UNIVERSIDADE FEDERAL DO PARÁ

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA AQUÁTICA E PESCA

KURT SCHMID

BAITED REMOTE UNDERWATER VIDEO, UMA PROMISSORA FERRAMENTA NÃO-DESTRUTIVA PARA AVALIAR ASSEMBLÉIAS DE PEIXES EM RIOS AMAZÔNICOS DE ÁGUA CLARA: TESTANDO EFEITOS DE ISCA E HÁBITAT

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Dissertação apresentada ao Programa de Pós-graduação em Ecologia Aquática e Pesca da Universidade Federal do Pará, como requisito parcial para obtenção do título de Mestre em Ecologia Aquática e Pesca.

Orientador: Prof. Dr. Tommaso Giarrizzo

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RESUMO

A inacessibilidade dos ecossistemas aquáticos e as dimensões quase continentais da bacia amazônica vêm dificultando historicamente a aquisição *in situ* de conhecimento científico sobre sua fauna de peixes altamente diversificada. Especificamente escassos são dados ecológicos quali-quantitativos e estudos sobre a ictiofauna reofílica que ocorre nas extensas áreas de corredeiras, típicas para rios amazônicos de água clara. Tais ambientes com elevada energia hídrica são difíceis de navegar e prejudicam métodos tradicionais invasivos de levantamento ictiofaunístico. No presente projeto de mestrado foi testado e adaptado um método não-invasivo que emprega técnicas de video subaquático remoto, o BRUV (*Baited Remote Underwater Video*), para estudar a íctiofauna reofílica em dois hábitats aquáticos distintos do médio rio Xingu. Comparou-se a estrutura da assembleia de peixes (I) entre os hábitats de praia e corredeira/pedral e (II) entre cinco tipos de isca de atração (sardinha, pescada-branca, comida de gato, milho doce e controle sem isca), testados para desenvolver um protocolo de amostragem com BRUV para tais ambientes lóticos em rios de água clara da região amazônica e, eventualmente, em outras regiões tropicais do mundo. Em 80 amostras de 120 minutos de vídeo subaquática, coletados em julho de 2014, forma registrados um total de 2.460 peixes de 56 taxa, pertencentes à 13 famílias. Diferenças na composição da assembleia, na riqueza de espécies e na abundância relativa foram detectadas entre os tipos de hábitats e entre as iscas. Os resultados sugerem que a sardinha (*Sardinella brasiliensis*) produz os melhores resultados e poderá ser recomendada como isca padrão para a amostragem com BRUV em rios amazônicos de água clara. Os dados e as imagens de vídeo digital refletem a ictiofauna única e altamente diversa do rio Xingu anterior aos previstos impactos ambientais irreversíveis e de larga escala, oriundos do represamento do rio e da futura operação da usina hidrelétrica de Belo Monte.

Palavras-Chave: Rio Xingu, Métodos não-extrativistas, Peixes reofílicos

ESTRUTURA DA DISSERTAÇÃO

A dissertação foi elaborada conforme a formatação do Programa de Pós-graduação em Ecologia Aquática e Pesca da Universidade Federal do Pará apresentando um artigo científico submetido, e proposto por dois revisores para publicação, em revista internacional (Hydrobiologia – The International Journal of Aquatic Sciences) e um texto introdutório e conclusões gerais da pesquisa realizada.

INTRODUÇÃO GERAL

Na bacia amazônica as dimensões quase continentais e a inacessibilidade de muitos de seus ecossistemas aquáticos vêm dificultando historicamente a aquisição *in situ* de conhecimento científico sobre sua fauna altamente diversificada de peixes neotropicais (Bohlke et al., 1978; Zuanon, 1999; Camargo, 2004, Camargo et al., 2005). A maior bacia hidrográfica do mundo, com uma área superior à 7,3 milhões de quilômetros quadrados, abriga mais que 2.500 espécies de peixes conhecidas e, estima-se, mais 1,000 novas espécies a serem ainda descobertas (Junk et al., 2007; Freitas et al., 2014). A escassez de conhecimento científico ao respeito desta diversidade íctica e seus ecossistemas aquáticos é um dos maiores obstáculos para sua conservação.

Especificamente escassos são dados sobre espécies reofílicas que habitam as extensas áreas de corredeiras (Bohlke et al., 1978; Helfman 1983; Zuanon et al., 1999), muitas vezes não navegáveis e impossíveis de amostrar com métodos convencionais. Na Amazônia, tais corredeiras ocorrem tipicamente nos rios de água clara (Sioli, 1984) como, por exemplo, os rio Trombetas e Xingu no qual essa pesquisa foi desenvolvida. Estes rios drenam topografias com desníveis acentuados, adquirindo elevados níveis de energia hídrica (potencial e cinética), tornando-os alvos de planos para o aproveitamento hidroenergético (Zuanon, 1999). Frente aos planos ambiciosos do governos brasileiro para o aproveitamento do grande potencial hidroenergético da bacia amazônica (PNE 2030) destaca-se ainda mais a necessidade de elevar a quantidade e qualidade de estudos sobre os peixes reofílicos desta região.

Atualmente, a integridade dos ecosistemas aquáticos e a diversidade íctica do rio Xingu estão ameaçadas pela construção e a futura operação de uma das maiores projetos de aproveitamento hidroenergético do mundo, a Usina Hidrelétrica de Belo Monte (UHE Belo Monte) (Szabaj, 2015; Winemiller et al., 2016). A usina foi planejado para a opreção em outono de 2015, reagendado para finalização da construção em 2016 (Szabaj, 2015; Winemiller et al., 2016) e o reservatório está sendo formado atualmente, em feveréiro de 2016. A alteração da vazão do rio, da qualidade da água e a perda e homogenização dos hábitats lóticos, tais como as cachoeira, corredeiras e pedrais impactam diretamente os peixes reofíicos e migradores que dependem de tais condições hidrológicas para seus processos de reprodução e recrutamento (Agostinho et al., 2001; Junk et al., 2007; Agostinho et al., 2008; Barbosa et al., 2015).

Para levantar dados de ictiofauna em estudos ecológicos e programas de monitoramento, porém, são empregados tradicionalmente técnicas de captura que

resultam na retirada e, muitas vezes, no sacrifício de elevados números de indivíduos de peixes (Cappo et al., 2004; Watson, 2004). Técnicas observacionais não-invasivas, como o censo visual subaquático ou *Underwater Visual Census* (UVC) em mergulho, tornaram-se os métodos preferidos para estudar peixes marinhos em águas rasas com boa visibilidade de água (Murphy & Jenkins, 2010), especificamente para estudos realizados em áreas de conservação ou com presença de espécies ameaçadas e/ou endêmicas (Wraith, 2007). A amostragem em UVC, porém, é limitada à profundidade e ao tempo de fundo máximos do mergulhador (Watson, 2004). Interações de peixes com o mergulhador, assim como, a identificação e contagem errônea de espécies ou a variabilidade entre observadores podem ainda prejudicar a precisão e acurácia dos resultados (Langlois et al., 2010).

Entretanto, muitas das desvantagens do UVC e dos métodos de captura acima expostas podem ser superadas pelo emprego de técnicas não-invasivas e não-destrutivos de filmagem subaquática como, por exemplo, o BRUV (*Baited Remote Underwater Video*) (Watson, 2004; Dorman et al., 2012). No método de BRUV são coletados vídeo-amostras da ictiofauna por meio da atração dos peixes com iscas no campo de visão de câmeras posicionados no substrato. Limitados unicamente pelas possibilidades técnicas, os sistemas de BRUV podem operar em grandes profundidades, sobre qualquer tipo de hábitat bentônico (Cappo et al., 2004) e sem necessidade de captura e sacrifício de peixes.

A grande maioria dos trabalhos com BRUV ainda se concentra nos ambientes recifais na costa da Austrália Ocidental, na Nova Zelândia e África do Sul. Pesquisas realizadas em águas continentais são os mais escassos. Até o presente momento somente duas pesquisas foram conduzidas, ambas no continente australiano, como indica a literatura. Ebner & Morgan (2013) aplicaram a amostragem com BRUV para estudar diferentes técnicas de amostragem com vídeo subaquático e compará-las com métodos tradicionais de captura (redes) em três lagos fluviais (*waterholes*) do Fortescue River na zona tropical do norte da Austrália. Na área estudada o BRUV demonstrou desempenho melhor que a captura com redes, especificamente na detecção espécies, e foi útil para identificar uma estruturação da assembléia de pexies relacionada à profundidade (Ebner and Morgan, 2013). Ainda Ebner et al. (2014) compararam o censo visual em mergulho livre (*snorkeling*) com o BRUV em ambientes de cachoeiras e sequências rasas da corredeiras e piscinas no rio Harvey Creek na Austrália. Eles concluíram que o segundo método foi especificamente efetivo para detectar predadores de topo e espécies com elevada capacidade olfativa como, por exemplo, enguias. Ainda, a amostragem com

BRUV aumentou a segurança do observador nesses ambientes aquáticos continentais com perigos potenciais (ex. crocodilios, enredamento, fortes correntezas) (Ebner et al., 2014).

Frente à inexistência de um único método tradicional de levantamento ictiofaunístico possível de ser aplicado em uma variedade de hábitats lóticos amazônicos com substratos complexos, empregou-se nesta dissertação o método de BRUV para estudar a assembleia de peixes neotropicais reofílicos que habitam os ambientes de praia e corredeira/pedral no médio rio Xingu.

Os desenhos amostrais de BRUV muitas vezes são desenvolvidas com base em estudos pré-existentes. Estudos em ambiente marinho apontam para a necessidade de fazer testes e desenvolver metodologias de BRUV adaptadas especificamente aos hábitats e condições ambientais locias (Gladtsone et al., 2012). Entre as maiores fontes de variabilidade de dados, notadamente dos resultados de abundância e diversidade relacionados aos níveis tróficos, foram identificados o tipo e a quantidade de isca empregada (Harvey et al., 2007; Wraith, 2007; Dorman et al., 2012; Hardinge et al., 2013). Pela ausência de estudos anteriores com essas técnicas de filmagem subaquática no continente sul-americano e, em específico, em águas continentais na região amazônica, integrou-se nessa pesquisa uma investigação metodológica com intuito de desenvolver uma metodologia da amostragem especificamente adaptada aos hábitats lóticos.

Definição dos objetivos

Os objetivos principais desta dissertação são:

(I) Investigar com o método BRUV a assembleia de peixes reofílicos em hábitats lóticos no trecho médio do rio Xingu, PA.

(II) Desenvolver um protocolo de amostragem com BRUV e de análise de vídeo adaptado aos rios amazônicos de água clara.

Os objetivos específicos são:

(1) Adquirir estimativas precisas de riqueza de espécies (S) e abundância relativa (N) da ictiofauna reofílica dos habitats lóticos do médio rio Xingu.

(2) Analisar diferenças na riqueza, abundância relativa e composição da assembleia de peixes amostrados com BRUV entre os dois hábitats lóticos, praia e corredeira/pedral, na área de estudo no médio rio Xingu.

(3) Testar cinco diferentes tipos de isca (sardinha, pescada, milho, comida de gato e um controle sem isca) quanto sua eficiência e eficácia de atração na amostragem de peixes reofílicos com BRUV, visando identificar a isca mais indicada para a padronização metodológica.

(4) Estabelecer e padronizar: tempo de amostragem, número de réplicas, tratamento e apresentação de isca, configurações de câmera, lançamento e posicionamento dos sistemas de BRUV, de forma que sejam adaptados às condições bióticas e abióticas de correnteza, visibilidade, densidade de peixes e substrato existentes.

(5) Avaliar a praticidade logística e o custo do uso das diferentes iscas para levantamentos em áreas remotas da bacia amazônica.

MATERIAL E MÉTODOS

Área de estudo

O rio Xingu é um dos principais afluentes do baixo rio Amazonas. Sua bacia hidrográfica estende-se sobre um território de aproximadamente 51 milhões de hectares nos estados do Pará e Mato Grosso (MMA, 2002; Isaac et al., 2015). Suas nascentes estão localizadas no estado de Mato Grosso e sua foz, ou confluência com o rio Amazonas, no município de Porto de Moz (PA).

A drenagem por rochas cristalinas antigas (pré-cambrianas) do escudo Brasileiro condicionou propriedades físico-químicos de pH entre 6,2 e 7,0; oxigênio dissolvido entre 6 e 7 mg/l e baixa carga de sedimentos e material orgânico em suspensão (Botelho & Camargo, 2010). São as características de um rio amazônico de águas claras (Sioli, 1984) com uma visibilidade de água que pode ultrapassar 4 metros (Barthem & Fabré, 2004), uma condição favorável ao uso de técnicas de filmagem subaquática. O nível da coluna d'água do rio Xingu acompanha a sazonalidade bem definida dos períodos de chuva intensa (enchente e cheia) de dezembro a maio, com uma vazão média do rio de 8.000 a 10.000 m^3/s, e de estiagem (vazante e seca) de junho a novembro com vazão média de 2.000 m^3/s (Camargo et al., 2004). A paisagem fluvial apresenta nas épocas da vazante e seca, período em que a visibilidade da água permite a amostragem com BRUVS, extensas áreas de corredeiras entrelaçados com praias fluviais e bancos de areia expostos.

Aproximadamente 470 espécies de peixes foram registradas no rio Xingu (Camargo et al., 2004). Estima-se, porém, que no total habitam em torno de 600 espécies as águas do Xingu (Isaac et al., 2002; Camargo et al., 2004). A grande biodiversidade íctica ainda conta com uma elevada taxa de endemismo (Camargo et al., 2012; Winemiler et al., 2016). As espécies endêmicas de maior importância econômica para a pesca ornamental no rio Xingu são a arraia de fogo *Potamotrygon leopoldi* Castex & Castello, 1970 e, principalmente, o acari zebra *Hypancistrus zebra* Isbrücker & Nijssen, 1991 ameaçado pela sobrepesca (Carvalho Júnior, 2009; Camargo et al., 2012) entre outras espécies.

