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INSTITUTO DE CIÊNCIAS BIOLÓGICAS  
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA AQUÁTICA E PESCA

**DA ECOLOGIA REPRODUTIVA AO PROTOCOLO DO TAMANHO DE  
PRIMEIRA MATURAÇÃO SEXUAL EM PEIXES: MODELOS PARA UM  
CURIMATÍDEO AMAZÔNICO**

Tese de doutorado apresentado ao programa de Pós - graduação em Ecologia Aquática e Pesca da Universidade Federal do Pará como requisito parcial para a obtenção do título de Doutor em Ecologia Aquática e Pesca.

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PRIMEIRA MATURAÇÃO SEXUAL EM PEIXES: MODELOS PARA UM  
CURIMATÍDEO AMAZÔNICO**

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*“Não sei o que possa parecer aos olhos do mundo, mas aos meus pareço apenas ter sido como um menino brincando à beira-mar, divertindo-me com o fato de encontrar de vez em quando um seixo mais liso ou uma concha mais bonita que o normal, enquanto o grande oceano da verdade permanece completamente por descobrir à minha frente.”*

**(Isaac Newton)**

*“You're gonna need a bigger boat”*

**(Brody - Jaws, 1975)**

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*Aos meus pais, meu irmão e ao Mauricio.*

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## RESUMO

Compreender os mecanismos reprodutivos adaptativos e os fatores ambientais que os regulam, é de grande importância para a elaboração de ações de conservação, manejo e uso dos estoques naturais. Considerando o exposto, esta tese de doutoramento teve como principais objetivos: i) descrever os aspectos reprodutivos de uma espécie de peixe detritívora *Cyphocharax abramoides* e sua relação com os períodos pluviométricos no baixo Rio Anapu, ii) comparar três diferentes técnicas para estimativa do tamanho médio de primeira maturidade sexual ( $L_{50}$ ), e iii) testar a eficácia do método Bayesiano para a estimativa do  $L_{50}$  mesmo com um pequeno número amostral para três diferentes espécies de peixes amazônicos (*C. abramoides*, *Ageneiosus ucayalensis* e *Anableps anableps*). Os dados pluviométricos e 886 exemplares do curimatídeo foram obtidos no baixo Rio Anapu entre maio de 2012 e abril de 2013. Os indivíduos capturados (471 fêmeas e 415 machos) foram medidos, pesados e eviscerados para a retirada e pesagem das gônadas, que foram identificadas quanto ao sexo e tiveram seu estágio maturacional determinado macroscopicamente e confirmado histologicamente. Observou-se que a população de *C. abramoides* não apresentou diferença na proporção sexual entre os períodos pluviométricos, e que tanto machos quanto fêmeas apresentaram crescimento polifásico, com machos apresentando crescimento alométrico positivo e fêmeas crescimento alométrico negativo nas duas fases de crescimento observadas. O  $L_{50}$  do *C. abramoides* foi estimado ainda através da identificação macroscópica das gônadas (fêmeas = 13.70; machos = 13.50), utilizando o IGS como *proxy* de maturidade sexual (fêmeas = 15.04; machos = 14.74) e através da relação massa comprimento – LWR (fêmeas = 13.85; machos = 13.06). O  $L_{50}$  mostrou que machos atingem a maturidade sexual antes das fêmeas e a avaliação do fator de condição, dos valores de IGS e da distribuição da frequência relativa dos indivíduos maduros indicam um período reprodutivo longo, desova parcelada e dois picos de maior atividade reprodutiva altamente correlacionados com as chuvas, indicando a importância desta em ambientes com características lacustres. Considerando aspectos como a facilidade de obtenção dos dados, mortalidade dos peixes e acurácia dos resultados, a LWR se mostrou eficaz e indicada para a estimativa do  $L_{50}$  em peixes com crescimento polifásico. Por fim, o  $L_{50}$  foi estimado através do método bayesiano para três espécies de diferentes ordens e os resultados confirmaram a eficácia do modelo.

**Palavras-chave:** Reprodução,  $L_{50}$ , Inferência Bayesiana, Peixes, Amazônia

## ABSTRACT

Understanding the adaptive reproductive mechanisms and environmental factors that regulate them is very importance for conservation actions, management and use of natural stocks. Considering the above, this PhD thesis aims to describe: i) the reproductive aspects of the detritivorous fish *Cyphocharax abramoides* and its relationship with the rainfall periods in the Lower Rio Anapu, ii) to compare three different techniques to estimate the average size of first sexual maturity ( $L_{50}$ ) and iii) to test the effectiveness of the Bayesian method to estimate the  $L_{50}$  even with a small sample size using three different species (*C. abramoides*, *Ageneyosus ucayalensis* and *Anableps anableps*) of Amazonian fish. The rainfall data were obtained and 886 individuals of the curimatidae were captured in the Lower Anapu River between May 2012 and April 2013, with 471 females and 415 males. Specimens were measured, weighed and gutted to remove and weigh the gonads, which were sexed and had their maturational stage determined macroscopically and histologically confirmed. The *C. abramoides* population presented no difference in the sex ratio between rainfall periods, and both males and females showed polyphasic growth, with a positive allometric growth for males and negative allometric growth for females in both phases. The  $L_{50}$  has shown that males reach sexual maturity earlier than females and the evaluation of the condition factor, the gonad development and distribution of the relative frequency of mature individuals indicate a long reproductive period, parceled spawning and two peaks of greater reproductive activity highly correlated with the rainfall, indicating the importance of local precipitation in an environment with lake characteristics. The  $L_{50}$  of the *C. abramoides* was estimated by macroscopic identification of the gonads (females = 13.70; male = 13.50) using GSI as sexual maturity proxy (females = 15.04; male = 14.74) and through the length – weight relationship - LWR (females = 13.85; male = 13.06). Covering aspects such as the easiness to obtaining data, mortality of fish and accuracy of the results, the LWR is effective and suitable for the estimation of  $L_{50}$  in fish with polyphasic growth. Finally,  $L_{50}$  was estimated using Bayesian method for three species of different orders confirming the effectiveness of the model.

**Keywords:** Reproduction,  $L_{50}$ , Bayesian inference, Fishes, Amazon

## APRESENTAÇÃO GERAL

### INTRODUÇÃO

Entender como indivíduos de uma espécie alocam recursos e tempo entre as atividades relacionadas à manutenção, crescimento e reprodução é fundamental para compreender a sua ecologia (Wootton 1992). Levando em consideração que o sucesso biológico é determinado pela capacidade de um indivíduo em estar geneticamente representado na próxima geração, a reprodução é um dos eventos de maior importância dentro do ciclo de vida de uma espécie (Lowe-McConnell 1999).

Adaptações a fatores bióticos (i.e. pressão por predação, disponibilidade de alimento, respostas a fatores de estresse) e abióticos (i.e. tipo de habitat, temperatura, fotoperíodo, pluviosidade, fluviometria) são responsáveis por diversas estratégias adotadas por peixes teleósteos quanto a sua biologia reprodutiva (Vazzoler 1996; Agostinho et al. 2004; Barbieri et al. 2004; Cossins & Crawford 2005; Alvarenga et al. 2006; Guerrero et al. 2009; Godinho et al. 2010).

Na região Amazônica, o pulso de inundação é um evento abiótico fundamental no ciclo de vida dos peixes, que são submetidos a grandes variações sazonais, resultando na necessidade de aperfeiçoar os ganhos energéticos na época mais favorável, muitas vezes a cheia (Goulding 1980). O aumento da área alagada permite a exploração de novos ambientes e um aumento do espectro alimentar (Lowe-McConnell 1999; Neves Dos Santos et al. 2008) enquanto que a diminuição das águas limita o espaço, obrigando os peixes a migrarem da várzea para outros locais como o canal principal dos rios ou para os lagos permanentes (Ruffino & Isaac 1995).

A precipitação local também é fundamental para a biota aquática, já que as chuvas podem conduzir material alóctone das florestas adjacentes em direção aos cursos d'água e gerar um aumento na quantidade de material orgânico nos corpos d'água e da



diversidade de fontes alimentares detritais (Tundisi et al. 1993; Pereira & Resende 1998) favorecendo o sucesso de espécies detritívoras (Bowen 1983; Winemiller 1990; Araújo et al. 2009).

A grande quantidade de nutrientes disponibilizados com o aumento das águas e das chuvas, estimula a produção primária e secundária (Balcombe et al. 2005) e pode resultar na melhora da condição corporal, do recrutamento e da sobrevivência dos peixes (Balcombe & Arthington, 2009; Beesley et al. 2012) pois tanto a precipitação local quanto o pulso de inundação disponibilizam maior quantidade de ambientes e alimentos que podem ser cruciais para a alimentação, desova, refúgio e berçário de peixes (Poizat & Corivelli 1997; Baber et al. 2002; Cunico et al. 2002).

Peixes são um grupo extremamente diverso e sujeitos a diversas alterações no meio aquático, sendo por isso ótimos modelos para estudos fisiológicos, especialmente quanto a reprodução, por ser um processo de alto custo energético e ocorrer somente quando os indivíduos estão em sua zona de conforto ambiental e metabólico, podendo servir como modelo de estudo para a compreensão da influência dos fatores ambientais, naturais ou antropogênicos, sobre os sistemas biológicos (Ribeiro & Moreira 2012). Portanto, compreender os mecanismos adaptativos e os fatores ambientais que os regulam é de grande importância para a elaboração de ações de conservação, manejo e exploração de estoques pesqueiros (Barbieri et al. 2004).

Dentre as principais informações para elaboração de ações de manejo pesqueiro de uma população a longo prazo, a estabilidade do ambiente, a capturabilidade da espécie e os fatores que determinam seu recrutamento são considerados vitais para estudos e aplicação de medidas de manejo, pois ajudam a garantir a sobrevivência de novos recrutas que alcançarão a maturidade na próxima geração (Beverton et al. 1984). Além disso, são importantes as informações sobre as características reprodutivas da população alvo como

tipo e período de desova, que também ajudam a definir períodos de defeso (proibição da pesca durante a desova das espécies) e uso de apetrechos adequados para não comprometer o tamanho médio da primeira maturação sexual ( $L_{50}$ ) (Vaz-dos-Santos et al. 2013).

