qual teve sua assimilação relativa maior do que a proporção consumida, indicando assim a onivoria de ambas as espécies;
- Notoriamente, nós comprovamos que a análise de conteúdo estomacal é importante para a identificação dos itens consumidos pelas espécies, sendo indispensável para se conhecer a biologia das espécies de peixes. Porém, quando possível, esta análise deve ser complementada com estudo de isótopos estáveis que nos dão uma maior compreensão da biologia alimentar das espécies, expandindo o conhecimento da ingestão mecânica dos itens alimentares ao patamar de assimilação de nutrientes. Isso reduz os erros de interpretação e classificação das espécies em suas respectivas guildas tróficas;
- A correta classificação trófica é muito importante para estabelecer os padrões ecológicos das espécies, principalmente neste estudo em que duas das espécies de Characidae foram recentemente descritas e *Bryconamericus* sp. é uma nova espécie.
- As espécies como um todo, parecem coexistir, principalmente, pelas diferenças morfológicas que possuem, pelo uso diferencial de microhabitats para alimentação e, o fato de que quando as espécies compartilham recursos, estes são abundantes no sistema aquático, como insetos aquático e material vegetal aquático e terrestre.