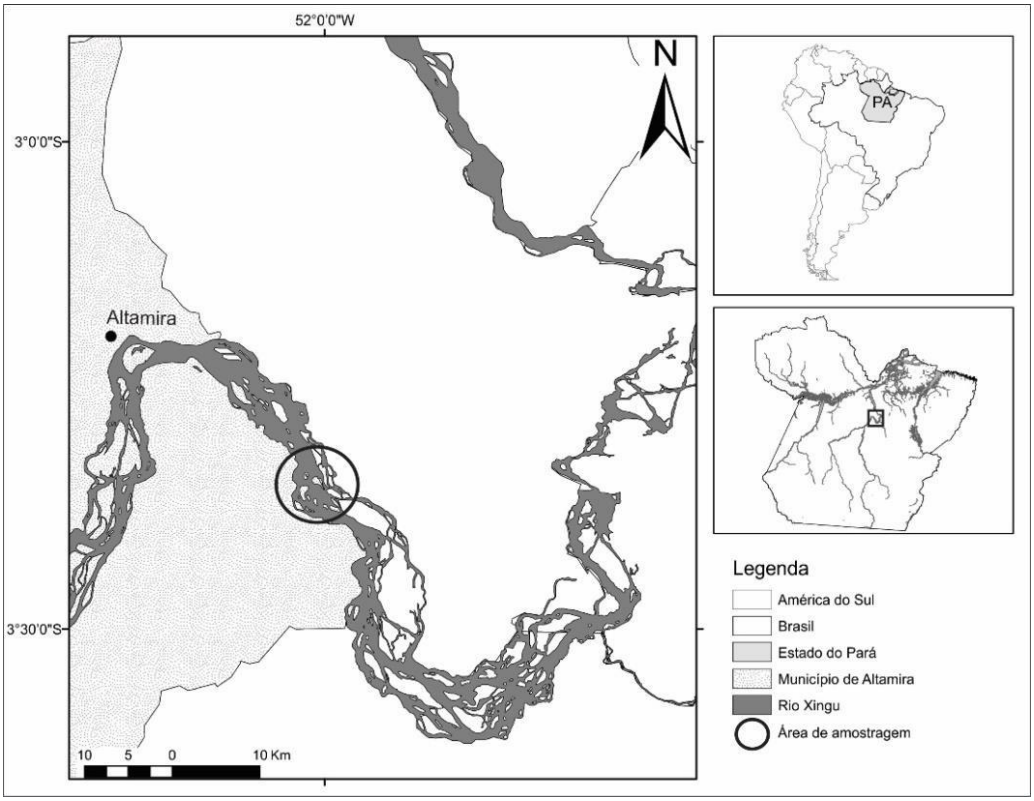

Figura 1: Mapa da área de estudo no baixo rio Xingu, aproximadamente 30 Km à jusante de Altamira.

Procedimentos em Campo

O estudo foi conduzido na região da Volta Grande no baixo rio Xingu (Sawakuchi et al., 2015), aproximadamente 30 km à jusante da cidade de Altamira. O estudo foi limitado a um trecho do rio com extensão de 4.3 km entre os pontos de lançamento mais distantes (03°19'58.9" S 52° 0'9.09" W and S 03°22'16.2" S 51°59'51.36" W – WGS84).

Usou-se câmeras digitais de alta definição GoPro Hero Black Edition 3+ (www.gopro.com) com distância focal fixada para infinito, em configurações de ângulo aberto (wide angle) de 1920 x 1080 pixels e filmagem em 30 imagens por segundo (30 fps). As câmeras foram posicionadas em estruturas estáveis de metal galvanizado, construídas com referência ao modelo de single-BRUV desenvolvido pelo Instituo de Ciências Marinhas da Austrália AIMS (*Australian Institute of Marine Science*). Boias com cordas flutuantes foram amarradas para posicionamento e recuperação dos sistemas.

Todos os lançamentos foram realizados de forma embarcada, padronizados para águas rasas com profundidade máxima de dois metros e uma distância mínima de 200 metros entre os locais de lançamento. Nenhum sistema de BRUV foi posicionado à jusante de outro na mesma linha de correnteza para evitar influências nos resultados causadas pela sobreposição das plumas de isca. A câmera e isca foram alinhadas com a

correnteza, evitando a atração de peixes na direção oposta, para atrás do campo de visão da câmera. A amostragem foi limitada para o período diurno, entre 8h30 e 17h30 horas, evitando quaisquer efeitos de comportamento crepuscular ou noturno de certas espécies de peixes (Willis & Babcock, 2000; Harvey et al., 2007; Ebner & Morgan, 2013; Wraith et al., 2013; Santana-Garcon et al., 2014). Antes de cada lançamento registraram-se as características físico-químicas da água no local. Temperatura da água (°C) e oxigênio dissolvido (mg/l) foram medidas com o oxímetro manual (Instrutherm MO-910), pH e condutividade elétrica (mS/cm) com sonda manual (Hanna Instruments HI-98129) e velocidade de correnteza (m/s) com correntômetro digital (Flowatch from JDC Electronic SA).

Para seleção dos locais de lançamento de BRUV identificaram-se as I) características de substrato para os dois tipos de hábitat lótico: praia (substrato não consolidado) e corredeira/pedral (substrato consolidado) e II) da paisagem fluvial para seleção dos locais de lançamento de BRUV. Entre estes lançamentos foram aleatorizados os cinco tipos iscas com oito réplicas para cada hábitat totalizando 80 amostras com aproximadamente 130 minutos de vídeo subaquático. Posteriormente, para a análise de vídeo padronizou-se todas as amostras para 120 minutos de, contando a partir do momento do assentamento da estrutura de BRUV no fundo.

Testou-se sardinha *Sardinella brasiliensis* (Steindachner, 1879) da marca Costa Sul Pescados S/A (www.costasul.com.br) descongelada, cortada e amassada; pescada branca *Plagioscion squamosissimus* (Heckel, 1840) comprada com pescadores do rio Xingu, cortada e amassada; comida de gato da marca Whiskas contendo salmão; milho doce enlatado da marca Goiás Verde (www.goiasverde.com.br) e “branco” ou controle sem isca. A seleção das iscas para o presente projeto baseou-se em informações obtidas à partir das referências bibliográficas, em critérios financeiros e de praticidade logística para estudos *in situ* na região amazônica, assim como, na constante disponibilidade e uniformidade da isca para garantir que os resultados podem ser comparados posteriormente em escala temporal. A sardinha foi escolhida pelas propriedades muito semelhantes aos da espécie *Sardinops sagax* (Jenyns, 1842). *S. sagax* tem sido a isca de atração preferencial em pesquisas com BRUV, principalmente na Austrália e Nova Zelândia por causa de sua carne mole com alto teor de óleos (Cappo et al., 2004; Hardinge et al., 2013; Wraith et al., 2013). Um estudo comparativo conduzido por Wraith et al. (2013) em recifes de pedra no parque marinho da Baía de Jervis (JBMP) em New South Wales, Austrália, revelou que o *S. sagax* produziu os mais consistentes resultados de riqueza de espécies e abundância. A pescada *P. squamosissimus* foi integrada ao

estudo por ser um peixe de carne mole, nativo e abundante na área de estudo, podendo servir potencialmente como alternativa econômica e sustentável à sardinha por ser adquirida localmente na área de estudo, ao contrário da sardinha comprada na cidade de Belém e transportada de forma congelada por vias aéreas até a cidade de Altamira. A comida de gato contendo peixe, segundo (Dorman et al., 2012) mostrou bom poder de atração de peixes recifais, amostrando a maior riqueza média nesse estudo conduzido na Austrália ocidental. Outro argumento pela escolha de comida de gato foi sua composição, contendo tanto carne de peixe, quanto componentes vegetais. Esperou-se que essa isca seria capaz de amostrar uma ampla gama de grupos tróficos: herbívoros, onívoros e carnívoros. Milho doce foi incluído no estudo pela abundância de espécies herbívoros e frugívoros na área de estudo e, como é o caso da comida de gato, pela praticidade logística e durabilidade em forma enlatada. Comida de gato foi comprada em sachês e milho doce de forma enlatada. Ao contrário das iscas de peixe, estes não necessitam de refrigeração durante toda a campanha de coleta in situ - ambos critérios importantes para pesquisas de campo na região amazônica.

Um total de 800 g de isca foi usado em cada lançamento e descartadas após recuperação dos dispositivos de BRUV. Os efeitos da quantidade de isca foram investigados por Hardinge et al. (2013) em um levantamento ictiofaunístico com stereo-BRUV na Five Fathom Bank, na costa leste da Austrália. Nesse ambiente marinho, a menor quantidade testada de 200 g comprovou ser tão efetiva quanto as quantidade maiores de 1000 g e 2000 g durante 60 minutos de tempo gravação. Levando em consideração o fluxo constante em um rio, a densidade desconhecida de peixes nos pontos amostrais e um tempo de amostragem maior, assumiu-se que a isca será lavada e esgotada numa taxa mais acentuada. Usou-se, portanto, 800 g de isca com a intenção de criar uma pluma de odor constante durante toda a amostragem e de poder excluir “quantidade de isca” como variável.

As iscas de peixes (sardinhas e pescadas) foram transportadas congeladas em caixas térmicas, cortadas em pedaços e amassadas no recipiente de isca imediatamente antes de cada lançamento para melhor liberação dos sucos e óleos (Willis & Babcock, 2000; Langlois et al., 2010; Fitzpatrick et al., 2012; Hardinge et al., 2013). O saco de isca foi construído com uma tela de PVC do tipo “galinheiro” com malha de 5 mm. Para os lançamentos de controle (branco) usou-se sacos de isca específicos, mantidos isolados das iscas ou de outros sacos de isca durante todo o estudo, para evitar quaisquer influência (Harvey et al., 2007). A malha menor foi empregada para evitar que a comida

de gato e o milho doce fossem lavadas para fora do recipiente já nos primeiros minutos após o lançamento por causa de sua granulometria inferior.

Os recipientes foram posicionados a 50 cm da lente da câmera devido à visibilidade reduzida e presença de numerosos peixes de pequeno porte, principalmente da família Characidae. Um distância maior de 80-120 cm, como estabelecida na maioria dos levantamentos com BRUV em ambientes marinhos e estuarinos (Harvey et al., 2007; Wraith, 2007; Langlois et al., 2010; Gladstone et al., 2012; Ebner & Morgan, 2013; Wakefield et al., 2013) inviabilizou ou reduziu as chances de identificação de espécies de forma drástica.

Procedimentos em Laboratório

Os arquivos das amostras de vídeo foram baixados em discos rígidos externos portáteis de 2 TB (Toshiba Canvio Basics 3.0 e Samsung M3 Portable), organizados e pré-analisados ainda em campo para identificar e repetir lançamentos que precisavam ser excluídas do conjunto de dados devido um mal-posicionamento ou bolhas de ar na caixa estanque na frente da lente da câmera, por exemplo.

As gravações foram analisadas com o programa livre VLC (www.videolan.org). Espécies de peixes foram identificadas até o menor nível taxonômico possível. Para espécies impossíveis de identificar descreveu-se e estabeleceu-se um morfotípo. Determinaram-se a hora da primeira chegada (avistagem/registro) ou first arrival time (FAT) de cada espécie a abundância relativa (MaxN); o número máximo de indivíduos de uma dada espécie contados dentro do mesmo campo de visão na mesma hora. MaxN é uma estimativa conservadora (Watson et al., 2005; Becker et al., 2010) porém evita a recontagem de um mesmo peixe (Willis & Babcock, 2000; Cappo et al., 2004; Harvey et al., 2007; Becker et al., 2010; Gladstone et al., 2012; Hardinge et al., 2013). Cada amostra de 120 minutos foi dividida em 12 subamostras de 10 minutos, repetindo a análise de MaxN em cada subamostra para gerar curvas de acumulação de espécies. Todas as amostras foram analisadas pela mesma pessoa. Especialistas taxonômicos somente foram consultados em casos duvidosos.

CAPÍTULO 1

Este capítulo (artigo científico) foi elaborado e submetido de acordo com as normas do periódico *Hydrobiologia,* disponíveis no *site*:

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(Fonte e espaçamento foram alterados aqui para facilitar a leitura)

Baited remote underwater video as a promising non-destructive tool to assess fish assemblages in clearwater Amazon rivers: testing the effect of bait and habitat type

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Abstract

Baited remote underwater video (BRUV) is being employed in marine ecosystems to cost effectively assess fish fauna non-extractively and with minimal species bias. The technique has had limited application in freshwater ecosystems. Reophilic fish assemblages of the Xingu River, an Amazonian clearwater river in Northern Brazil, were sampled with BRUV systems. Two-hour video recordings were collected using five different bait treatments (sardine, freshwater croaker, cat food, sweet corn and no bait) from two lotic habitat categories (rock and sand bottom). A total of 2460 fish from 56 different taxa, belonging to 13 families, were counted from the 80 BRUV deployments. Significantly different fish assemblage, species richness and abundances were detected between habitat types and bait treatments. Our results suggest that crushed sardine produces optimal results and should be used as a standardized bait for BRUV sampling in clearwater Amazon rivers. Crushed sardines attracted reophilic fish more efficiently and yielded the highest values of species richness, relative abundance and number of exclusively sampled species. The results reflect the Xingu River's unique fish diversity prior to the expected large-

scale environmental degradations from the upcoming operation of the Belo Monte hydroelectric power plant.