O tamanho médio de primeira maturação sexual descreve o comprimento ou classe de comprimento no qual 50% da população inicia o período reprodutivo (Vazzoler 1996) e o conhecimento desta estimativa pode auxiliar na definição do tamanho mínimo de captura e conseqüentemente fatores como o tamanho mínimo de malha das redes de pesca ou no tamanho mínimo dos anzóis para a pesca (Shephard & Jackson 2005; Wilberg et al. 2005; Binohlan & Froese 2009; Kinas & Andrade 2010; Schill et al. 2010; Stark 2012)

Devido a sua importância, diversos métodos têm sido utilizados para estimativa do  $L_{50}$ , sendo métodos frequentistas baseados em uma proporção de indivíduos maduros por classe de comprimento o mais comum (Chen & Paloheimo 1994; Dadebo et al. 2003; Lewis & Fontoura 2005; Fontoura et al. 2009). Para isso, a identificação dos indivíduos maduros dentro de uma população pode ser feita através da observação macroscópica e/ou microscópica (histológica) das gônadas, ou utilizando o índice gonadossomático (IGS) como *proxy* de maturidade sexual (Vazzoler 1996; Núñez & Duponchelle 2009; Fontoura et al. 2009).

Considerando que as demandas energéticas para reprodução podem se refletir no padrão de crescimento causando uma mudança na taxa de crescimento que pode indicar o tamanho no qual a população atinge o  $L_{50}$  (Quince et al. 2008; Fontoura et al. 2010) o valor pode ser estimado através de dados de comprimento e massa dos indivíduos, seja através de uma adaptação da fórmula da relação peso-comprimento de Huxley (1924) proposta por Bervian et al. (2006) na qual o crescimento de um indivíduo que pode ser

dividido em duas ou mais fases é considerado; ou através de novos modelos como o “Modelo genérico bifásico” que assume que o crescimento pós-maturação apresenta um formato de Von Bertalanffy (Quince et al. 2008).

Por fim, ao contrário da inferência frequentista usada na equação logística, o método bayesiano permite um resultado acurado da estimativa mesmo quando um pequeno número amostral é utilizado (Kontkanen et al. 1997), o que levou à um aumento do uso desse método em estudos de manejo pesqueiro (Chen et al. 2013; Mäntyniemi et al. 2005; Wilberg et al. 2005; He & Bence 2007; Chen et al. 2010; Doll & Lauer 2013).

Considerando a importância que as variações pluviométricas têm no aumento sazonal das fontes de alimento detritais e a importância do estudo sobre as características reprodutivas de peixes para seu manejo, esta tese teve como principais objetivos: (1) descrever os aspectos reprodutivos de uma espécie de peixe detritívora amazônica, o curimatídeo *Cyphocharax abramoides*, e sua relação com os períodos pluviométricos na Amazônia Oriental, (2) comparar três diferentes técnicas para estimativa do tamanho médio de primeira maturidade sexual em peixes utilizando o *C. abramoides* como estudo de caso e por fim, (3) testar o modelo bayesiano para estimativa do tamanho médio de primeira maturação sexual em peixes usando como estudo de caso três espécies de ordens distintas, *C. abramoides*, *Ageneyosus ucayalensis* e *Anableps anableps*.

Para melhor apresentação, a caracterização da área de estudo e os métodos de coleta foram abordados na apresentação geral, e cada objetivo citado foi trabalhado em forma de artigo, sendo o primeiro intitulado "*Reproductive seasonality of the detritivorous fish Cyphocharax abramoides (Kner, 1958) (Characiformes: Curimatidae) in flooded rivers of the Eastern Amazon*"; que conta com a co-autoria dos pesquisadores Rossineide Martins da Rocha (UFPA) e Luciano Fogaça de Assis Montag (UFPA). Esse artigo está submetido à "*Environmental Biology of Fishes*" e tem como objetivo descrever

os parâmetros reprodutivos do *Cyphocharax abramoides* a fim de compreender a influência das variações pluviométricas sobre o ciclo de vida de peixes detritívoros amazônicos, provendo informações importantes sobre a importância das chuvas locais para a reprodução de peixes em ambientes semi-lacustres.

O capítulo II é intitulado "*Comparing three methods to estimate the average size at first maturity (L<sub>50</sub>): A study case on Curimatidae exhibiting polyphasic growth*"; e contou com a coautoria dos pesquisadores Bruno Eleres Soares (UFRJ), Roberta Danniely Oliveira Raiol (UNAMA), Kyle Logan Wilson (University of Calgary) e Luciano Fogaça de Assis Montag (UFPA). Esse artigo está submetido à revista "*Canadian Journal of Fisheries and Aquatic Sciences (CJFAS)*" e utilizando o *C. abramoides* como espécie alvo, teve como objetivo comparar três métodos para a estimativa do tamanho médio de primeira maturidade sexual (L<sub>50</sub>) em peixes, e levando em consideração fatores como facilidade de acesso dos dados, a mortalidade de peixes e acurácia dos resultados, buscou-se gerar um compilado de informações para facilitar a elaboração de futuros trabalhos, além de resultados promissores para o manejo de estoques pesqueiros.

Um terceiro capítulo intitulado "*Estimating the average size at first maturity (L<sub>50</sub>) in fish by Bayesian method*" com coautoria dos pesquisadores Valéria de Albuquerque Oliveira (UNAMA), Bruno Spacek Godoy (UFPA) e Luciano Fogaça de Assis Montag (UFPA). Este artigo será submetido à revista "*Journal of Applied Ichthyology*" e teve como objetivo testar a eficácia do método Bayesiano como ferramenta para estimar o L<sub>50</sub> em peixes, usando três espécies de ordens diferentes como estudo de caso (*C. abramoides*, *A. ucayalensis* e *A. anableps*), auxiliando a estimar um número mínimo de dados a serem utilizados a fim de obter resultados acurados, facilitando assim a busca de dados para estimativa de L<sub>50</sub> mesmo com uma pequena unidade amostral.

E por fim, são apresentadas as considerações finais da tese.

## MATERIAL E MÉTODOS

Para a realização dos capítulos I e II foram utilizados dados de *C. abramoides* coletados durante a tese no Baixo Rio Anapu como caracterizado nos tópicos a seguir. Para o capítulo III, além dos dados de *C. abramoides* coletados, foram utilizadas informações de *A. ucayalensis* (V. Oliveira, Informação pessoal) também coletado no Baixo Rio Anapu, e *A. anableps* coletados no Rio Maracanã (Oliveira et al. 2011).

### *Área de estudo*

O Rio Anapu é um rio de água preta que nasce nas proximidades da cidade de Carvalho (PA) e deságua na foz do Rio Pará, na Baía de Portel. Na área do Baixo Rio Anapu ocorre um alargamento chamado de Baía de Caxiuanã e Baía dos Botos, que apresenta diversas “baías” com formação característica de bocas de rios alagados. Nessa região, foi fundada a primeira Floresta Nacional (FLONA) da Amazônia Legal Brasileira, a FLONA de Caxiuanã, situada nas coordenadas 1°42’30”S e 51°31’45”W (Figura 1), nos Municípios de Melgaço e Portel no estado do Pará, a aproximadamente 350km da capital Belém. Apesar da região em torno da baía de Caxiuanã não ser um verdadeiro lago, esta apresenta uma forte semelhança com ambientes lacustres (Montag & Barthem, 2003)

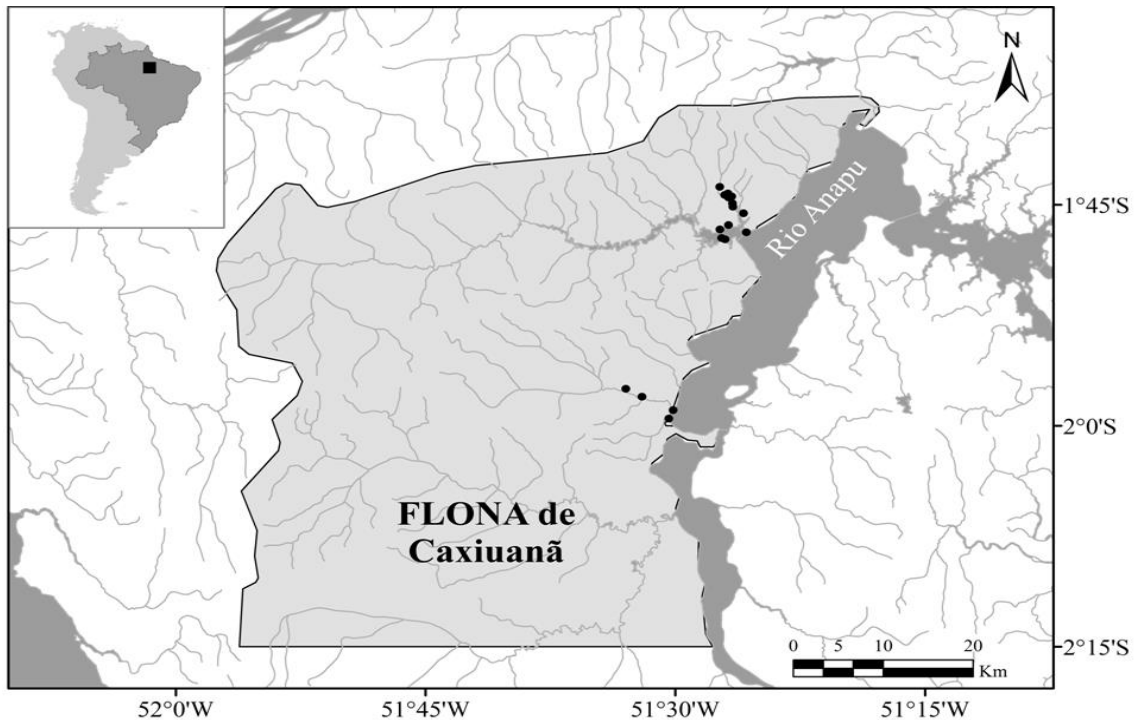


Figura 1 – Área de estudo apresentando os pontos de captura do curimatídeo *Cyphacharax abramoides* no Baixo Anapu, dentro da Floresta Nacional de Caxiuanã - Amazônia Oriental.

Dentro da FLONA de Caxiuanã, encontra-se a Estação Científica Ferreira Penna (ECFPn), que dá suporte para diversas atividades de pesquisa e serviu como base para a realização deste estudo. Segundo os dados da estação meteorológica da ECFPn, o clima da região é tropical úmido com tipo climático Am (de acordo com a classificação de Köppen). A região tem sazonalidade bem definida, com quatro períodos hidrológicos distintos apresentando períodos mais chuvosos entre março e abril, podendo chegar a 460 mm de pluviometria, e períodos de estiagem entre agosto e novembro, com pluviosidade inferior a 50 mm.

O nível da água na região apresentou variação anual de 50 cm no período de coleta, com padrões fluviométricos altamente correlacionados com a pluviometria; e variação diária de aproximadamente 30 cm com estreitas áreas alagáveis devido a reduzida influência da maré (Montag et al. 2008) (Figura 2).

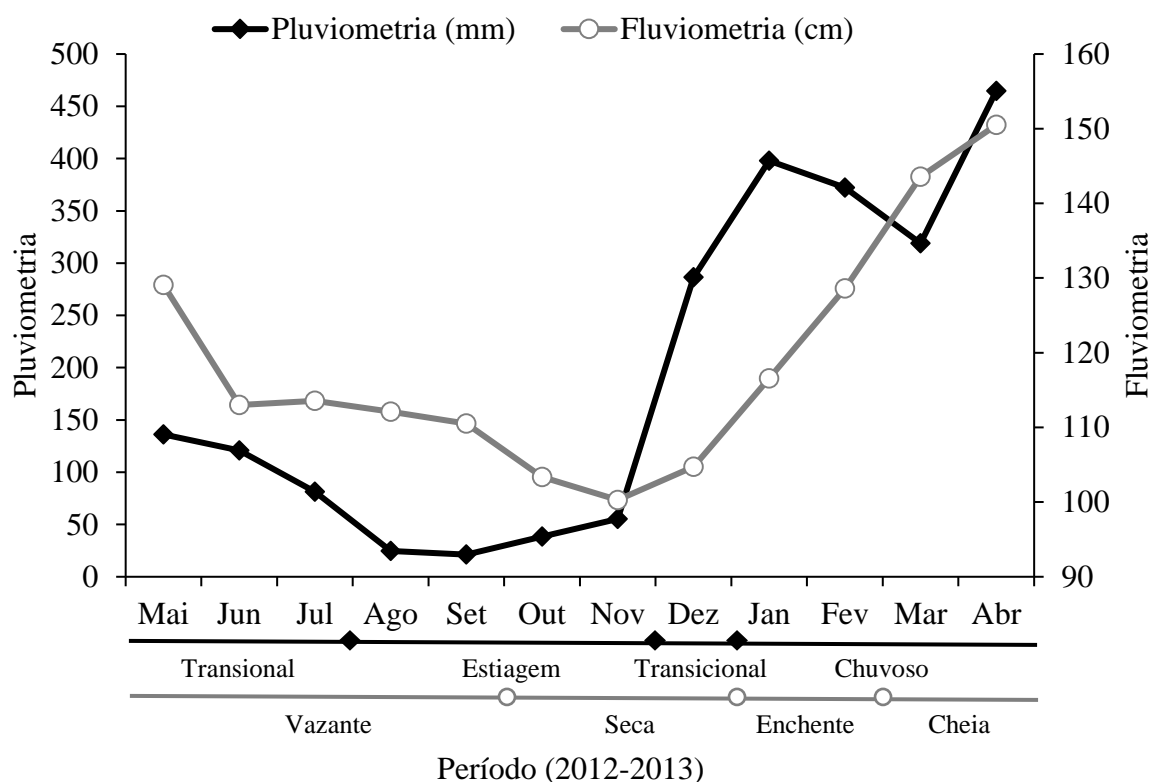


Figura 2 – Variação Pluviométrica e Fluviométrica dos rios da região do Baixo Anapu na Floresta Nacional de Caxiuanã com seus respectivos períodos hidrológicos para o período de coleta - Amazônia Oriental entre Maio de 2012 e Abril de 2013. Fonte: Agência Nacional das Águas (ANA) e Experimento em Grande Escala da Biosfera - Atmosfera na Amazônia (ESECAFLOR/LBA) e *Tropical Ecology, Assessment and Monitoring Initiative* (TEAM).

### *Procedimento de campo*

A coleta dos indivíduos de *C. abramoides* teve duração de um ano (maio de 2012 a abril 2013), e ocorreu mensalmente nos rios e igarapés no baixo rio Anapu, dentro da área da FLONA de Caxiuanã. Foram utilizadas redes de emalhe (Figura 3) de malhas 2, 3, 4, 8, 10 e 12 cm entre nós opostos, cujas diferentes malhas foram estendidas aleatoriamente, a fim de formar duas baterias de aproximadamente 200 m de comprimento e 1,5 m de altura, que ficaram submersas entre 16h e 22h.



Figura 3 – Rede de espera sendo armada no Baixo Rio Anapu na Floresta Nacional de Caxiuanã, Amazônia Oriental para a captura dos exemplares de *Cyphocharax abramoides*.

Mensalmente foram coletados aproximadamente 100 indivíduos de tamanhos variados de *C. abramoides*, que após coleta foram levados ao Laboratório da Estação Científica Ferreira Penna, mensurados para obtenção do comprimento total em centímetros (Ct) com 0.1 cm de precisão e pesados para determinação da massa total em gramas (Mt) com 0,001 g de precisão (Figura 4). As gônadas de todos os espécimes foram retiradas a partir de uma incisão ventro-longitudinal, pesadas (precisão 0,001g) e classificadas macroscopicamente quanto ao sexo e estágio maturacional. Após a pesagem



e identificação, as gônadas foram fixadas em formol a 10% por até 24 horas e posteriormente preservadas em álcool 70% para posterior procedimento laboratorial.



Figura 4 – Exemplos de *C. abramoides* sendo medidos para obtenção do comprimento total (Ct) e massa total (Mt) na Estação Científica Ferreira Penna (ECFPN), coletados no Baixo Rio Anapu, Amazônia Oriental.

Espécimes testemunhos foram depositados no acervo da coleção ictiológica do Museu Paraense Emílio Goeldi (MPEG), na cidade de Belém, Pará, Brasil, sob os registros MPEG 30477 e MPEG 30478.

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## **CAPÍTULO I**

**REPRODUCTIVE SEASONALITY OF THE DETRITIVOROUS FISH**  
***Cyphocharax abramoides* (KNER, 1958) (CHARACIFORMES: CURIMATIDAE)**  
**IN FLOODED RIVERS OF THE EASTERN AMAZON**

Capítulo submetido à revista *Environmental Biology of Fishes* (ISSN: 0378 - 1909)

**Reproductive seasonality of the detritivorous fish *Cyphocharax abramoides* (Kner, 1958) (Characiformes: Curimatidae) in flooded rivers of the Eastern Amazon**

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**Abstract:** The Lower Anapu River, Eastern Amazon, has a discrete flood pulse with narrow flooded areas due to the reduced influence of tides. In this environment, local precipitation might act as an important factor in the input of detrital food, which can serve as a trigger for fish reproductive period. Therefore, this paper aims to determine the effects of local precipitation on the reproductive ecology of an abundant detritivorous fish in flooded rivers. Between May 2012 and April 2013, 886 specimens were captured monthly using gill nets; 471 females and 415 males. Individuals were measured, weighed, sexed, and the gonadal development stage was defined macroscopically and confirmed histologically. Sex ratio, length-weight relationship (LWR), condition factor (k), Gonadosomatic Index (GSI), relative frequency of maturity stages, and size at first gonadal maturity ( $L_{50}$ ) were estimated and tested in relation to rainfall periods. There was

no difference between the expected sex ratio (1:1), and both males and females showed polyphasic growth. The condition factor indicated higher values at the beginning of the rainy season and lower values during the rainy/dry transitional season for both sexes. GSI and relative frequency of mature individuals indicated a long reproduction period with multiple spawning events along the year, and two peaks with higher activity during the rainy/dry and rainy seasons. The  $L_{50}$  value showed that males reach sexual maturity before females. Results indicated a reproductive pattern strongly influenced by rainfall, corroborating the importance of local precipitation for lacustrine environments.

**Key words:** Amazon,  $L_{50}$ , Rainfall periods. Spawning season, Teleostei

## **Introduction**

The Flood Pulse Concept (FPC) proposed by Junk et al. (1989) is the most widely accepted theory on the functioning of floodplains, and it states that annual and predictable flood pulse is the main driving force maintaining biodiversity and production of river-floodplain ecosystems (Tockner et al. 2000). Predictability, duration, and extent of flood pulses are considered the main factors determining the use of floodplains for spawning and growth of fish (Welcomme & Halls 2004; Balcombe & Arthington 2009).

This concept shows that regular and predictable processes can be major factors influencing fish populations in floodplains. Lakes, however, receive much less heterogeneous sources of water, dissolved solids, and allochthonous organic matter than rivers, and can reflect regional rainfall patterns better than rivers (Wantzen et al. 2009) since local precipitation can mobilize terrestrial organic matter and increase the already high concentration of nutrients in reservoirs (Geraldes & Boavida 2004).