Key Words: Freshwater ecology; Neotropical fish; lotic freshwater habitats; Xingu River; Brazil; Amazon region; hydroelectric power plants

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Introduction

Conservation management and monitoring of the status of indicator species or assemblages requires the use of non-extractive methods (Pelletier et al., 2011) which can provide both accurate (small bias) and precise (low uncertainty) estimates of the metrics of interest (counts of the numbers of individuals or species diversity of fish) (Andrew et al., 1987; Underwood et al., 2000; Jones et al., 2015). Yet every method used to estimate the abundance of fishes, including non-extractive underwater video sampling, has its own particular limitations and biases that need to be taken into consideration (Lincoln Smith, 1989). Pilot studies are important to address such method-related biases as they assist with the planning and optimization of sampling methods through the development of standardized protocols (Gladstone et al., 2012).

Observational fish fauna sampling techniques have become frequently employed alternatives for sampling shallow water marine fishes in areas with good water visibility (Murphy and Jenkins 2010). The main categories include visual census performed by divers (UVC), hydroacoustics (remote acoustic sensing) and underwater video from static and mobile camera systems such as remotely operated vehicles (ROVs) equipped with video cameras, and finally baited remote underwater video (BRUV) (Bortone et al., 1986; Cappo and Brown, 1996; Watson, 2004). Comparisons of data collected from traditional underwater visual census (UVC) by SCUBA divers have been made to data collected by divers on rebreathers (Lindfield et al., 2014), to data collected by diver operated underwater stereo-video systems (Harvey et al., 2002a,b, 2004, Watson et al., 2005, Holmes et al., 2013) and baited remote underwater video (BRUV) (Watson et al., 2005). These comparisons have highlighted some of the biases and limitations of UVC approaches. Along with the diver's physical restrictions (depth and bottom time) and biases related to fish behavior towards a SCUBA diver (Watson et al., 2005), UVC revealed a lack of precision in length estimation of reef fish when compared to video systems (Harvey et al., 2002a; Holmes et al., 2013). Further, data collected during UVC cannot be validated, contrarily to permanent video samples obtained with BRUV (Holmes et al., 2013).

BRUV sampling techniques have rapidly been adopted for assessing the composition, richness and habitat preferences and associations of marine fishes (Murphy and Jenkins 2010, Mallet and Pelletier 2014)). BRUVs have primarily been deployed in reef habitats (e.g., Willis and Babcock 2000; Langlois et al., 2010; Dorman et al., 2012). But also across sand (Schultz et al., 2012) and multiple habitats (Fitzpatrick et al. 2012, Harvey et al., 2013a), both during the day and at night (Harvey et al., 2012; Fitzpatrick et al., 2013). Studies have been conducted from very shallow to deeper mesophotic habitats across the continental shelf (Fitzpatrick et al., 2012) and down the continental slope to depths of 1500m and greater (Zintzen et al., 2012). They have also been used to evaluate fishes inhabiting pelagic habitats (Heagney et al., 2007; Santana Garcon et al., 2014a, b) and to a lesser extent, in estuarine ecosystems (Gladstone et al., 2012; Lowry et al., 2012). BRUVs have been shown to be cost effective (Watson et al., 2005; Langlois et al. 2010) and effective in detecting wide ranges of species and functional groups (Cappo et al., 2003; Harvey et al., 2007).

BRUV sampling designs are often developed from previous studies rather than optimizing the design for local environmental conditions and habitats. The absence of a specifically developed sampling protocol (i.e. bait type, soak time, replication, etc.) potentially compromises the value of the acquired data (Gladstone et al., 2012). For instance, the type and volume of bait were identified as a major source of variation in experimental outcomes, mainly on trophic level-related abundance and diversity results (Harvey et al., 2007; Wraith, 2007; Dorman et al., 2012; Hardinge et al., 2013).

BRUV methods have rarely been applied to sample freshwater fish assemblages. To our knowledge, only two surveys have been conducted, both in Australia. Ebner and Morgan (2013) applied BRUV sampling to study different video deployment techniques and compared species richness to conventional netting techniques in three Fortescue River waterholes, in the tropical north-western Australia. In the studied area underwater video outperformed conventional netting techniques, specifically in detecting species, and were useful to identify depth-related structuring of fish assemblages (Ebner and Morgan, 2013). Further, in clear tropical rainforest streams, Ebner et al.

(2014) compared BRUV stations to active visual surveys of aquatic vertebrates and invertebrates in pool habitats and concluded that the former were particularly effective in detecting top predators and olfactory-driven species. BRUV sampling improved observer safety in freshwater environments with potential hazards (e.g. crocodiles, entanglement, extreme flows).

Conducting *in situ* field studies on neotropical freshwater fish assemblages has historically been a major challenge in the Amazon river basin (Bohlke et al., 1978; Zuanon, 1999; Camargo et al., 2005). With an area exceeding 7.3 million km^2, the world's largest river basin is home to more than 2,500 known fish species, and another estimated 1,000 new species are yet to be discovered (Junk et al., 2007). The shortage of scientific knowledge regarding the Amazon river basin and the extraordinary fish diversity it contains is a major obstacle to conservation and management of this important ecosystem. A network of more than 1,000 tributaries with distinct limnologic characteristics (related to geomorphological factors) play a fundamental role in the patterns of biodiversity found in the Amazon basin (Lowe-McConnell, 1987). These water bodies are generally inaccessible and make biodiversity assessments logistically and financially challenging (Bohlke et al., 1978; Zuanon, 1999; Camargo et al., 2005). Studies on reophilic freshwater fish in topographically complex lotic habitats, such as fast flowing rock riffle and waterfall areas (Bohlke et al., 1978; Helfman, 1983; Zuanon et al., 1999), found principally in Amazon clearwater rivers (*sensu* Sioli, 1984), are particularly rare and yet are needed to understand basic aspects of their biology and ecology and habitat use.

Sampling tools for fish studies in Amazonian freshwater ecosystems are usually extractive capture techniques involving nets, fish traps or hook-and-line fishing techniques (Barthem et al., 1991; Camargo, 2004; Camargo et al., 2004; Botelho & Camargo, 2010; Giarrizzo et al., 2015). However, strong currents and complex bottom structures in the lotic habitats of Amazonian clearwater rivers hamper the use of such traditional methods and hold high risks of equipment damage. Potentially dangerous animals like caimans (*Melanosuchus niger* and *Caiman* spp.), freshwater stingrays (*Potamotrygon* spp. and *Paratrygon* spp.), piranhas (*Serrasalmus* spp. and *Pygocentrus* spp.) and electrical eels (*Electrophorus* spp.) make the direct observation by humans (*e.g.*, visual

census) hazardous. We propose that, under such conditions, BRUV could be a versatile, useful and cost effective method to sample the diverse fish fauna in these unique Amazonian freshwater ecosystems.

We used BRUV methods to assess fish fauna composition, species richness and relative abundance in the Xingu River, a South American freshwater system. The main goal was to test the effectiveness and feasibility of using BRUVs to assess fish assemblages in lotic habitats of a clearwater Amazon river. We specifically tested the effects of five different bait treatments (i.e., no bait; sweet corn; cat food with salmon; crushed freshwater croaker and crushed sardine*)* on fish fauna composition in two distinct lotic freshwater shallow habitats (i.e., rock and sand bottom). We were interested in which bait would maximize the species richness (S) and relative abundance (N) estimates (including species accumulation). Further we aimed to determine the number of replicates and the sampling time required to stabilize sampling precision and species accumulation and to provide directives towards an optimal BRUV sampling methodology for such lotic habitats.

Elevating the quantity and quality of studies on reophilic amazon fish is critical considering the Brazilian Government's ambitious plans (PNE 2030) to explore the Amazon basin's large hydropower potential. The Xingu River's ecological balance and its unique and diverse ichthyofauna are currently threatened by one of the world's biggest hydroelectric power plant projects, the Belo Monte dam (UHE Belo Monte). Planned for operation in fall 2015 and rescheduled for completion in 2016 (Szabaj, 2015; Winemiller et al., 2016), the reservoir is currently being formed and ready by February of 2016.

The alteration of flow speed, water quality and the loss and homogenization of lotic habitats such as rapids and riffles is a direct threat to reophilic and migratory fish which depend on such hydrological conditions for reproduction and recruitment processes (Agostinho et al., 2001; Junk et al., 2007; Agostinho et al., 2008; Barbosa et al., 2015). Below the dam (reservoir), the discharge reduction has the potential to dramatically change the availability of habitats for different species and to isolate fish populations though loss of habitat connectivity (Barbosa et al., 2015). Scientists alert for unparalleled biodiversity

loss due to the chosen site's exceptionally high fish species endemism (Szabaj, 2015; Winemiller et al., 2016); i.e. the popular Zebra pleco (armored catfish) *Hypancistrus zebra* (Isbrücker & Nijssen, 1991) and the White-blotched river stingray *Potamotrygon leopoldi* (Castex & Castello, 1970). These species are already undergoing drastic population declines since they are highly sought after by the international ornamental fish trade.

Material and methods

Study area

The Xingu River is one of the main tributaries (approximately 2,000 km in length) of the southern lower Amazon (Fig. 1). Its headwaters are located in the border area between the states of Mato Grosso and Pará. At the Xingu River's lower section, where the study was conducted, water visibility frequently exceeds 4 m (Barthem & Fabré, 2004), allowing the use of underwater survey methods.

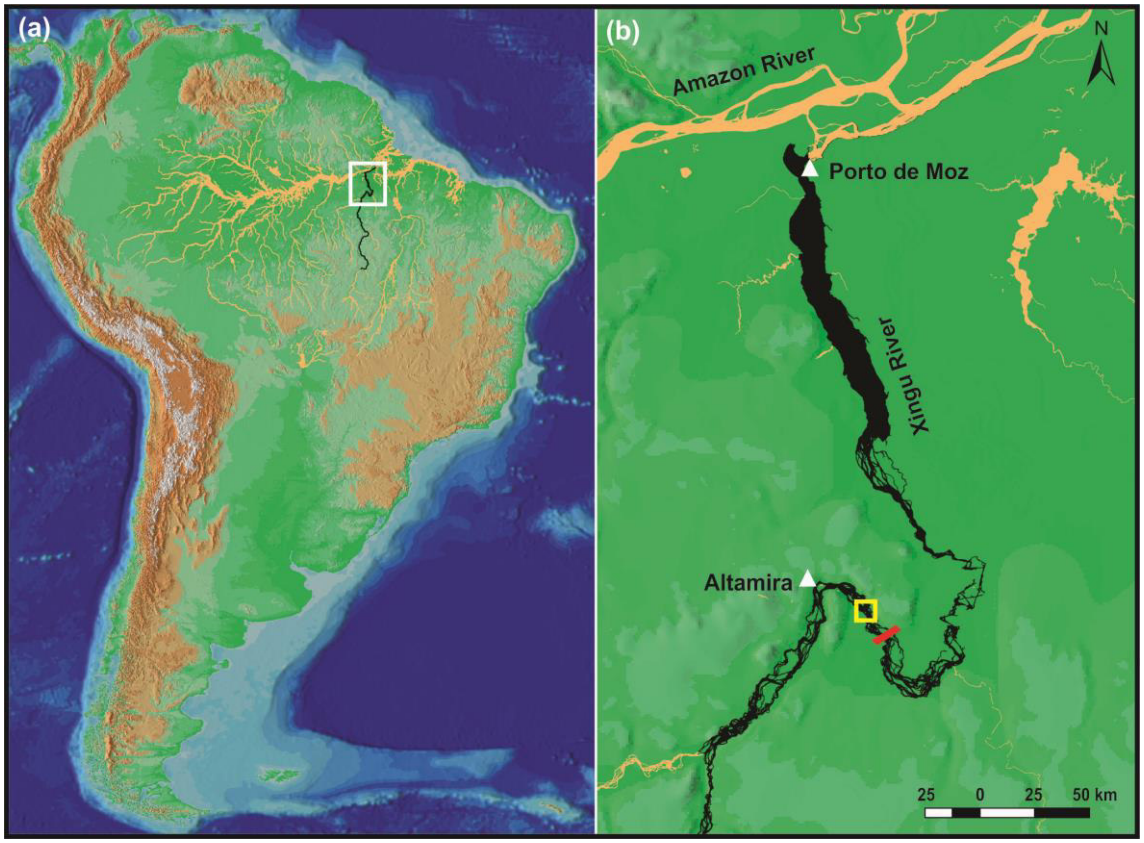


Fig. 1 Study area location at the lower Xingu River, State of Pará, Brazil and examples of sand and rock bottom habitats

The riverscape in the study area reflects a series of geological events which structured the Xingu river basin's geomorphology. Numerous waterfalls, rapids, river beaches and islands, separated by often strong currents, form an extensive heterogeneous spatial array of distinct freshwater habitats. These conditions have driven the evolution and distribution of the Xingu River's unique and highly diverse fish fauna resulting in a large number of endemic species (Camargo et al., 2004). Approximately 470 fish species have been recorded, yet estimations suggest fish diversity could be as high as 600 species (Camargo et al., 2004; Isaac et al., 2002) making this one of the world's richest freshwater fish assemblages.

Sampling procedure

A total of 80 BRUV deployments were performed in July 2014 during the Receding Water period (Barbosa et al., 2015) which marks the beginning of the dry season, when the increased water visibility favors the use of underwater video techniques. Sampling was conducted approximately 40 km downstream from the city of Altamira, at the Volta Grande area in the -Xingu River's lower section (Sawakuchi et al., 2015). The study site was limited to a river stretch of 4.3 km extending between the two most distant points of deployment (03°19'58.9" S 52° 0'9.09" W and S 03°22'16.2" S 51°59'51.36" W – WGS84). Single GoPro Hero 3+ Black Edition digital high definition (HD) cameras (www.gopro.com) were used with fixed focal length, 30 frames per second (fps) and a wide angle field of view (FOW) with 1080 x 1920 pixels. These high definition action cameras are a cost effective, yet well performing alternative to handheld cameras commonly used in BRUV studies (Struthers et al., 2015). The cameras worked with lithium batteries (1180 mAh), allowing approximately 130 minutes of recording, which was adopted for every deployment. The entire system was protected in a watertight case. After recovery, during video analysis, all samples were standardized to 120 minutes of footage, counted from the moment the device was positioned on the river bed. Our cameras were mounted on galvanized iron BRUV structures (Cappo et al. 2003, 2004). Bait containers were positioned 50 cm from the video system to attract fish closer to the camera and thus improve the identification of smaller species. The cameras were mounted on a small platform at a height of 30 cm off the BRUV systems

base. Buoys with floating ropes were attached to deploy and recover BRUV systems.

Sample locations were selected by i) visually identifying the substrate characteristics as either consolidated (rock) or unconsolidated (sand) substrate, and ii) considering the spatial extent and continuousness of at least 300 m for these characteristics in the surrounding area, thus to avoid biases originated by bait plume expansion over, downstream habitats. All deployments were boat-based, standardized to shallow-water (2 m maximum depth) and dropped randomly with a minimal lateral distance of 200 m between BRUVs. No device was positioned immediately downstream of another to avoid bias from overlapping bait plume. BRUVs were placed with camera and bait container facing downstream to maximize fish detection and to avoid fish attraction behind the camera's field of view (FOV). All surveys were limited to daylight period, from 0830 to 1700 hours, to exclude effects from crepuscular or nocturnal behavior patterns of many fish species (e.g., Willis & Babcock, 2000; Harvey et al., 2007; Ebner & Morgan, 2013) as well as avoid biases due to limitations imposed by the decrease of day light.

Experimental design

Five different bait treatments; i) crushed sardine, ii) crushed freshwater croaker, iii) fish containing cat food, iv) sweet corn and v) no bait (control) were randomly assigned between two distinct habitat types (i.e., rock and sand bottom). Eight replicate BRUVs were deployed for each bait treatment within each habitat type. Ungutted Sardine, *Sardinella brasiliensis* (Steindachner 1879), was chosen due to its similar bait characteristics to pilchard *Sardinops* spp. – high oil content and soft flesh (Cappo et al., 2003, 2004; Langlois et al., 2010; Harvey et al., 2013b; Wraith et al., 2013),. Freshwater croaker, *Plagioscion squamosissimus* (Heckel 1840), was included to compare a locally available and abundant fish species as a cost efficient and logistically more practical potential alternative to sardine. Sweet corn (brand: Goiás Verde) was included due to the high diversity of frugivorous fish species inhabiting the Xingu River. Cat food (brand: Whiskas), as successfully used in a BRUV study in marine environment by Dorman et al. (2012) was tested due to its composition of fish meat (Salmon)

and vegetal components, to attract fish from a variety of trophic groups. Further, canned cat food and sweet corn were employed due to the durability with no need for cold storing and its logistical practicality, both important criterions for bait choice during field studies in the Amazon Region. For every deployment, 800 g of bait was placed in a bag of 15 x 30 cm made of 5 mm plastic square mesh. Frozen sardines and freshwater croaker were kept on ice in field, thawed prior to use, cut in small pieces of approximately 2-3 cm, weighed and thoroughly crushed in the bait bag immediately before every deployment for more effective liberation of oils and juices during sampling (e.g., Langlois et al., 2010; Fitzpatrick et al., 2012; Hardinge et al., 2013). Empty bait containers were used as control treatments which had never been in contact with any bait during the entire study, and were intended to act as a control group (Harvey et al., 2007, Hardinge et al. 2013).

Environmental data

Environmental data were measured at every location. Water temperature (resolution: 0.1°C) and dissolved oxygen (resolution: 0.1 mg/l) were measured with a handheld oximeter (Instrutherm MO-910) pH (resolution: 0.01 pH) and electrical conductivity (resolution: 0.01 mS/cm) were measured with a Hanna Instruments HI-98129 and the current flow speed (0.1 m/s) was determined with the digital current measuring device (Flowatch from JDC Electronic SA).

Video footage analysis

Footage was analyzed using the free software VLC (www.videolan.org) and fish were identified to the lowest possible taxonomic level. Some unidentified species, such as small characids, which showed distinct patterns of other confidently identified species of the respective families were grouped into different morphotype categories; *Characidae* sp. 1*; Characidae* sp. 2; *Creagrutus* spp.*; Hemigrammus* sp.*; Pterygoplichthys* sp. and *Loricariidae* n.i.. Morphotypes for new, not yet described species of the Xingu River were named with sp. 1 and sp. 2 after the species or genus name (i.e. *Hypomasticus megalepis* sp. 1 or *Teleocichla* sp.1). First arrival time (FAT) of any species and MaxN, based on the maximum number of individuals for a given species counted within the field of view at the same time, were the quantitative methods

used (Willis & Babcock, 2000; Cappo et al., 2003, 2004; Harvey et al., 2007; Gladstone et al., 2012; Harvey et al., 2013b). Video analysis was completed by one person, consulting taxonomic specialists only for difficult to identify species. Fish taxa were assigned to 6 feeding categories following Camargo (2009) (Table 5 - Appendice 1 and Table 6 - Appendice 2).

Statistical analysis

Univariate analysis

Differences in species richness (S) and relative abundance (MaxN) among baits and between habitats were tested using a two factor univariate permutational analysis of variance (PERMANOVA), with bait considered as a fixed factor with five levels (sardine, crushed freshwater croaker, cat food, sweet corn and no bait) and habitat as a fixed factor with two levels (rock and sand bottom). Univariate PERMANOVA tests were run on Euclidean distances matrices with 9999 permutations (Anderson, 2001). For statistically significant ($P \leq 0.05$) main effects, post hoc pairwise comparisons were evaluated with the PERMANOVA t statistic.

Multivariate analysis

Multivariate PERMANOVA used Bray-Curtis similarity matrix of fourth-root transformed abundance data (MaxN) with 9999 permutations (Anderson, 2001) to assess differences in species composition among baits and habitats. Fourth-root transformation was applied to balance the contribution of abundant and rare species to (Underwood & Chapman, 1998; Clarke & Warwick, 2001). The PERMANOVA design was identical to the one used for univariate analysis. Patterns in fish assemblage data of 80 BRUV samples were explored visually by an unconstrained ordination using Principal Coordinates Analysis (PCO). The PCO is an ordination procedure that is consistent with the PERMANOVA approach by providing a direct projection of the points (e.g. samples) in the space defined by the actual dissimilarities (Anderson et al 2008). Therefore, the PCO provides additional insights into the relative sizes and directions of effects in complex experimental designs. A Pearson correlation >0.3 was used as an arbitrary limit to display potential correlations between individual taxa

abundances and the canonical axes scores. A second PCO was obtained by calculating the centroids of the two-way interaction cell groupings in the full multivariate Bray-Curtis space, followed by calculating the distances among them (Anderson et al., 2008). The PCO performed with the distances among centroids is very useful in unraveling patterns among levels of factors in complex designs and can be used to further elucidate and interpret significant interaction. All statistical analysis were run in the software package PRIMER-E v6 (Clarke & Gorley, 2006) with the PERMANOVA extension (Anderson et al., 2008).

Sampling precision

The relationship between soak time and the number of species observed by the five bait treatments was evaluated by plotting species accumulation curves (randomised richness plot against the 15 minute time intervals up to 120 min of soak time) (Santana-Garcon *et al*. 2014).

Multivariate precision analysis based on abundance values was calculated for sardine, the bait which achieved the highest cumulative species richness and plotted for sampling size (number of replicates) and soak time (60, 90 and 120 min intervals), using the R software (R Core Team (2013) as for the above species accumulation curves. We used the pseudo multivariate dissimilarity-based standard error (MultSE), as proposed by Anderson & Santana-Garcon (2015) for ecological studies with multivariate assemblage data when dissimilarity-based analyses are intended to follow.

Results

Environmental characteristics

Apart from the dissolved oxygen (DO) and electrical conductivity (EC) levels, water variables were consistent throughout the sampling period and between deployment locations. Overall pH ranged from 6.65 to 7.90, and water temperature from 29.5 to 31.1 °C. Rock habitats presented higher levels of DO (mean ± standard error: 8.17 ± 0.215 mg/l) and EC (16.25± 0.316 µS/cm) than sand (DO: 7.56 ± 0.279 mg/l; and EC: 15.1 ± 0.234 µS/cm). The current flow speed varied between 0.05 and 0.6 m/s showing no specific pattern between sampling locations and habitat types. Horizontal water visibility during the sampling period (July 2014) was estimated at approximately 1.5 m.

Assemblage Composition

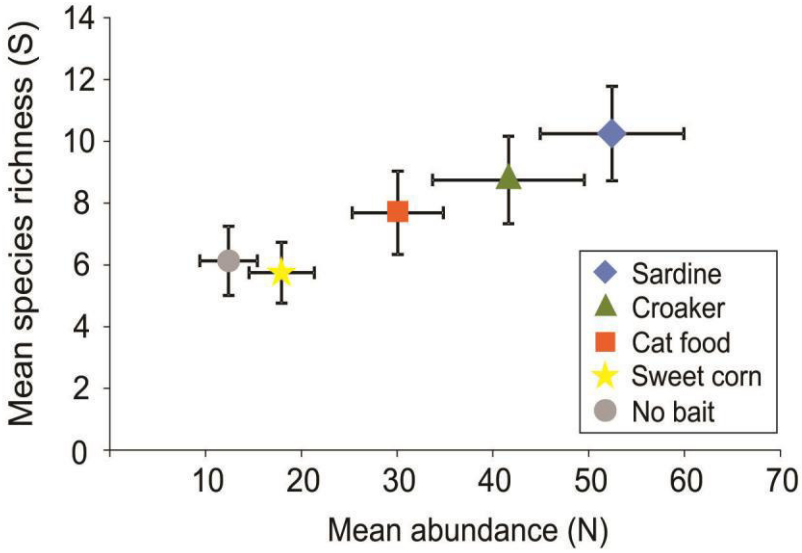
A total of 2460 individuals (1592 for rock and 868 for sand) were observed from a total of 80 120 min BRUV samples. A total of 56 taxa (49 for rock and 32 for sand habitat) belonging to 13 families were identified. Characidae, Cichlidae and Anastomidae accounted for the highest species richness per family, with 12, 9 and 9 taxa, respectively. Overall, 24 taxa were detected exclusively in rock, 7 only in sand and 25 in both substrate type habitats. From the 56 taxa 43 were identified at the species level, 8 to genus and 3 only to family level.

Only 4 species were recorded in both habitats by all bait treatments: *Boulengerela cuvieri*, *Brycon pesu* sp. 1; *Bryconops alburnoides* and *Hemiodus unimaculatus*. Crushed sardine yielded the highest average species richness (mean S ± SE) (10.3 ± 1.5) and number of exclusive species (7): *Acestrorhynchus microlepis* (ΣMaxN = 1), *Anostomoides passions* (ΣMaxN = 1), *Electrophorus electricus* (ΣMaxN = 1), *Hypomasticus megalepis* sp. 2 (ΣMaxN = 1), *Loricaria birindellii* (ΣMaxN = 1), *Moenkhausia heikoi* (ΣMaxN = 38) , *Teleocichla* sp. 1 (ΣMaxN = 1).. Freshwater croaker (8.8 ± 1.4) detected 3 taxa exclusively; *Centromochlus heckelii* (ΣMaxN = 1), *Knodus heteresthes* (ΣMaxN = 1), *Pterygoplichthys* sp. (ΣMaxN = 1). Cat food (7.7 ± 1.3) attracted *Baryancistrus niveatus* (ΣMaxN = 1) and *Bivibranchia velox* (ΣMaxN = 4) *exclusively.* Two taxa, *Squaliforma emarginata* (ΣMaxN = 1) and *Teleocichla*

377 *centrarchus* (ΣMaxN = 1) were detected only with Sweet corn (5.8 ± 1.0) as bait.
378 No bait video samples (6.1 ± 1.1) recorded *Semaprochilodus brama* (ΣMaxN =
379 2) exclusively (Figure 2).

380 BRUVs baited with sardine or freshwater croaker sampled approximately 64%
381 of all predatory fish recorded during this study. This pattern was more prevalent
382 in sand habitat (77%) than in rock habitat (59%). Larger predatory fish, such as
383 the peacock bass *Cichla melaniae* and the freshwater stingray *Potamotrygon*
384 *leopoldi* (both commercially targeted endemic species) as well as the black
385 piranha *Serrasalmus rhombeus*, were recorded mainly over rock bottom. One
386 exception was the orange tailed pike characin *Boulengerella cuvieri* which was
387 equally distributed between the two habitats.

388 Overall the highest mean MaxN ± standard error per bait were also recorded on
389 BRUVs baited with crushed sardine (52.4 ± 7.5), followed by freshwater croaker
390 (41.6 ± 7.9), cat food (30.1 ± 4.8), sweet corn (17.9 ± 3.4) and no bait (12.4 ±
391 3.0) (Fig. 2). PERMANOVA test results for total MaxN and S showed significant
392 differences among the factor levels for bait treatments and for habitat types
393 (Tabel 1, both $P < 0.0001$) with highest values attributed to rock habitat.