The Lower Anapu River region, in the Eastern Amazon, is an area with specific characteristics regarding its hydrography, since it has an extension called Caxiuanã Bay with formation of several flooded rivers featuring a predominantly lacustrine environment. Moreover, despite the similarity to lake environments, the region around the Caxiuanã Bay still has a discrete flood pulse with narrow flooded areas due to the reduced influence of tides (Montag & Barthem 2006).

In this environment, local precipitation might act as an important factor in detrital food input, since rain can carry allochthonous material from the adjacent forests towards streams and generate an increased amount of organic matter in water bodies (Tundisi et al. 1993). In general, internal organic matter cycles and nutrients coming from both water and terrestrial environments lead to nutrient uptake, resulting in a system that operates at a higher trophic level than expected (Junk 1999), which can influence the dominance of certain fish guilds.

For instance, the vast majority of fish species occupies higher trophic levels and uses invertebrates as their link to the detritivorous base of the food chain (Bowen 1983); however, in Neotropical and South American rivers with large floodplains there is a high proportion of detritivorous fish associated to the abundance of detritus and to their successful assimilation (Bowen 1983; Pereira & Resende 1998). The Curimatidae family is a highly specialized consumer of detritus (Bowen 1983), and in a study by Montag & Barthem (2006) in FLONA of Caxiuanã, *Cyphocharax abramoides* (Kner 1958) was one of the most dominant species. Detritivorous habit is one of the major pathways for the conversion of plant material into animal biomass (Bowen 1983), and it facilitates organic matter decomposition, accelerating nutrient recycling process and increasing environmental productivity (Gneri & Angelescu 1951)

Therefore, the reproductive period of several fish species can be related to hydrological periods, and considering an environment with subtle flood pulse and lacustrine characteristics, our paper aims to determine the effects of local precipitation on the reproductive ecology of an abundant detritivorous fish (*C. abramoides*) in flooded rivers of the Lower Anapu River. We expect to find that local precipitation shows a positive correlation with spawning season, body condition, sexual proportion, and size at first maturity of fish, caused by a larger amount of environments and detrital food availability, which can be crucial for feeding, spawning, shelter, and nursery (Poizat & Corivelli 1997; Baber et al. 2002; Cunico et al. 2002).

## **Material and methods**

### *Study Area*

The Lower Anapu River is part of the first National Forest (FLONA of Caxiuanã) in the Brazilian Legal Amazon (1°42'30"S e 51°31'45"W) and its black water rivers and creeks are naturally dammed (Figure 5) (Montag & Barthem 2006). The Anapu River has an enlargement called Caxiuanã Bay, and whereas the region around the Bay is not a real lake, it has a strong resemblance to lacustrine environments or "ria lakes" (Montag & Barthem 2006).

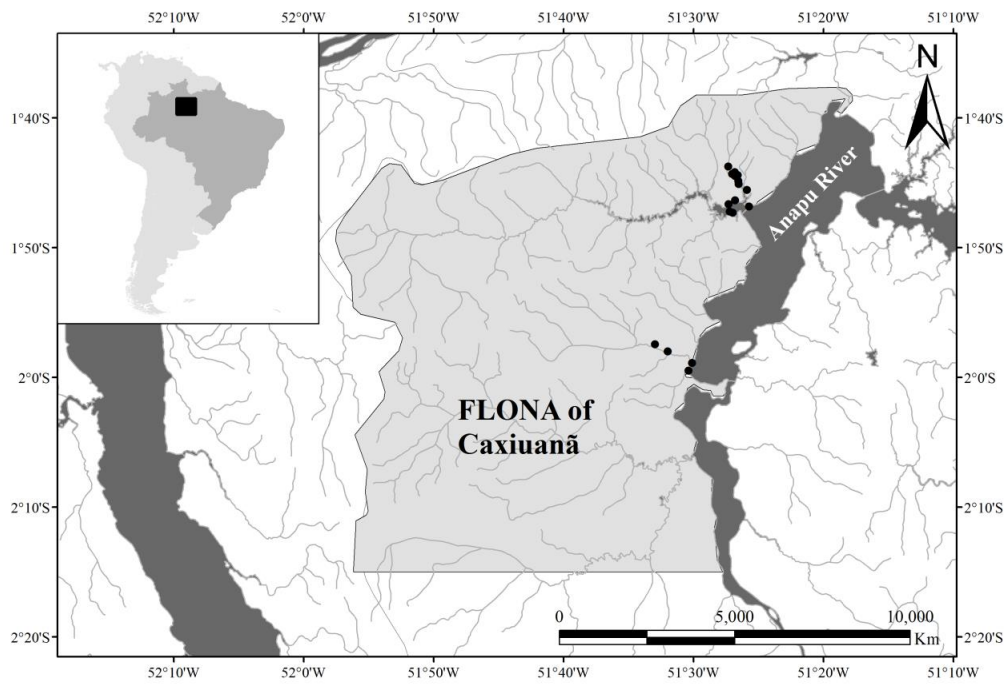


Figure 5 – Study area and data location inside FLONA of Caxiuanã, Lower Anapu River, Pará State, Brazil.

According to Köppen’s classification, the climate is tropical humid, subtype “Am”, with average rainfall around 2,020mm (Moraes et al. 2009). During the sampling period, the region had a well-defined seasonality with four distinct hydrological periods (Rainy, Rainy/Dry, Dry, and Dry/Rainy) with the rainiest seasons between March and April, reaching 460 mm of rainfall, and dry seasons between August and November, with rainfall lower than 50 mm (Figure 6). The annual water level oscillation is not remarkable and varies with rainfall, and the rivers have a 30cm daily tide with well-defined edges and narrow floodplains (Montag & Barthem 2006).

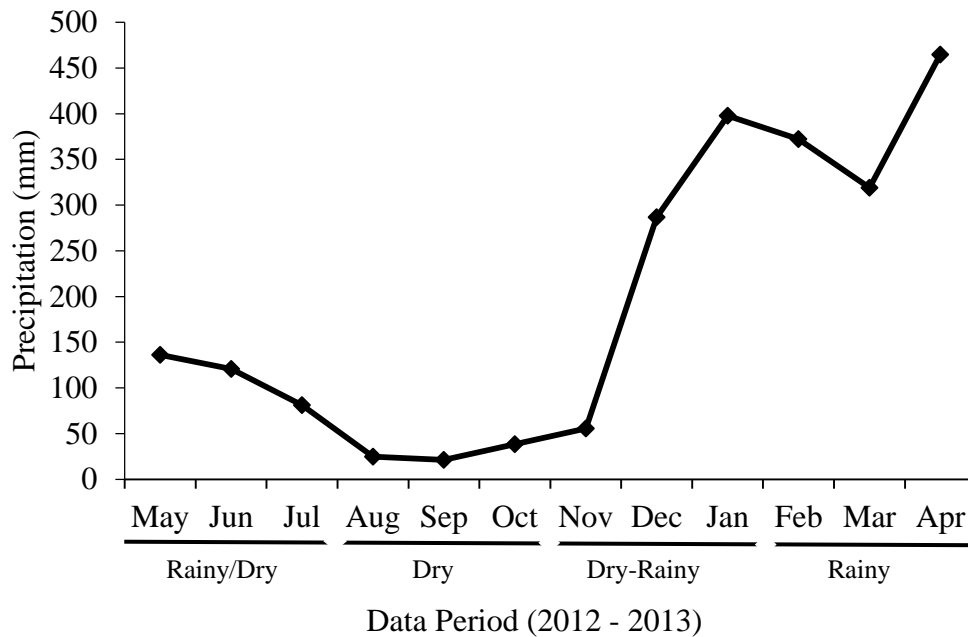


Figure 6 – Local rainfall variation with its respective hydrological periods identified over the study period in the Lower Anapu River, Eastern Amazon. Source: The Large Scale Biosphere-Atmosphere Experiment in Amazonia (ESECAFLOR / LBA) and Tropical Ecology, Assessment and Monitoring Initiative (TEAM).

*Field Procedures and laboratorial analysis*

Fishery procedures occurred monthly between May 2012 and April 2013 in the rivers and streams of Caxiuanã National Forest. Sampling was conducted using gill nets (knot-to-knot meshes of 15, 20, 25, 30, 35, and 40 mm and total length of 200 meters), submerged between 4 p.m. and 10 p.m. Captured specimens were identified, measured to the nearest 0.1 cm to obtain Total Length, and weighed to the nearest 0.1 g to determine total mass (TM).

Gonads were removed from all specimens and weighed fresh. Individuals were sexed and gonadal development was classified macroscopically according to Núñez & Duponchelle (2009) in immature (A), maturing at maturity (B), mature (C), and spawned

or spermiated (D). Gonads were then fixed in formalin (10%) for 24 hours, and then, preserved in alcohol (70%) for subsequent analysis.

Macroscopic classification of gonads was used in the statistical analysis, and in order to confirm its effectiveness, approximately five monthly samples of each maturation stage were examined on a microscope to confirm the stages, whenever possible. After fixation, the samples went through a dehydration process in solutions with increasing amounts of ethyl alcohol (70% - 100%), cleared in xylene (I and II), and subsequently, embedded in paraffin for subsequent 5- $\mu$ m-thick cuts. The slides were stained in hematoxylin-eosin (H.E) for observation under a photomicroscope and were classified according to the methodology proposed by Nunez & Duponchelle (2009); (i) immature, (ii) maturing, (iii) mature, (iv) spent, and (v) resting.

Voucher specimens were deposited in the ichthyological collection of Museu Paraense Emílio Goeldi - MPEG (Belém, Pará, Brazil) under MPEG 30477 and MPEG 30478 codes.

### *Statistical Analysis*

Sex ratio (females: males) was calculated based on the absolute frequency of males and females per rainfall season for each length class. Based on the null hypothesis that a 1:1 ratio would be expected regardless of length class and season, significance deviation was tested using Chi-square (Sokal & Rohlf 1995).