394

395 **Fig. 2 -** Mean (± SE) values of species richness (S) and abundance (MaxN) for
396 different types of bait

Table 1 - PERMANOVA results for relative abundance (total MaxN) and species richness (S)

	Relative abundance				Species richness		
Source	**df**	**MS**	**Pseudo-F**	**P(perm)**	**MS**	**Pseudo-F**	**P(perm)**
Habitat	1	6315.4	**35.261**	**<0.0001**	614.43	**117.26**	**<0.0001**
Bait	4	4313.4	**24.083**	**<0.0001**	56.647	**10.811**	**<0.0001**
Habitat × Bait	4	153.93	0.85945	0.4946	36.502	0.69662	0.5901
Residual	69	179.1			52.399		
Total	78						

Except for sardine versus freshwater croaker, all other pairwise tests revealed that the mean relative abundance differed significantly between bait treatments. Differences in species richness were not significant between no bait *vs*. sweet corn, cat food *vs*. croaker, as well as, sardine *vs*. croaker, while all other pairwise bait comparisons showed significant differences ($P < 0.05$) (Table 2).

Table 2 PERMANOVA pairwise test results for relative abundance (total MaxN) and species richness (S)

	Relative abundance			Species richness		
Groups	**T**	**P(perm)**	**perms**	**T**	**P(perm)**	**perms**
No bait × Cat food	61.656	**<0.0001**	9828	24.057	**0.0249**	9809
No bait × Sweet corn	2.404	**0.0227**	9851	0.3540	0.7288	9837
No bait × Croaker	58.287	**<0.0001**	9822	34.669	**0.0024**	9833
No bait × Sardine	75.804	**<0.0001**	9840	41.279	**0.0005**	9823
Cat food × Sweet corn	43.658	**0.0003**	9702	35.005	**0.0023**	9555
Cat food × Croaker	2.263	**0.0323**	9835	15.034	0.1444	9539
Cat food × Sardine	41.861	**0.0003**	9792	26.993	**0.0083**	9679
Sweet corn × Croaker	48.635	**<0.0001**	9827	45.648	**0.0003**	9279
Sweet corn × Sardine	67.419	**<0.0001**	9814	49.284	**<0.0001**	9567
Croaker × Sardine	16.194	0.1108	9808	14.803	0.1541	4924

Multivariate PERMANOVAs for assemblage composition

Considering the abundance data (MaxN) for the fish fauna composition matrix, highly significant differences were detected for the two factors (bait, $P < 0.0001$; habitat, $P < 0.0001$) (Table 3). Pairwise tests from assemblage composition showed significant differences between samples with no bait or sweet corn

413 tested against sardine and freshwater croaker, whilst compositions did not differ
414 significantly for sardine *vs*. freshwater croaker, as well as cat food *vs*. all bait
415 treatments except no bait (Table 4).

416 **Table 3** PERMANOVA results on abundance data (MaxN) for the fish
417 assemblage composition

Assemblage composition				
Source	**Df**	**MS**	**Pseudo-F**	**P(perm)**
Habitat	1	38203	**16.622**	**<0.0001**
Bait	4	5433.6	**23.642**	**<0.0001**
Habitat × Bait	4	2407.8	10.76	0.3815
Residual	69	2298.3		
Total	78			

418

419 **Table 4** PERMANOVA pairwise (bait type) test results on abundance data
420 (MaxN) for assemblage composition

Assemblage composition			
Groups	**T**	**P(perm)**	**Perms**
No bait × Cat food	17.889	**0.0013**	9927
No bait × Sweet corn	14.901	**0.0196**	9928
No bait × Croaker	20.453	**0.0003**	9929
No bait × Sardine	22.132	**<0.0001**	9922
Cat food × Sweet corn	10.376	0.3822	9925
Cat food × Croaker	0.72768	0.843	9939
Cat food × Sardine	11.335	0.2334	9923
Sweet corn × Croaker	14.626	**0.0204**	9947
Sweet corn × Sardine	17.946	**0.0011**	9936
Croaker × Sardine	0.55612	0.9595	9938

421

422 Principal Coordinates Analysis for fish fauna composition supported the
423 PERMANOVA results and showed strong separation of samples between both
424 lotic freshwater habitats, but also a consistent gradient in the y axis in bait. The
425 frugivorous serrasalmid *Myleus setiger* and the omnivorous characid *Bryconops*
426 *alburnoides* characterized the sand bottom habitats. A more diverse fish
427 assemblage composition belonging to three different trophic groups and families

characterized rock bottom habitats: the iliophagous anastomid species *Leporinus maculatus* and *Hypomasticus julii*; from the cichlidae family the omnivorous *Geophagus argyrostictus* and the piscivorous *Cichla melaniae*; and from the loricarid family, the iliophagous *Baryancistrus xanthellus* (Fig. 3a),. Both, C. *melaniae* and B. *xanthellus* are endemic to the Xingu River. The PCO analysis among centroid distances of the fish fauna composition for the different combinations of bait and habitat revealed similar patterns of segregation over both habitats in a symmetric gradient, with greater centroid distances between habitat types (PCO1) than between bait types (PCO2) (Fig. 3b). In total, 78% of data variation was explained by PCO1 and PCO2 axes (Fig. 3b). A grouping effect was evident among sardine, freshwater croaker and cat food. In rock and sand habitat, the dissimilarity of centroid distances between the 3 baits within this group formed by the fish baits and fish containing cat food was smaller than to either no bait or sweet corn.

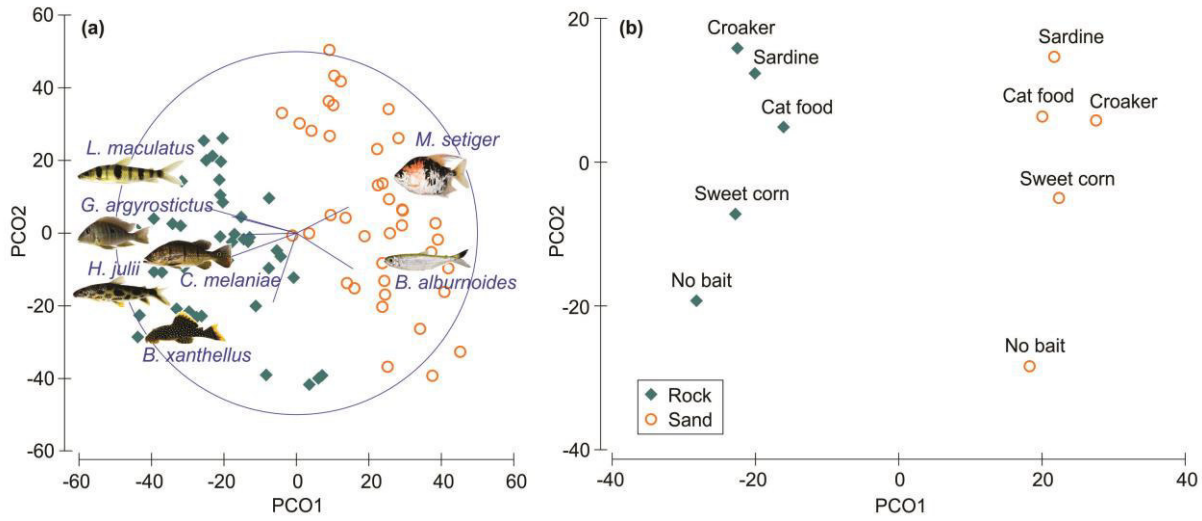


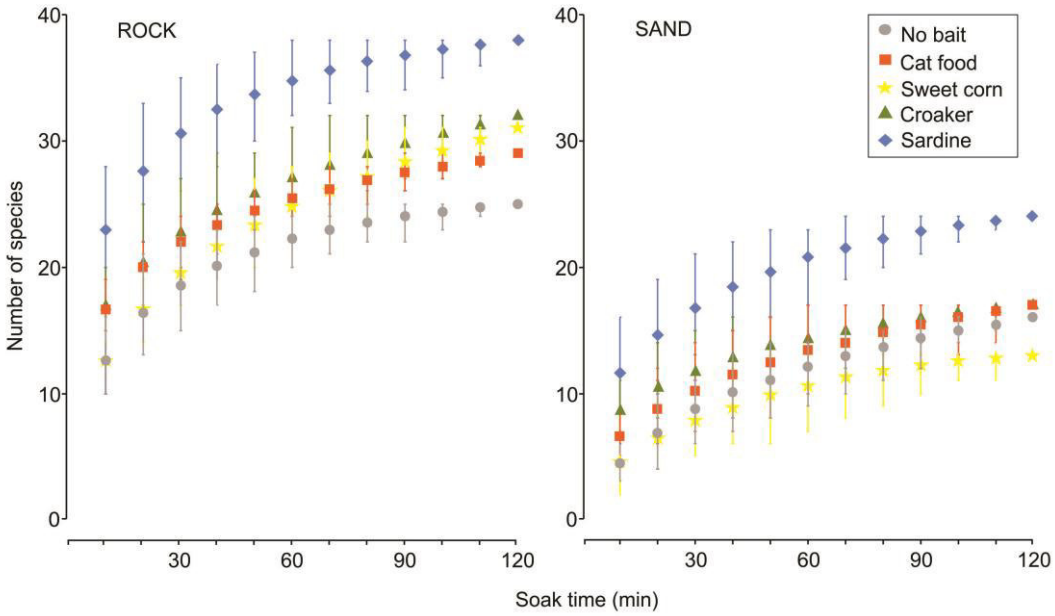
Fig. 3 Principal coordinates ordinations (PCO) of the fish assemblages. Symbols represent (a) individual BRUV deployments in sand and rock bottom, with species in vectors showing high correlation (> 0.3) with axes and (b) centroids of the two-way interaction of bait and habitat

Species accumulation

The shortest average FATs, over sand substrate, where achieved with the two fish baits: sardine (0.64 ± 0.57 SD mins) and freshwater croaker (1.61 ± 2.28 SD mins). Over rock substrate, average FATs were shorter for all bait

treatments - all within the first 60 seconds of soak time, including no bait (1.0 ± 1.0 SD mins). In this habitat type, freshwater croaker and cat food produced the shortest average FAT's with 0.15 ± 0.11 SD mins and 0.17 ± 0.19 SD mins closely followed by sweet corn (0.24 ± 0.17 SD mins) and sardine (0.35 ± 0.42 sec). Generally, different taxa of characins (*Brycon* spp.; *Moenkhausia* spp.; *Creagrutus* spp. and others), as well as cichlids (*Teleocichla centrarchus*; *Crenicichla* sp. 1 and *Geophagus altifrons*) were the first fish to arrive in the field of view (Table 5 – Appendice 1).

In both habitats BRUVs baited with sardines sampled the highest and freshwater croaker the second highest mean species richness in all time intervals, as well as cumulative along the entire soak time (Fig. 4). And 92% of the species richness in rock and 87.5% in sand habitats were detected with sardine within the first 60 minutes of sampling, corresponding to 34 and 21 taxa respectively. In rock habitats the mean cumulative species richness for sardine was approximately 22.6% higher than for freshwater croaker after 60 min and still 16% higher after the complete sampling time of 120 min. In sand habitats the differences were even bigger and more constant; 31.5% higher after 60 min and 29.5% after 120 min. All real bait types produced higher species richness results in rock habitat and thus steeper curve slopes, more apparent during the initial 60-70 minutes of soak time. Cat food species accumulation in rock habitat reduced earlier and produced, in the last 40 minutes, lower mean species numbers than sweet corn. In rock habitat species accumulation for sweet corn appeared more continuous than for other baits during the entire 120 minutes, but reduced the most in sand habitat, performing even weaker than no bait.

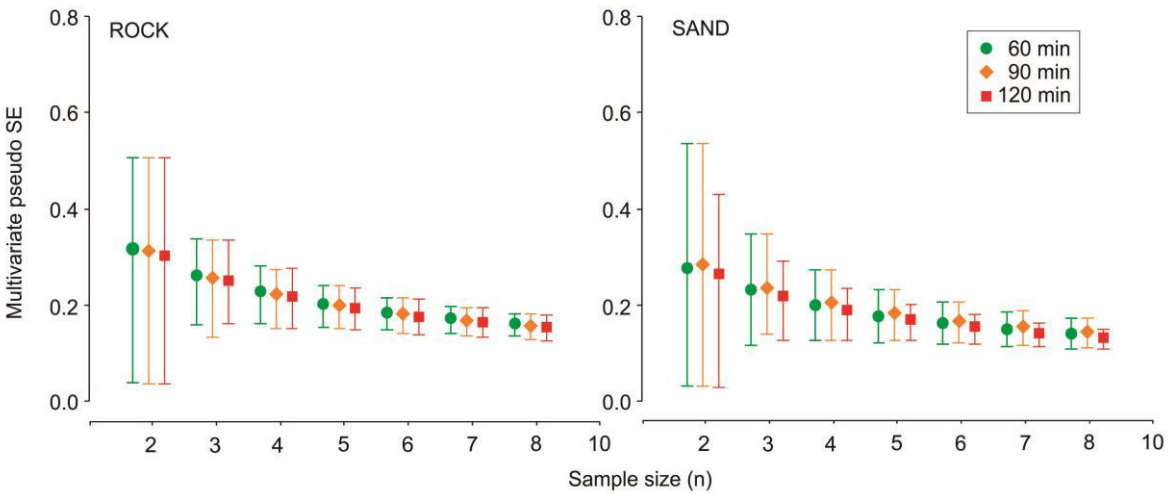


475

476 **Fig. 4** Species accumulation curves for the five bait treatments in rock and sand
477 habitats in 15 minute time intervals for soak times up to 120 minute. Error bars
478 represent standard error

479 Precision analysis

480 Multivariate precision for sardine was generally very similar between both
481 habitats and showed little variation between soak time intervals (60, 90 and 120
482 min). Improvement of precision was gained predominantly by increasing the
483 number of replicate samples. Highest precisions were consequently achieved in
484 both habitats by a sampling size of n=8 and 120 minutes of soak time, but a
485 leveling-off in MultSE occurs for sample sizes between 5 and 6 replicates (Fig.
486 5).



487

488 **Fig. 5** Precision analysis (± MultSE) in a) rock and b) sand habitat for sampling
489 intervals of 30, 60 and 120 minutes and number of replicates sampled with
490 sardine

Discussion and conclusions

Habitat

Substrate complexity of aquatic habitats has a direct influence on the fish assemblage compositions in different marine and freshwater ecosystems (Luckhurst and Luckhurst, 1978; Willis et al., 2005; Junk et al., 2007; Giakoumi and Kokkoris, 2013). Structurally more complex rock habitats have been confirmed to produce a diverse pattern of microhabitats and thus sustain higher fish density (Smith et al., 2015), more diverse and functionally more complex fish communities in comparison to habitats with unstable unconsolidated substrate (Cruz et al., 2013). Rock formations, riffles and rapids are the dominant aquatic environments in our study area at the lower Xingu River section and offer a greater substrate variety and complexity, explaining the remarkably different assemblages of reophilic fish between both habitat types. The relative abundance estimates in consolidated substrate (rock) habitats were 83% and the species richness 53% higher than in unconsolidated substrate (sand) habitats. Our results corroborate the positive relationship between habitat complexity and neotropical fish species density and/or species richness found in the Cinaruco River a tributary of the Orinoco River in Venezuela, (Willis et al., 2005) and the Jaú River in Brazil, a Rio Negro tributary (Kemenes and Forsberg, 2014), both located in the Amazon region.

The strong grouping effect and the clear separation of sample data between rock and sand habitat, as visualized in the PCO graphs, positively reflects the BRUV's capacity to detect such habitat associated fish composition differences. We consider this a promising result, given the habitats with consolidated substrate are very predominant in the studied area. Further we assume that our sampling locations were chosen adequately, habitat-representative and with sufficiently homogeneous characteristics throughout the majority or the entire area covered by each bait plume. Small sand patches occur even within extended areas of rock rapids, i.e. in current shadows of big boulders in our study area. If chosen based solely on its on-the-spot substrate characteristics without considering the spatial continuousness, such sampling location would

potentially reflect the fish assemblage characteristics of the surrounding rock habitat rather than of the given small sand deposit micro-habitat.

Bait

Significant differences between bait treatments for S and N confirm that the use of bait, especially fish bait, increases species richness and abundance sampling results in our study area. Sardine and freshwater croaker were the most similar bait types tested in this study and produced the most similar data on reophilic fish fauna of the medium Xingu River. PERMANOVA pairwise comparison showed no significant differences for composition, species richness and relative abundance between this two fish baits. Specifically when compared for MaxN, all of the other bait type comparisons produced significantly different results. Yet contrarily to these univariate and multivariate PERMANOVA results, species accumulation curves in both rock and sand habitats clearly highlight sardine as the preferable choice of bait for BRUV sampling in our study area. The cumulative species curve slope of sardine developed steeper and above all other bait treatments with a significant distance, including to freshwater croaker. In rock bottom habitats, with an overall higher fish density and species richness than over sand, cat food initially accumulated equally high numbers of species as freshwater croaker. Yet 30-40 minutes into sampling its curves flattened suddenly, as the soak time necessary to attract new species within the cameras field of view increased. This may indicate that the liquid components of the cat food used in this study contained most of the odors which effectively attracted fish in the initial phase, but have been washed out of the bait container quickly. Furthermore we observed that the difference in attraction efficiency between sardine and all other bait treatments is more apparent in sand habitat, even between both fish baits; sardine and freshwater croaker. The naturally higher fish density and species richness encountered in rock habitats (Smith et al., 2015) probably increases chances of fish detection, even with less efficient bait types or no bait. Whilst in the “poorer” and less diverse shallow water sand bottom habitat (Cruz et al., 2013) - referring to the daytime period only - the efficiency differences between baits appear to be less camouflaged.

For most of the species sampled exclusively with either one of the bait types only a single individual was registered (ΣMaxN = 1) overall. This is insufficient data to conclude that the given species are attracted mainly or even exclusively to the respective bait type. Still, the count of exclusively sampled species per bait reveals the same pattern as the mean S, mean MaxN and species accumulation results. Sardine also ranked highest with 7 species, followed by freshwater croaker with 3 and cat food and sweet corn both with 2 species. This may indicates that sardine is more efficient in attracting a wider range of trophic guilds and consequently fish species than the other tested baits.

A comparison study conducted by Wraith et al. (2013) showed that pilchard, with its similarity to sardine, yielded the most consistent outcomes of species richness and abundance data. Our results lead us to conclude that sardine, due to its high oil content and soft flesh, worked particularly well in constant, unidirectional water flow. We understand, that this fluvial condition allows the bait plume to efficiently develop to its maximum volume until water dilution reduces the concentration of fish attracting odors to an ineffective level at a certain distance from the BRUV.

Sardine was also the most cost efficient bait tested in this study with approximately 2.40 US$ per deployment. The downside of using sardine or any other fish as bait is its poor *in situ*-practicality. The need to transport and maintain sardines frozen during a field study in the tropical-equatorial climate of the amazon region is a major logistical challenge and demands considerable quantities of ice and cool boxes. Canned cat food, or as used in this study, in packets (sachets) required no specific maintenance other than being stored away from wild animals and direct sunlight. The price of approximately 5 US$ per deployment for cat food exceeded that of all other bait types. Additionally, results of this study, notably for species accumulation, did not support the use of this bait in our study area as soak times above 20-30 minutes are required. At last, any canned or packaged bait, including sweet corn, produce large amounts of waste when multiplied with a high number of deployments. *In situ* at the Xingu River all litter had to be transported both ways by boat.

584 Although trophic group analysis on the sampled fish assemblages was no
585 subject to this study and should be investigated separately, some observations
586 were made. Overall approximately 70% of all detected fish were omnivorous
587 species. The second most important trophic group with approximately 18% was
588 formed by iliophagous, followed by frugivorous fish (approx. 8%). Only about
589 4% of all fish were predatory, with sardine and freshwater croaker accounting
590 for the majority of all registered piscivorous and carnivorous specimens; close
591 to 60% in rock and 80% in sand habitat. Habitat differences were mainly
592 detected between the two most representative groups; omnivorous species
593 corresponded for over 60% of fish individuals sampled in rock and over 80% in
594 sand habitat; iliophagous species for 24.4% in rock but only 6.4% in sand
595 habitat. The difference was driven mainly by various iliophagous species from
596 the Anastomidae family, sampled in abundance over rock bottom.

597 Sampling regime

598 According to Gladstone et al. (2012) the ideal sampling regime, regarding soak
599 time and number of replicates, is a balance between precision of sampling
600 results and cost effectiveness. Effects of sampling time on precision and cost
601 effectiveness have specifically been investigated by Gladstone et al. (2012) in
602 the marine ecosystem of the Wagonga Inlet, New South Wales, Australia where
603 90 minutes of soak time with 10 replicates revealed as the most precise but
604 most expensive and five replicates with 90 minutes the most practical option.
605 Santana-Garcon, et al. (2014) recorded 180 minutes of underwater video
606 footage in a BRUV study of pelagic fish species in the Ningaloo Marine Park,
607 Western Australia. Willis & Babcock (2000) found consistency in estimates of
608 relative fish density for the most cost effective soak time of 30 minutes. In
609 freshwater, Ebner & Morgan (2013) adopted 60 minutes as the adequate time
610 while sampling fish assemblage and species richness in the Fortesque River,
611 Western Australia. In our study area, best multivariate precision results were
612 achieved with the longest soak time (120 min) and the biggest sampling size
613 (n=8). But examination of the plots (Fig. 5) reveals that in both habitats the
614 multivariate precision did not improve substantially anymore after 5 or 6
615 replicate samples. Therefore, 5 or 6 samples and 60 min soak time appear to
616 be adequate, especially when the main goal is to detect temporal and spatial

617 changes in fish assemblages, i.e. in conservation and monitoring programs
618 where cost efficiency is paramount. Precision improved mainly with increasing
619 replicate numbers. Soak time had no important influence on precision of
620 estimates and could thus be reduced in order to achieve better cost efficiency
621 during sampling and video analysis. A shorter sampling period also reduces the
622 required quantity of bait, thus cuts down the sampling costs.

623 Effects of bait quantity on stereo-BRUV fish assemblage sampling have been
624 investigated by Hardinge et al. (2013) in the Indian Ocean at the Five Fathom
625 Bank, Western Australia. During 60 minutes of sampling time, the smaller
626 quantity of 200 g of crushed pilchard showed to be as effective as higher
627 quantities, such as 1000 g or 2000 g. Considering constant water flow and
628 unknown fish density in our study area, combined with 120 minutes sampling
629 time, we expected the bait to be depleted or washed out at a faster rate,
630 justifying the use of 800 g of bait to create a constant plume. During the
631 recovery we observed yet considerable quantities of oily liquid draining from the
632 bait bag and forming oil films on the water surface. Hence we assume that 800
633 g of crushed sardine would also be suitable for longer sampling regimes and
634 most certainly still yield higher values of N and S. For shorter soak times, i.e. 60
635 minutes, we assume that bait quantity can be reduced, eventually to 400 – 500
636 g. This assumption was additionally sustained by video analysis. Fish actively
637 feeding on the bait were predominantly small species from the Characidae and
638 Serrasalmidae families, thus no bait has ever been depleted or even notably
639 reduced in quantity. But further investigation is necessary as the effects of bait
640 quantity were not a subject of our study. On the contrary, we intentionally used
641 a sufficient amount of bait in order to exclude “bait quantity” as an additional
642 source of variation.

643 Performance of BRUV

644 Our results highlight that BRUVs were effective in attracting and detecting high
645 diversities of fish species and quantifying their relative abundances from
646 habitat-based fish assemblages. Baited remote underwater video, like most
647 other sampling techniques, has specific biases and limitations. For instance;
648 species identification to the lowest taxonomic level may be limited if solely

649 based on video footage samples (Pelletier et al., 2011; Cappo et al., 2004),
650 mainly for small individuals with similar size and body shape. In our study the
651 identification problems predominantly occurred with small characids as from the
652 *Moenkhausia* or *Hemigrammus* genera. The BRUVs predominantly detected
653 mobile reophilic fish (pelagic and demersal species) showed low capacity in
654 attracting and detecting small cryptic species, i.e. loricarids.This armoured
655 catfish are very abundant in the study area (Camargo et al., 2004), although
656 spend most of their lives hidden in crevices and other rock hideaways
657 (Camargo et al., 2012). BRUV's weaker performance in detecting cryptic living
658 species, compared to conventional UVC sampling, has already been highlighted
659 in reef habitats (Lowry et al., 2012). Further, the relative abundance metric
660 MaxN potentially underrepresents the true abundance and its changes
661 (Campbell et al., 2015; Schobernd et al., 2015). Field and laboratory based
662 experiments both revealed a non-linear relationship for MaxN with true
663 abundance and increasing underestimation for high abundances and mobility
664 patterns (Campbell et al., 2015; Schobernd et al., 2015). We therefore assume
665 that in our study the high abundances of different small schooling and highly
666 mobile characid species have likely been underestimated. Suboptimal water
667 visibility conditions during the sampling period additionally increased this
668 problem. Choosing the ideal period for *in situ* BRUV sampling campaigns in
669 amazonian freshwater ecosystems should therefore not only consider the
670 seasonality of precipitation and water levels based on long term meteorological
671 data. Ideally a sampling period is scheduled according to "up to date" local
672 information on the development of the water level and transparency.

673 The devices used in this first study were deployed over a variety of complex
674 rock structures with no greater difficulties. The maximum current flow speed
675 sampled in this study was 0.6 m/s, stronger currents might require heavier
676 structures to avoid BRUVs from tipping over. This should always be tested and
677 adapted previously to actual sampling. The HD action cameras (GoPro Hero 3+
678 Black Edition) performed well under the conditions in the study area. Limitations
679 during fish species identification were associated to either the extraordinary
680 similarity between species or water visibility, never to the cameras resolution or
681 range. BRUVs can provide former inaccessible data on the reophilic fish fauna

682 of amazon clearwater streams and rivers, and thus contribute to the
683 conservation of their lotic habitats. Additionally we underline that the use of this
684 non-extractive sampling method is highly desirable in the Xingu River due the
685 large variety of endemic species present.

686 We conclude that similar sampling regimes would also be suitable to assess
687 freshwater fish assemblages with baited remote underwater video in other lotic
688 systems with sufficient visibility. In tropical regions few minor adjustments such
689 as bait quantity and distance to the camera or soak time might be sufficient.
690 Based on our results, an oily fish as bait (i.e. pilchard and sardine species)
691 appear to maintain their fish attracting odors well in the constant water flow and
692 perform well in riverine habitats. Yet in lower or higher latitudes the choice of
693 bait should be specifically investigated at first hand, as in our study due to the
694 lack of previous research in different freshwater ecosystems (Gladstone et al.,
695 2012; Ebner and Morgan, 2013).

696 Recommendations

697 Inevitably the Xingu River's aquatic ecosystems and reophilic fish will soon
698 suffer dramatic changes due to the environmental impacts caused by the Belo
699 Monte hydroelectric power plant (Szabaji, 2015; Winemiller et al.,2016). Our
700 results provide a suitable base to establish the BRUV method in a fish fauna
701 monitoring program for the lotic habitats of the Xingu River, specifically in the
702 area directly impacted by the dam. Regarding its limitations, notably to detect
703 cryptic fish, we reinforce that BRUV would be best employed in combination
704 with other sampling techniques, if possible non-extractive, wherever the aims of
705 a research and the habitat characteristics allow it. Additionally, the obtained
706 results and the video footages can serve as important permanent records. The
707 present study enabled us to acquire footage of fish species rarely or never
708 filmed previously in their natural environment, such as the endangered endemic
709 serrasalmid species *Ossubtus xinguense* and others.