To characterize growth patterns and identify potential differences between sexes, the length-weight relationship (LWR) was calculated for each sex, based on the equation proposed by Huxley (1924):  $TW = a.TL^b$ , where TW is the total mass (g); a is the coefficient of proportionality; TL is the total length (cm); and b is the coefficient of allometry. Then, proportional residue ((Observed weight - expected weight) / Observed



weight) was plotted as a function of TL to identify possible trends in the data and Huxley's allometric equation was adjusted using the *Solver* routine (Microsoft Excel®).

Since proportional residue was not randomly distributed along the y-axis, LWR is not suited to a regular potential equation, suggesting a polyphasic growth. We used linear regression and an adjusted equation to confirm if there is a residue trend according to individual lengths, considering that a biphasic LWR was used according to the methodology proposed by Bervian et al. (2006).

A Student *t-test* was performed for females and males to determine if the b value was significantly different from 3, at a significance level of 5% (Zar 1999), assuming that, when  $b = 3$ , growth is isometric, i.e., length increases at the same rate as mass, and when b is significantly larger or smaller than 3, growth is allometric, i.e., body length and mass grow at different rates.

The allometric condition factor was estimated for males and females based on the equation:  $K = \frac{TW}{TL^b}$ , where k is the condition factor and b is the coefficient of allometry obtained in the LWR (Braga 1986).

Reproductive periods were established through monthly distributions of the relative frequencies of maturation stages and through monthly variations in average gonadosomatic index (GSI) for males and females (Santos 1978), according to the formula:  $GSI = \frac{Gonad\ Weigh}{Total\ body\ mass} * 100$  (Nikolsky 1969). This method provides a more accurate reproductive season, eliminating the variation in average individual weight.

GSI values and condition factor (k) were assessed regarding statistical testing assumptions and the variation between rainfall periods (rainy, rainy/dry, dry and dry/rainy) was tested using Kruskal-Wallis test (*H*) with 5% significance level, followed by Nemenyi's post hoc test (Zar 1999).


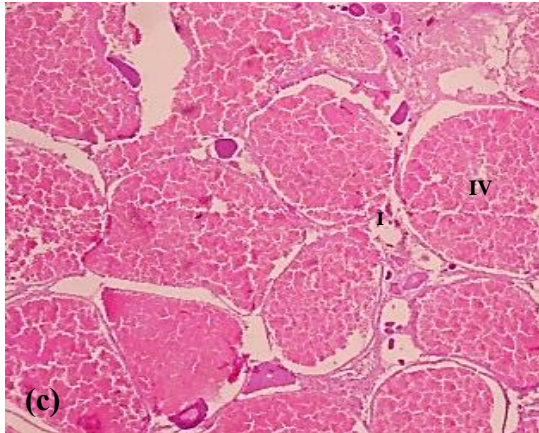
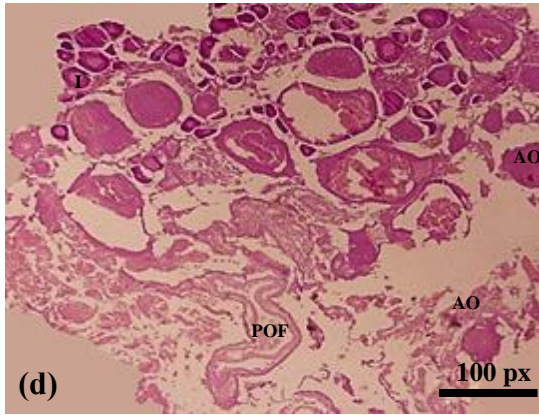
Size at first maturity ( $L_{50}$ ) can be defined as the size where 50% of individuals in the population actively enter into sexual maturation (Vazzoler 1981), and it was determined based on the number of mature individuals (maturate stages B, C, and D) per length class, according to the formula  $P = \frac{1}{1 + \exp[-r(TL - La)]}$ , where P is the proportion of mature individuals; r is the curve slope; TL is the total length (cm), and La is the average length (cm) of sexual maturity.

## Results

A total of 886 *C. abramoides* individuals were captured, with 471 females and 415 males, a sex ratio slightly biased towards females, 1.13:1 ( $\chi^2 = 3.53$ ;  $p > 0.05$ ), and there were no significant differences between rainfall periods (Rainy/Dry:  $\chi^2 = 0.001$ ,  $p = 0.025$ ; Dry:  $\chi^2 = 0.534$ ,  $p = 0.5$ ; Dry/Rainy:  $\chi^2 = 0.124$ ,  $p = 0.75$ ; and Rainy:  $\chi^2 = 1.236$ ,  $p = 0.075$ ). The average size of females, with a mean length of 17.4 cm ( $\pm$ SD 3.8) and average weight of 84 g ( $\pm$ SD 30.5), was higher than the average male, with mean length of 16.4 cm ( $\pm$  SD 1.9) and weight of 63g ( $\pm$  SD 21.3). An average of 39.2 females (min = 17; max = 58) and 34.5 males (min = 13; max = 75) were analyzed monthly.

Gonads of both sexes of *C. abramoides* are paired, tubular structures. The paired ovaries are approximately the same size and have different patterns of color, size, consistency, and vascularization, depending on the gonadal development stage. Histological analyses were performed on approximately 100 males and 100 females, representing all maturation stages; five different stages were observed for females and four stages were observed for males (Table 1).

Table 1 - Description of histological maturation stages observed for males and females of *C. abramoides* in the Lower Anapu River, Eastern Amazon.

		Female	(Figure	8
Maturation Stages	Male (Figure 7)			
				
				
Immature	Spermatogonia surrounded by connective tissue	<p>I) Ovigerous lamellae containing oogonia (small cells adhered to the lamellae wall)</p> <p>II) Pre-vitellogenic oocytes (with basophils and homogeneous cytoplasm, central nucleus, and evident nucleolus)</p>		

Maturing	<p>Numerous and well-organized seminiferous tubules with spermatogonia</p> <p>(St) Spermatocytes</p> <p>(Sd) Spermatids</p> <p>Presence of a small amount of free sperm (z) in the lumen</p>	<p>(II) Few pre-vitellogenic oocytes</p> <p>(III) Oocytes in early vitellogenesis (featuring cortical alveoli in the cell periphery)</p> <p>(IV) Oocytes in advanced vitellogenesis (featuring a central core, acidophilus cytoplasm filled with yolk, no obvious nucleus, and cortical alveoli)</p>
Mature	<p>Seminiferous tubules of irregular sizes, completely filled with sperm (z)</p>	<p>Presence of all cell types with predominance of vitellogenic oocytes (IV)</p>
Spent or Spawned	<p>Presence of empty tubules and a small amount of other cells (spermatogonia, residual sperm)</p>	<p>Few I, II, and III oocytes</p> <p>Presence of post-ovulatory follicles (POFs) and atretic oocytes (AO)</p>
Resting		<p>Predominance of pre-vitellogenic oocytes (I)</p>

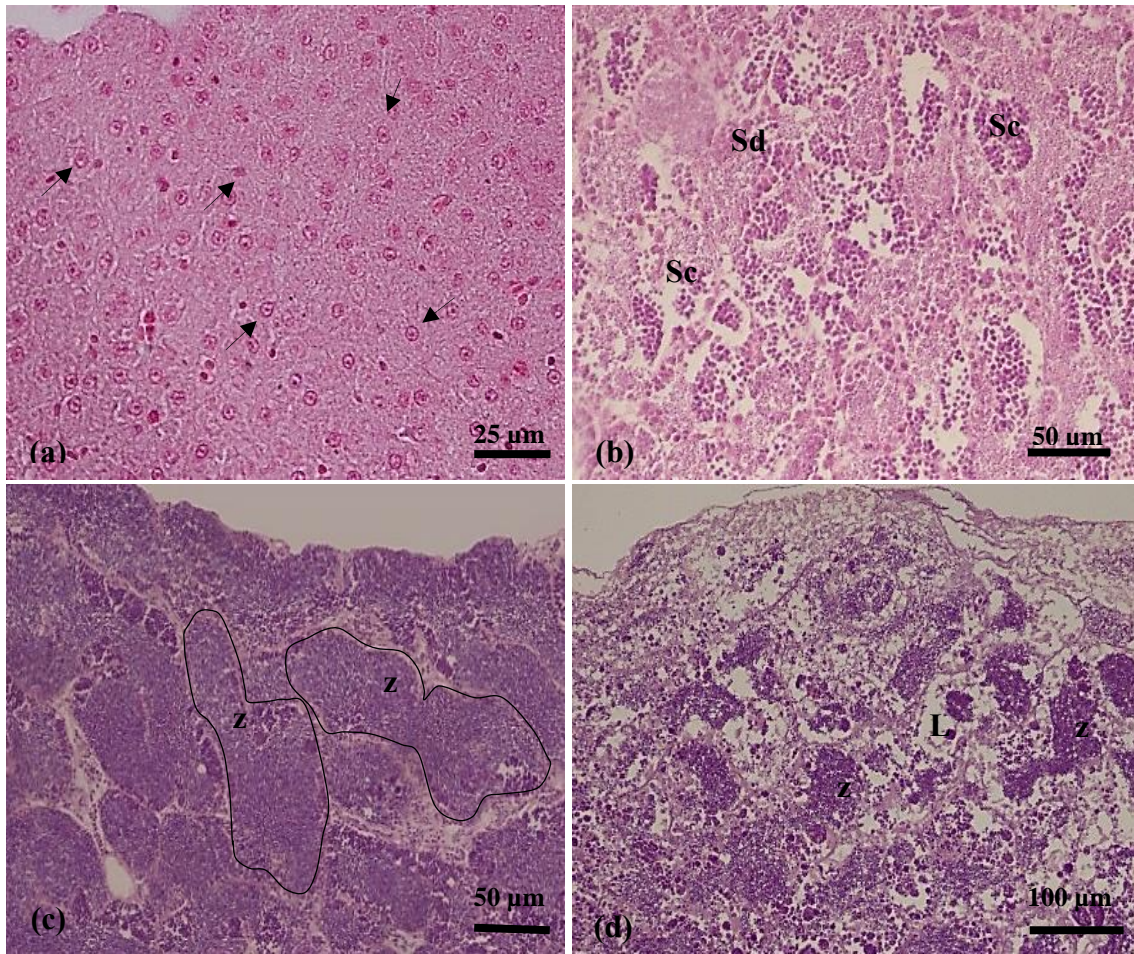


Figure 7 – Photomicrograph of gonadal development in males of *Cyphocharax abramoides* at different stages of maturation. (a) Immature – seminiferous tubules containing spermatogonia (arrow); (b) Maturing – with spermatogonia, spermatocytes (St), and spermatids (Sd); (c) Mature – Anastomosing seminiferous tubules (line) filled with sperm (z); (d) Spent – Tubules with lumen (L) containing residual sperm.



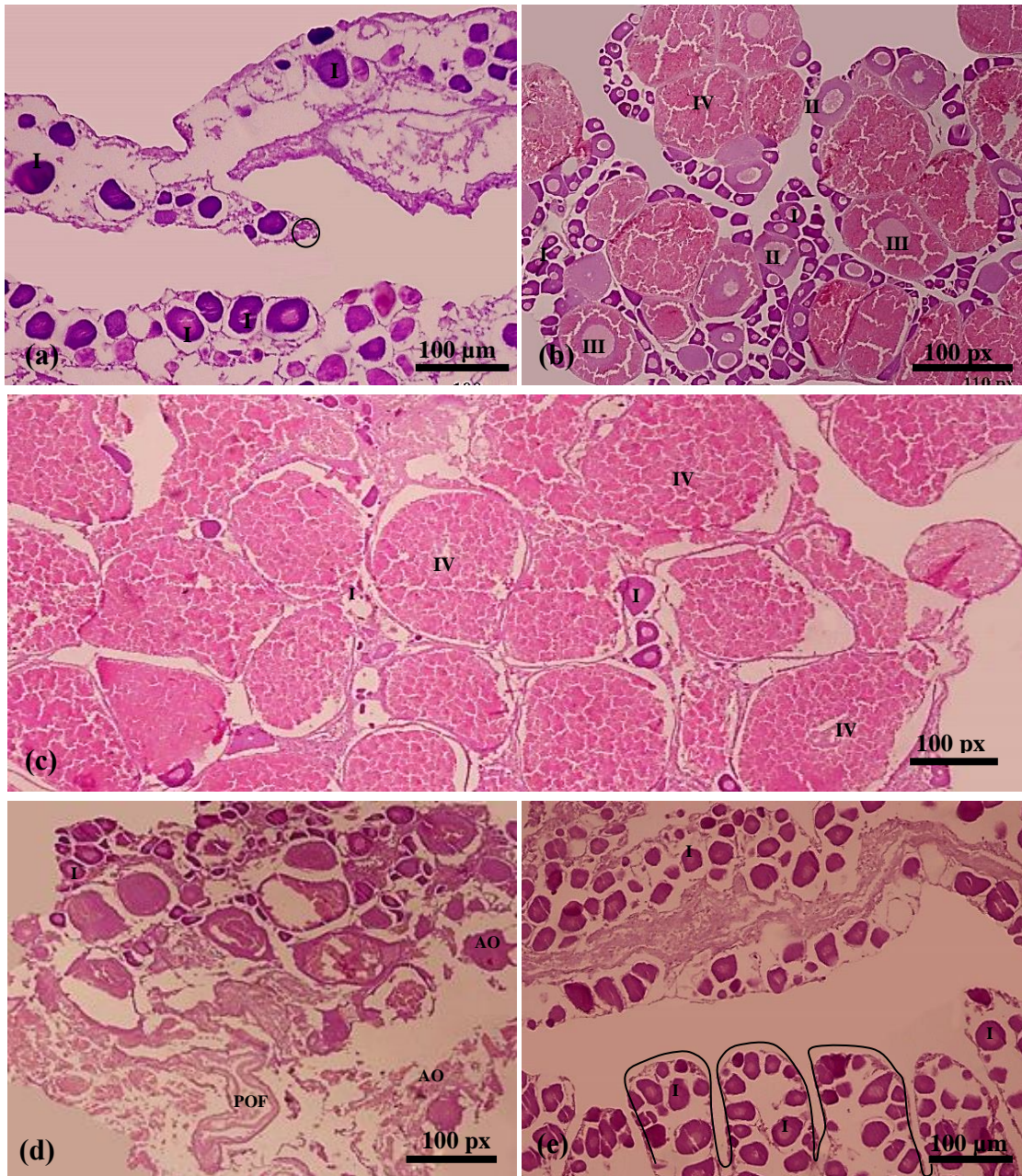


Figure 8 – Photomicrograph of gonadal development in females of *Cyphocharax abramoides* at different stages of maturation: (a) Immature – with type I oocytes (I) and oogonial nests (circle); (b) Maturing – abundance of type II, type III, and type IV oocytes; (c) Mature – predominance of type IV oocytes; (d) Spawning – post-ovulatory follicles (POF), atretic oocytes (AO) and type I oocytes; (e): Resting - ovigerous lamellae (line) containing type I oocytes.

The adjusted LWR for fish with polyphasic growth showed positive allometric growth for males (phase 1: 3.211; phase 2: 3.064) and negative allometric growth for females (phase 1: 2.733; phase 2: 2.590) in both phases. After the adjustment and analysis of proportional residuals, the model proved to be adequate (Figure 9;  $p > 0.05$ ).

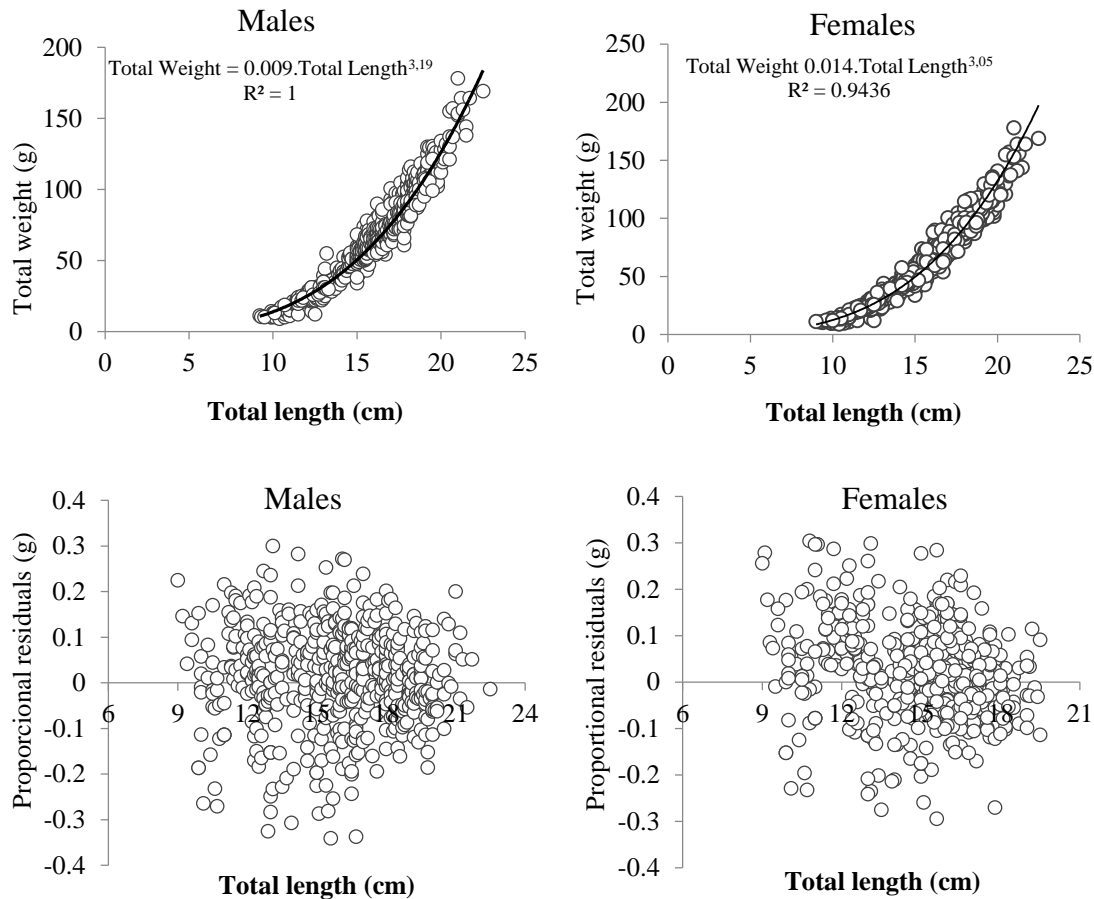


Figure 9 – Length-Weight Relationship and adjusted proportional residual distribution using polyphasic model for males and females of *Cyphocharax abramoides* collected at the Lower Anapu River, Eastern Amazon.

The condition factor ( $k$ ) indicated that males ( $k: H_{(11,367)} = 100.536, p < 0.005$ ; Figure 10a) and females ( $k: H_{(11,421)} = 45.318, p < 0.005$ ; Figure 10b) differ seasonally,

with higher values at the beginning of the rainy season and lower values during the rainy/dry transitional season for both sexes.