710

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Tables

Figure captions

Figures and appendices

Table 5 (Appendice 1). Average (± SD) first arrival time (mins)

		Average (± SD) first arrival time (mins)									
		ROCK					SAND				
		No bait	Cat food	Sweet corn	Fw. Croaker	Sardine	No bait	Cat food	Sweet corn	Fw. Croaker	Sardine
Characiformes	**Trophic group**										
Acestrorhynchidae											
Acestrorhynchus microlepis (Jardine 1841)	Piscivorous					5.19 ±(13.74)					
Anostomidae											
Anostomoides passionis[E] Santos & Zuanon, 2006	Iliophagous					11.7 ±(30.94)					
Hypomasticus julii[E] (Santos, Jégu & Lima 1996)	Iliophagous	6.12 ±(9.18)	19.22 ±(30.77)	3.74 ±(9.82)	18.92 ±(29.86)	18.38 ±(34.88)			14.76 ±(39.05)		
Leporellus vittatus (Valenciennes 1850)	Iliophagous				10.17 ±(26.92)	9.22 ±(24.39)					
Leporinus fasciatus (Bloch 1794)	Iliophagous	6.51 ±(16.78)	12.85 ±(27.17)	8.17 ±(14.82)	3.4 ±(4.77)	24.3 ±(26.95)		0.56 ±(1.48)			8.37 ±(22.15)
Leporinus desmotes Fowler 1914	Iliophagous		6.96 ±(12.86)	4.58 ±(12.12)		0.69 ±(1.67)					
Hypomasticus megalepis sp. 1	Iliophagous			6.76 ±(17.88)	5.09 ±(13.47)	0.28 ±(0.74)					
Hypomasticus megalepis sp. 2	Iliophagous					9.82 ±(25.99)					
Leporinus maculatus Müller & Troschel 1844	Iliophagous	0.29 ±(0.38)	17.38 ±(23.22)	15.14 ±(38.91)	0.76 ±(0.9)	5.33 ±(9.01)		0.41 ±(1.09)	10.84 ±(28.67)		0.08 ±(0.2)
Leporinus tigrinus Borodin 1929	Iliophagous	4.49 ±(11.87)	4.71 ±(12.45)	3.71 ±(9.82)	14.25 ±(23.77)	3.1 ±(6.61)					
Characidae											
Brycon pesu sp. 1	Omnivorous	0.32 ±(0.7)	15.17 ±(39.52)	0.26 ±(0.28)	3.01 ±(6.27)	14.61 ±(23.24)	<0.01 ±(0.01)	3.99 ±(8.39)	8.3 ±(18.11)	0.43 ±(0.42)	14.69 ±(33.12)
Brycon pesu sp. 2	Omnivorous				13.51 ±(34.61)	25.44 ±(44.54)		7.13 ±(13.85)		7.93 ±(14.03)	6.11 ±(12.25)
Brycon falcatus Müller & Troschel 1844	Omnivorous				0.71 ±(1.87)	0.33 ±(0.88)				6.48 ±(11.54)	9.46 ±(16.92)
Bryconops alburnoides Kner 1858	Omnivorous	11 ±(28.45)	0.58 ±(1.08)	11.81 ±(31.24)	0.11 ±(0.19)	2.54 ±(4.67)	8.84 ±(20.64)	12.98 ±(30.01)	1.28 ±(2.34)	1.14 ±(1.45)	5.92 ±(13.64)
Characidae sp. 1	Omnivorous						8.63 ±(22.82)		2.17 ±(5.74)		
Characidae sp. 2	Omnivorous	2.98 ±(7.76)	0.04 ±(0.07)	11.22 ±(29.01)	15.23 ±(39.08)	0.28 ±(0.43)	0.34 ±(0.89)	0.01 ±(0.03)			12.04 ±(31.84)
Creagrutus spp.	Omnivorous							9.78 ±(25.87)	1.04 ±(2.74)	0.13 ±(0.34)	3.00 ±(7.94)
Hemigrammus sp.	Omnivorous						8.34 ±(22.08)				0.28 ±(0.73)
Knodus heteresthes (Eigenmann 1908)	Omnivorous				3.86 ±(10.21)						
Moenkhausia celibela Marinho & Langeani 2010	Omnivorous	0.02 ±(0.04)	12.32 ±(27.92)		0.56 ±(0.61)	0.08 ±(0.15)		1.54 ±(3.57)		0.13 ±(0.34)	12.12 ±(32.06)

		Average (± SD) first arrival time (mins)									
		ROCK					SAND				
		No bait	Cat food	Sweet corn	Fw. Croaker	Sardine	No bait	Cat food	Sweet corn	Fw. Croaker	Sardine
Moenkhausia lepidura (Kner 1858)	Omnivorous		2.88 ±(3.54)	0.16 ±(0.44)	0.13 ±(0.23)	4.91 ±(8.45)	<0.01 ±(0.01)	7.82 ±(20.7)		5.9 ±(12.15)	17.66 ±(32.93)
Moenkhausia heikoi[E] Géry & Zarske 2004	Omnivorous					0.07 ±(0.12)					
Characidium fasciatum Reinhardt 1867	Iliophagous	9.29 ±(16.53)		4.42 ±(10.31)	1.25 ±(3.3)						
Boulengerella cuvieri (Spix & Agassiz 1829)	Piscivorous	9.73 ±(17.21)	10.5 ±(27.77)	10.72 ±(19.53)	22.26 ±(32.34)	18.12 ±(31.71)	15.18 ±(26.95)	18.51 ±(32.06)	13.76 ±(36.4)	9.33 ±(16.24)	38.64 ±(33.66)
Hemiodontidae											
Argonectes robertsi Langeani 1999	Iliophagous	10.17 ±(26.91)	13.23 ±(19.9)	11.22 ±(27.7)		6.35 ±(16.8)	2.5 ±(6.63)	23.26 ±(42.24)	2.57 ±(6.79)	17.21 ±(37.33)	6.98 ±(18.25)
Bivibranchia velox (Eigenmann & Myers 1927)	Iliophagous							7.77 ±(20.56)			
Hemiodus unimaculatus (Bloch 1794)	Iliophagous	11.86 ±(14.48)	23.29 ±(34.01)	10.71 ±(19.9)	3.37 ±(8.29)	3.88 ±(8.96)	9.61 ±(18.4)	25.3 ±(44.07)	27.16 ±(41.43)	7.9 ±(20.08)	19.89 ±(19.96)
Hemiodus vorderwinckleri (Géry 1964)	Iliophagous	6.7 ±(6.48)	17.45 ±(23.96)	6.76 ±(15.62)	16.36 ±(12.65)	7.36 ±(12.99)	3.49 ±(9.23)		16.96 ±(36.79)		
Prochilodontidae											
Prochilodus nigricans Spix & Agassiz 1829	Detritivores	3.39 ±(8.96)				6.55 ±(17.34)	11.24 ±(29.73)	9.74 ±(25.78)		14.59 ±(25.9)	3.04 ±(8.05)
Semaprochilodus brama (Valenciennes 1850)	Detritivores						10.38 ±(23.63)				
Serrasalmidae											
Myleus setiger Müller & Troschel 1844	Frugivorous		7.79 ±(17.14)	0.32 ±(0.85)		5.72 ±(11.74)	36.76 ±(48.02)	6.02 ±(10.82)	28.97 ±(35.72)	24.04 ±(18.24)	18.79 ±(32.68)
Myloplus arnoldi (Ahl 1936)	Frugivorous	11.23 ±(29.7)	25.09 ±(42.57)		2.76 ±(7.3)				3.16 ±(8.37)	6.12 ±(14.76)	5.95 ±(12.54)
Myleus schomburgkii (Jardine 1841)	Frugivorous		16.67 ±(29.33)	3.16 ±(8.36)		17.16 ±(32.18)				0.42 ±(1.11)	10.51 ±(27.58)
Ossubtus xinguense[E,VU] Jégu 1992	Omnivorous	1.40 ±(3.7)	13.37 ±(35.38)	10.03 ±(26.53)	7.93 ±(20.97)		6.71 ±(17.76)				
Serrasalmus rhombeus (Linnaeus 1766)	Piscivorous		3.25 ±(7.82)		3.18 ±(7)	25.57 ±(36.82)				9.04 ±(23.91)	8.07 ±(14.06)
Tometes spp.[E]	Frugivorous	25.28 ±(36.06)	0.51 ±(0.68)	15.28 ±(22.86)	2.68 ±(5.55)	11.22 ±(22.78)		0.59 ±(1.57)		0.53 ±(1.39)	0.44 ±(1.16)
Gymnotiformes											
Electrophoridae											
Electrophorus electricus (Linnaeus 1766)	Piscivorous					0.35 ±(0.91)					
Myliobatiformes											
Potamotrygonidae											
Potamotrygon leopoldi[E] Castex & Castello 1970	Carnivorous		4.74 ±(12.55)	22.89 ±(39.65)		1.23 ±(3.24)					
Perciformes											
Cichlidae											
Cichla melaniae[E] Kullander & Ferreira 2006	Piscivorous	24.97 ±(39.88)	8.30 ±(21.95)	4.52 ±(11.96)	33.14 ±(42.22)	2.54 ±(4.49)					9.63 ±(25.49)

		Average (± SD) first arrival time (mins)									
		ROCK					SAND				
		No bait	Cat food	Sweet corn	Fw. Croaker	Sardine	No bait	Cat food	Sweet corn	Fw. Croaker	Sardine
Crenicichla lugubris Heckel 1840	Piscivorous	4.46 ±(11.79)	1.83 ±(3.42)	9.36 ±(17.73)	10.68 ±(25.41)	11.07 ±(12.71)					
Crenicichla sp. 1	Piscivorous	4.42 ±(11.69)			1.08 ±(2.86)						
Geophagus altifrons Heckel 1840	Omnivorous	1.56 ±(4.13)	17.06 ±(30.91)		6.17 ±(16.32)	11.25 ±(26.89)	2.01 ±(5.31)	4.74 ±(12.53)			
Geophagus argyrostictus[E] Kullander 1991	Omnivorous	6.39 ±(14.07)	5.56 ±(11.44)	8.89 ±(22.74)	13.31 ±(20.23)	1.07 ±(2.84)					
Retroculus xinguensis[E] Gosse 1971	Omnivorous		2.93 ±(7.74)	14.64 ±(32.13)							
Teleocichla centrarchus Kullander 1988	Iliophagous			2.36 ±(6.23)							
Teleocichla cinderella Kullander 1988	Iliophagous	1.72 ±(3.5)	28.00 ±(41.36)	8.79 ±(23.26)	9.72 ±(16.07)	10.04 ±(23.29)	9.71 ±(25.7)		1.91 ±(5.05)		2.48 ±(6.55)
Teleocichla sp. 1	Iliophagous					11.57 ±(30.61)					
Siluriformes											
Auchenipteridae											
Centromochlus heckelii (De Filippi 1853)	Omnivorous									10.96 ±(28.99)	
Loricariidae											
Baryancistrus niveatus[E] (Castelnau 1855)	Iliophagous		9.04 ±(23.92)								
Baryancistrus sp. "verde"[E]	Iliophagous			6.88 ±(18.21)	10.63 ±(28.11)						
Baryancistrus xanthellus[E] Rapp Py-Daniel, Zuanon & Ribeiro de Oliveira 2011	Iliophagous	23.89 ±(24.97)	16.21 ±(28.15)	5.49 ±(13.92)		12.46 ±(22.27)					8.23 ±(21.76)
Hypostomus sp.	Iliophagous			8.08 ±(21.36)		6.3 ±(16.68)					
Loricaria birindellii[E] Thomas & Sabaj Pérez 2010	Iliophagous										13.74 ±(36.36)
Loricariidae n.i.	Iliophagous	10.46 ±(27.66)			15.98 ±(27.76)	14.76 ±(25.55)					
Pterygoplichthys sp.	Iliophagous				10.11 ±(26.76)						
Squaliforma emarginata (Valenciennes 1840)	Iliophagous			14.36 ±(38)							

Legend: E = Endemic fish species to the Xingu River; VU = Vulnerable species (IUCN Red List); gr. = group and aff. = affins; sp. 1 and sp.2 = morphotypes for new, not yet described species; (fam.) and (org.) = Common name for family or order if not available for species

Table 6 (Appendice 2): Mean (± SD) relative abundance (MaxN)