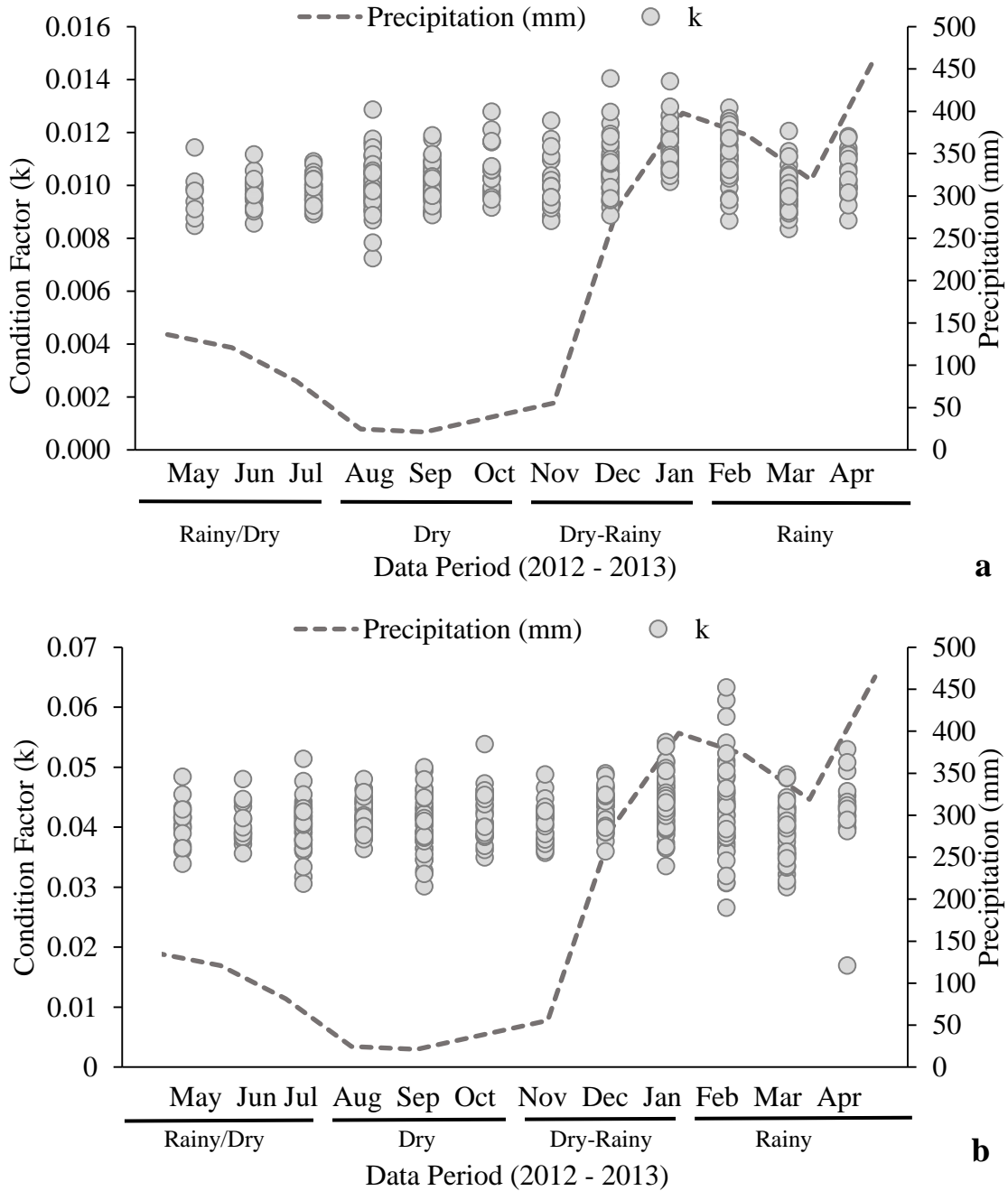
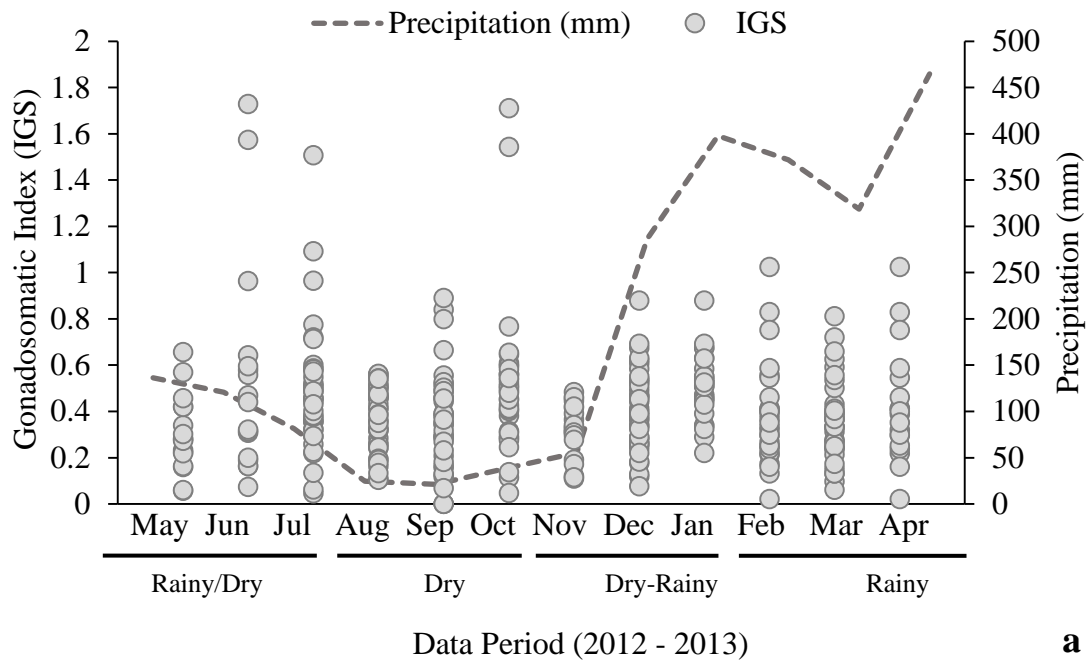


Figure 10 – Variation in the total conditional factor (k) of males (a) and females (b) of *C. abramoides* captured in the Lower Anapu River, Eastern Amazon.



GSI variation in males showed greater activity during the Rainy/Dry and dry seasons and in the Rainy season ( $H_{(11,361)} = 37.260$   $p < 0.005$ ) (Figure 11a), which was corroborated by the seasonal variation in maturation stage frequencies, revealing a higher proportion of mature males at the beginning and end of the rainy season (Figure 12a). A similar pattern was observed in females, with higher GSI values during the dry season and at the beginning of the rainy season ( $H_{(11,411)} = 147.645$ ,  $p < 0.005$ ; Figure 11b), corroborated by monthly maturation frequencies that also show females in all maturation stages throughout the sampling period (Figure 12b).



**a**

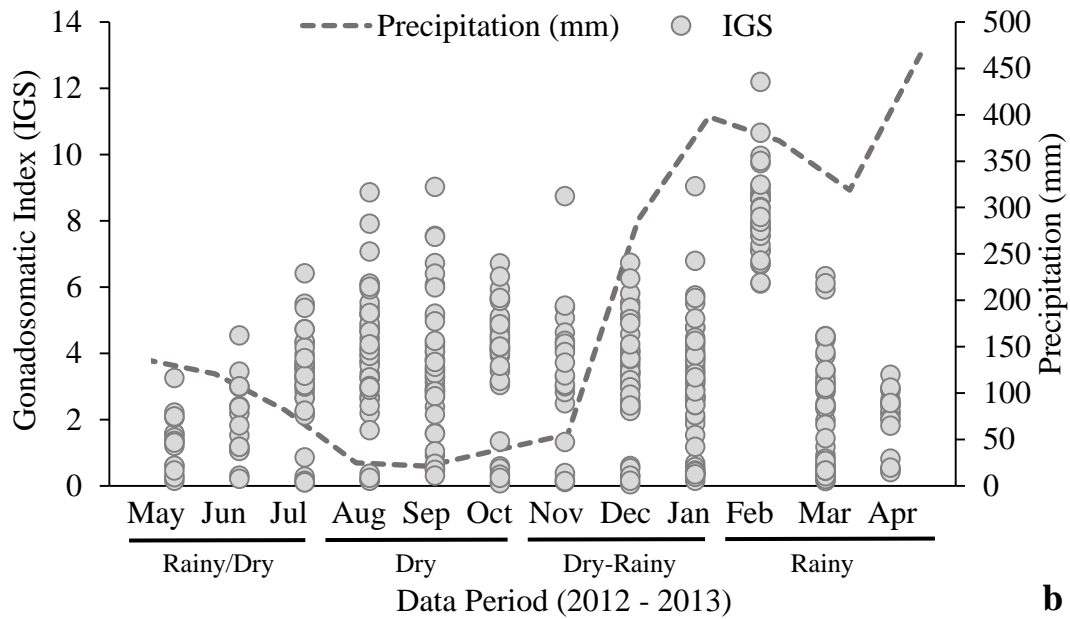
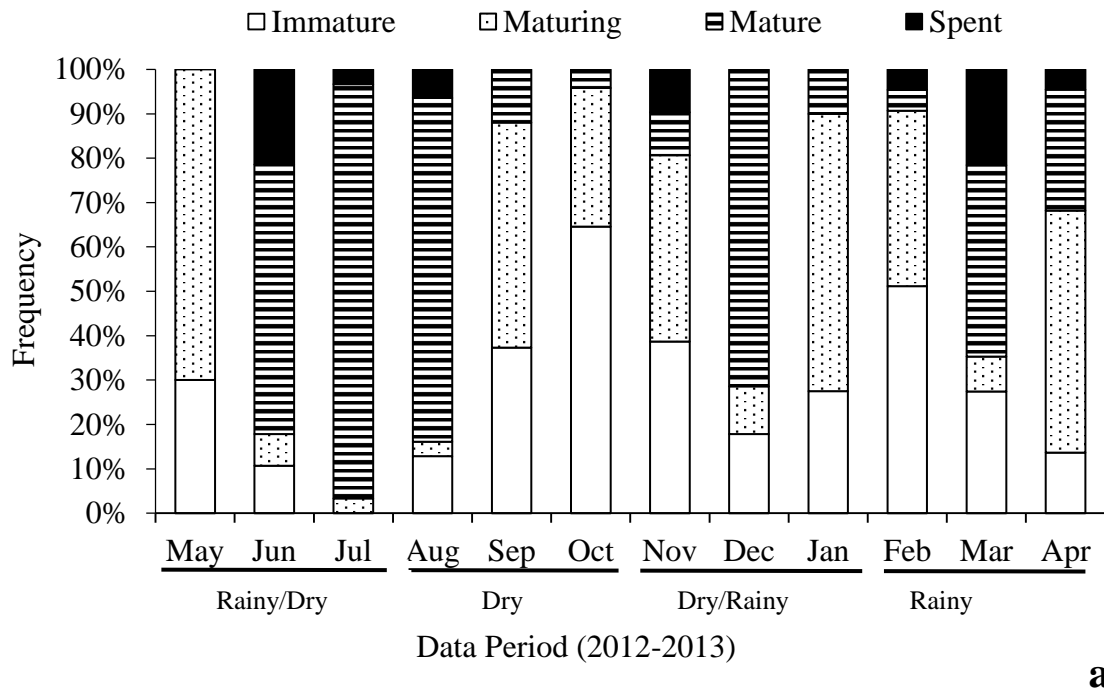