		Mean (± SD) relative abundance (MaxN)									
		ROCK					SAND				
		No bait	Cat food	Sweet corn	Fw. Croaker	Sardine	No bait	Cat food	Sweet corn	Fw. Croaker	Sardine
Characiformes	**Trophic group**										
Acestrorhynchidae											
Acestrorhynchus microlepis (Jardine 1841)	Piscivorous					0.13 (± 0.33)					
Anostomidae											
Anostomoides passionis[E] Santos & Zuanon, 2006	Iliophagous					0.13 (± 0.33)					
Hypomasticus julii[E] (Santos, Jégu & Lima 1996)	Iliophagous	1.00 (± 1.00)	1.00 (± 1.58)	0.63 (± 1.32)	2.63 (± 2.18)	2.38 (± 1.41)			0.13 (± 0.33)		
Leporellus vittatus (Valenciennes 1850)	Iliophagous				0.13 (± 0.33)	0.13 (± 0.33)					
Leporinus fasciatus (Bloch 1794)	Iliophagous	0.50 (± 0.71)	0.50 (± 0.50)	0.38 (± 0.48)	1.13 (± 1.05)	1.38 (± 0.99)		0.13 (± 0.33)			0.13 (± 0.33)
Leporinus desmotes Fowler 1914	Iliophagous		0.25 (± 0.43)	0.13 (± 0.33)		0.50 (± 0.71)					
Hypomasticus megalepis sp. 1	Iliophagous			0.25 (± 0.66)	0.13 (± 0.33)	0.25 (± 0.66)					
Hypomasticus megalepis sp. 2	Iliophagous					0.13 (± 0.33)					
Leporinus maculatus Müller & Troschel 1844	Iliophagous	1.88 (± 2.47)	2.00 (± 2.40)	1.00 (± 1.94)	3.50 (± 2.87)	1.50 (± 1.73)		0.13 (± 0.33)	0.50 (± 1.32)		0.13 (± 0.33)
Leporinus tigrinus Borodin 1929	Iliophagous	0.38 (± 0.70)	0.13 (± 0.33)	0.13 (± 0.33)	0.38 (± 0.48)	0.75 (± 1.09)					
Characidae											
Brycon pesu sp. 1	Omnivorous	0.63 (± 1.11)	7.63 (± 6.63)	4.25 (± 5.93)	15.88 (± 5.90)	13.75 (± 8.60)	0.38 (± 0.99)	9.00 (± 8.56)	5.00 (± 4.09)	12.88 (± 13.94)	14.88 (± 7.90)
Brycon pesu sp. 2	Omnivorous				0.50 (± 1.00)	0.38 (± 0.70)		2.13 (± 3.22)		0.75 (± 1.09)	1.13 (± 1.96)
Brycon falcatus Müller & Troschel 1844	Omnivorous				0.13 (± 0.33)	0.13 (± 0.33)				1.00 (± 1.50)	0.25 (± 0.43)
Bryconops alburnoides Kner 1858	Omnivorous	1.88 (± 3.22)	3.38 (± 4.88)	1.25 (± 2.17)	3.00 (± 4.00)	9.38 (± 13.13)	2.25 (± 3.56)	2.75 (± 2.86)	0.50 (± 1.00)	7.63 (± 7.14)	13.13 (± 11.19)
Characidae sp. 1	Omnivorous						0.13 (± 0.33)		0.13 (± 0.33)		
Characidae sp. 2	Omnivorous	1.25 (± 2.17)	4.00 (± 6.24)	7.50 (± 7.33)	8.00 (± 9.10)	10.63 (± 13.55)	0.25 (± 0.43)	2.13 (± 5.62)			0.63 (± 1.65)
Creagrutus spp.	Omnivorous							0.63 (± 1.65)	1.75 (± 3.38)	0.25 (± 0.66)	0.13 (± 0.33)
Hemigrammus sp.	Omnivorous						0.38 (± 0.99)				1.13 (± 2.98)
Knodus heteresthes (Eigenmann 1908)	Omnivorous				0.13 (± 0.33)						
Moenkhausia celibela Marinho & Langeani 2010	Omnivorous	0.25 (± 0.66)	3.38 (± 5.51)		5.57 (± 6.98)	3.25 (± 5.91)		1.13 (± 1.96)		0.25 (± 0.66)	1.00 (± 2.65)
Moenkhausia lepidura (Kner 1858)	Omnivorous		3.50 (± 5.05)	0.25 (± 0.66)	0.88 (± 1.17)	0.63 (± 0.99)	0.25 (± 0.66)	0.25 (± 0.66)		0.50 (± 1.00)	2.00 (± 2.96)
Moenkhausia heikoi[E] Géry & Zarske 2004	Omnivorous					4.75 (± 9.90)					

		Mean (± SD) relative abundance (MaxN)									
		ROCK					SAND				
		No bait	Cat food	Sweet corn	Fw. Croaker	Sardine	No bait	Cat food	Sweet corn	Fw. Croaker	Sardine
Characidium fasciatum Reinhardt 1867	Iliophagous	0.38 (± 0.70)		0.38 (± 0.70)	0.13 (± 0.33)						
Boulengerella cuvieri (Spix & Agassiz 1829)	Piscivorous	0.25 (± 0.43)	0.13 (± 0.33)	0.25 (± 0.43)	0.50 (± 0.50)	0.38 (± 0.70)	0.25 (± 0.43)	0.25 (± 0.43)	0.13 (± 0.33)	0.25 (± 0.43)	1.38 (± 0.86)
Hemiodontidae											
Argonectes robertsi Langeani 1999	Iliophagous	0.13 (± 0.33)	0.63 (± 0.86)	0.25 (± 0.43)		0.13 (± 0.33)	0.13 (± 0.33)	0.25 (± 0.43)	0.25 (± 0.43)	0.63 (± 0.86)	0.25 (± 0.43)
Bivibranchia velox (Eigenmann & Myers 1927)	Iliophagous							0.50 (± 1.32)			
Hemiodus unimaculatus (Bloch 1794)	Iliophagous	1.13 (± 1.62)	1.00 (± 1.32)	0.38 (± 0.70)	0.50 (± 1.00)	1.25 (± 2.63)	0.75 (± 1.09)	0.25 (± 0.43)	0.63 (± 0.99)	1.13 (± 1.69)	1.25 (± 1.20)
Hemiodus vorderwinckleri (Géry 1964)	Iliophagous	2.13 (± 1.54)	2.25 (± 1.85)	2.13 (± 2.09)	5.13 (± 7.01)	2.13 (± 1.90)	0.38 (± 0.99)		0.38 (± 0.70)		
Prochilodontidae											
Prochilodus nigricans Spix & Agassiz 1829	Detritivores	0.25 (± 0.66)			0.13 (± 0.33)	0.63 (± 1.65)	0.13 (± 0.33)	0.13 (± 0.33)		0.38 (± 0.70)	0.13 (± 0.33)
Semaprochilodus brama (Valenciennes 1850)	Detritivores						0.25 (± 0.43)				
Serrasalmidae											
Myleus setiger Müller & Troschel 1844	Frugivorous		0.63 (± 1.32)	0.13 (± 0.33)		0.38 (± 0.70)	0.38 (± 0.48)	0.25 (± 0.43)	1.25 (± 1.39)	1.75 (± 1.79)	2.88 (± 4.01)
Myloplus arnoldi (Ahl 1936)	Frugivorous	0.25 (± 0.66)	0.38 (± 0.48)		0.13 (± 0.33)				0.13 (± 0.33)	0.25 (± 0.43)	0.88 (± 1.36)
Myleus schomburgkii (Jardine 1841)	Frugivorous		0.50 (± 0.87)	0.13 (± 0.33)		0.50 (± 0.87)				0.38 (± 0.99)	0.63 (± 1.32)
Ossubtus xinguense[E VU] Jégu 1992	Omnivorous	0.13 (± 0.33)	0.13 (± 0.33)	0.13 (± 0.33)	0.13 (± 0.33)		0.13 (± 0.33)				
Serrasalmus rhombeus (Linnaeus 1766)	Piscivorous		0.25 (± 0.43)		0.38 (± 0.48)	0.75 (± 0.97)				0.13 (± 0.33)	0.25 (± 0.43)
Tometes spp.[E]	Frugivorous	1.75 (± 0.97)	3.38 (± 4.99)	2.25 (± 2.17)	2.75 (± 1.92)	2.88 (± 1.76)		0.13 (± 0.33)		0.13 (± 0.33)	0.25 (± 0.66)
Gymnotiformes											
Electrophoridae											
Electrophorus electricus (Linnaeus 1766)	Piscivorous					0.13 (± 0.33)					
Myliobatiformes											
Potamotrygonidae											
Potamotrygon leopoldi[E] Castex & Castello 1970	Carnivorous		0.13 (± 0.33)	0.25 (± 0.43)		0.13 (± 0.33)					
Perciformes											
Cichlidae											
Cichla melaniae[E] Kullander & Ferreira 2006	Piscivorous	0.38 (± 0.48)	0.13 (± 0.33)	0.13 (± 0.33)	0.50 (± 0.50)	0.38 (± 0.70)					0.13 (± 0.33)
Crenicichla lugubris Heckel 1840	Piscivorous	0.25 (± 0.43)	0.38 (± 0.48)	0.38 (± 0.48)	0.50 (± 0.71)	0.75 (± 0.66)					
Crenicichla sp. 1	Piscivorous	0.13 (± 0.33)			0.13 (± 0.33)						

		Mean (± SD) relative abundance (MaxN)									
		ROCK					SAND				
		No bait	Cat food	Sweet corn	Fw. Croaker	Sardine	No bait	Cat food	Sweet corn	Fw. Croaker	Sardine
Geophagus altifrons Heckel 1840	Omnivorous	0.38 (± 0.70)	0.38 (± 0.70)		0.25 (± 0.66)	0.25 (± 0.43)	0.13 (± 0.33)	0.13 (± 0.33)			
Geophagus argyrostictus[E] Kullander 1991	Omnivorous	0.50 (± 0.71)	0.88 (± 1.54)	0.38 (± 0.70)	0.63 (± 0.86)	0.13 (± 0.33)					
Retroculus xinguensis[E] Gosse 1971	Omnivorous		0.13 (± 0.33)	0.75 (± 1.64)							
Teleocichla centrarchus Kullander 1988	Iliophagous			0.13 (± 0.33)							
Teleocichla cinderella Kullander 1988	Iliophagous	0.38 (± 0.70)	1.00 (± 0.71)	0.25 (± 0.66)	0.50 (± 0.71)	0.25 (± 0.43)	0.13 (± 0.33)		0.13 (± 0.33)		0.13 (± 0.33)
Teleocichla sp. 1	Iliophagous					0.13 (± 0.33)					
Siluriformes											
Auchenipteridae											
Centromochlus heckelii (De Filippi 1853)	Omnivorous									0.13 (± 0.33)	
Loricariidae											
Baryancistrus niveatus[E] (Castelnau 1855)	Iliophagous		0.13 (± 0.33)								
Baryancistrus sp. "verde"[E]	Iliophagous			0.25 (± 0.66)	0.13 (± 0.33)						
Baryancistrus xanthellus[E] Rapp Py-Daniel, Zuanon & Ribeiro de Oliveira 2011	Iliophagous	0.63 (± 0.70)	0.38 (± 0.70)	0.38 (± 0.70)		0.25 (± 0.43)					0.13 (± 0.33)
Hypostomus sp.	Iliophagous			0.13 (± 0.33)		0.13 (± 0.33)					
Loricaria birindellii[E] Thomas & Sabaj Pérez 2010	Iliophagous										0.13 (± 0.33)
Loricariidae n.i.	Iliophagous	0.25 (± 0.66)		0.13 (± 0.33)	0.25 (± 0.43)	0.38 (± 0.48)					
Pterygoplichthys sp.	Iliophagous				0.13 (± 0.33)						
Squaliforma emarginata (Valenciennes 1840)	Iliophagous			0.13 (± 0.33)							

Legend: E = Endemic fish species to the Xingu River; VU = Vulnerable species (IUCN Red List); gr. = group and aff. = affins; sp. 1 and sp.2 = morphotypes for new, not yet described species; (fam.) and (org.) = Common name for family or order if not available for species

CONCLUSÕES GERAIS E CONSIDERAÇÕES FINAIS

A pesquisa ecológica revelou que os peixes reofílicos no rio Xingu demonstram forte associação com os habitat aquáticos, notadamente com o tipo de substrato, mesmo em uma escala espacial reduzida. Os ambientes de praia e corredeira/pedral são agrupados de forma muito heterogenea na paisagem fluvial no trecho médio do rio Xingu. Mesmo assim, o BRUV possibilitou detectar essas diferenças significativas na riqueza de espécies, abundância relativa e na composição da assembléia de peixes na área de estudo.

O desenvolvimento do protocolo da amostragem específico para os habitats lóticos foi bem sucedido. A partir dos resultados obtidos determinou-se e/ou adequou-se: a isca mais eficiente (sardinha), o tempo de gravação e o número de réplicas mais econômico que ainda asseguram a obtenção de um conjunto de dados estatisticamente robusto baseado em estimativas acuradas de riqueza e abundância. Ainda foram refinados as configurações das câmeras digitais, a preparação e apresentação da isca, a distância entre isca e câmera e outros aspectos relacionados às técnicas de filmagem subaquática, à preparação e ao uso do equipamento de BRUV.

Os hábitats lóticos de rios amazônicos de água clara impedem o emprego de métodos tradicionais de levantamento ictiofaunístico. Extensas áreas de corredeiras, pedrais e cachoeiras com fortes correntezas impõem sérias limitações e riscos para o censo visual subaquático em mergulho livre e causam perda e/ou danificação de apetrechos de pesca (e.g., tarrafas). Na região amazônica ainda devem ser levados em consideração os potenciais riscos de acidentes com animais potencialmente perigosos como jacarés, serpentes, arraias de água doce e poraqués, entre outros. Ebner & Morgan (2013) apontam também para o perigo de ataques de crocodilos e tubarões cabeça-chata *Carcharhinus leucas* (Müller & Henle, 1839) em países tropicais.

Testar e confirmar a viabilidade do método BRUV para habitats lóticos de rios de água clara da região amazônica e desenvolver uma metodologia adaptada às condições biótica e abióticas complexas desses ambiente que prejudicam outros métodos foi importante pela necessidade de contribuir para o conhecimento sobre sua diversificada íctiofauna. Tais rios são e serão futuramente ainda mais sujeitos à alterações ambientais dramáticas impostas pelos projetos ambiciosos de

aproveitamento hídroenergético na região amazônica. As filmagens subaquáticas adquiridas com os BRUVS poderão ainda servir futuramente como registros valiosos da íctiofauna e dos habitats aquáticos amostrados.

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