Figure 11 – Monthly variation in Gonadosomatic Index (GSI) in males (a) and females (b) of *C. abramoides* captured at the Lower Anapu River, Eastern Amazon.



**a**

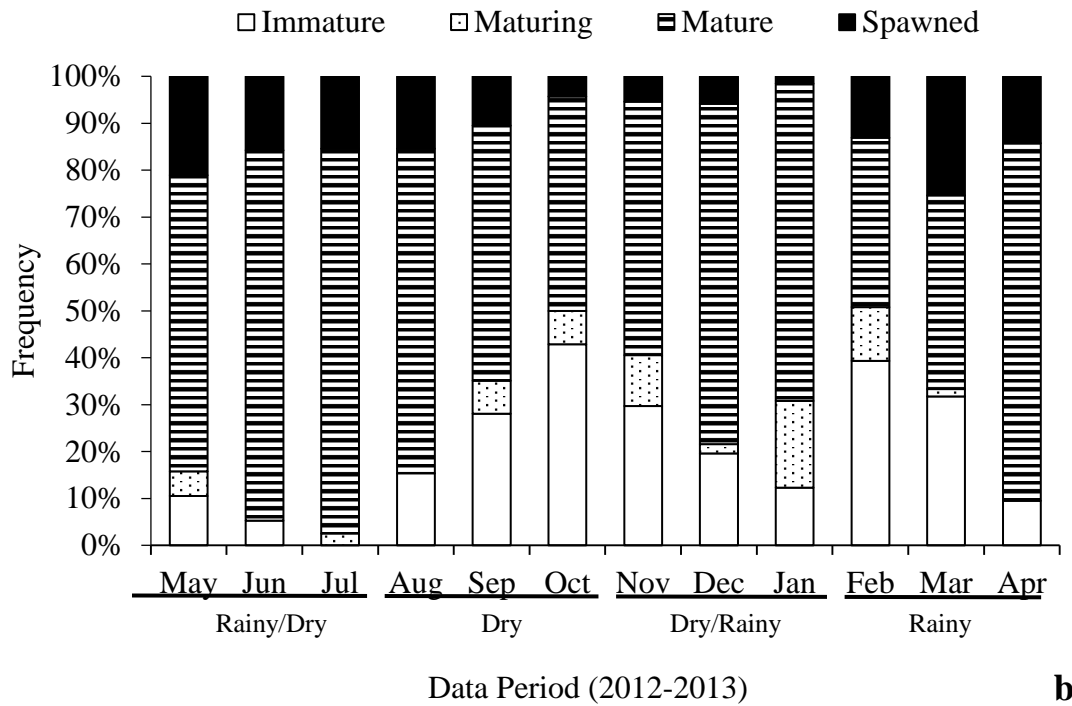
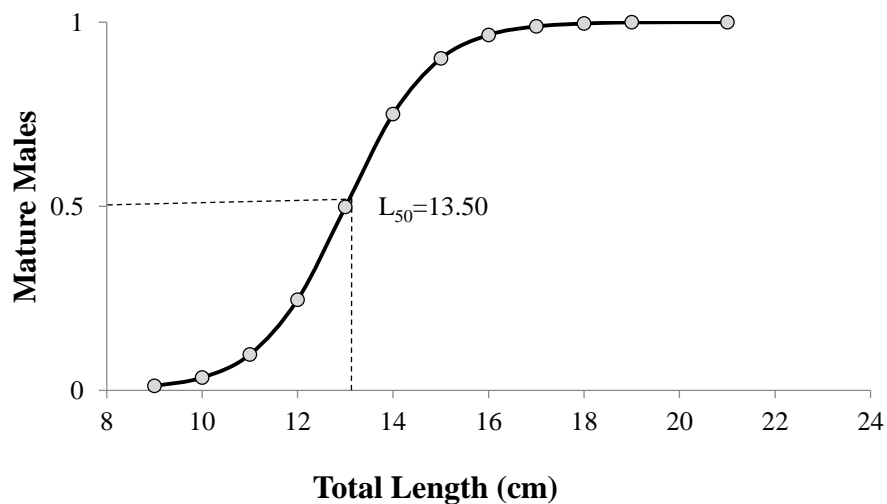


Figure 12 – Monthly variation in the frequency of gonadal maturation stages in males (a) and females (b) of *C. abramoides* captured in the Lower Anapu River, Eastern Amazon.

The mean length at first sexual maturity ( $L_{50}$ ) estimated for males ( $L_{50} = 13.50$ ) was lower than the mean female length ( $L_{50} = 13.79$ ) (Figure 13).



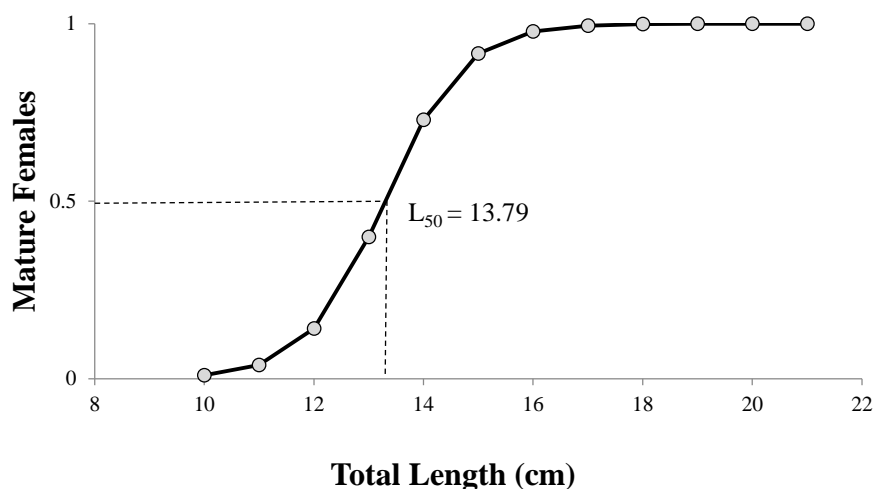


Figure 13 – Estimated length at first sexual maturity ( $L_{50}$ ) for males and females of *C. abramoides* by body size (total length) in the Lower Anapu River, Eastern Amazon.

## Discussion

Regarding the reproductive aspects of the curimatidae fish *Cyphocharax abramoides* of the Lower Anapu River, no differences between the proportions of males and females were found and both sexes showed polyphasic and allometric growth. Males reached sexual maturity before females and Condition factor ( $k$ ), Gonadosomatic Index (GSI), and relative frequency of mature individuals indicated a long reproductive period with multiple spawning events throughout the year, and two peaks with greater activity during the rainy/dry season and the rainy season.

For most fish species, sex ratio is 1:1 and varies according to food availability (Nikolsky 1963). In this study, there were no differences in the sex ratio of *C. abramoides* over the seasons, and although this is a very common result in the Characiform order (Thomé et al. 2005; Vitule et al. 2008; Nunes et al. 2015), a different result was observed for *Cyphocharax gilbert* in the Lower Paraíba do Sul River, with a higher percentage of

both sexes, depending on the area and length size, but with a proportional sex ratio during the periods of high reproductive activity (Menezes & Caramaschi 2007).

Differences in sex proportion are observed throughout the life cycle of fish and can be attributed to factors such as growth rate, mortality, behavior, or even the selectivity of the capture method, and can help in the characterization of population structure, as well as in the basing assessment on reproductive potential and stock size estimate studies (Vazzoler, 1996; Raposo & Gurgel 2001). For instance, when food supply is adequate, females tend to excel over males (Nikolsky 1963) as a strategy to accelerate population growth (Vasconcelos et al. 2011).

The growth type of a fish population can be used in ecological studies, since the length-weight relationship (LWR) might be used to predict weight-for-age in order to observe seasonal variations in fish growth, and is used in stock evaluation models as well as in comparisons of morphological and life history traits between different species, groups, or regions (Gonçalves et al. 1997; Richter et al. 2000; Jellyman et al. 2013). In *C. abramoides*, LWR indicated a polyphasic growth divided in two phases, with positive allometric growth for males and negative allometric growth for females in both phases. Most of the growth studies carried out on the *Cyphocharax* genus focus on detailing growth parameters (Hartz & Barbieri 1993; Benedito-Cecilio et al. 1997; Blanco et al. 2005) or are part of LWR studies (Teixeira-de Mello et al. 2009; Giarrizzo et al. 2011; Teixeira-de Mello et al. 2011; Antonetti et al. 2014), which do not consider growth as divided in two phases. This approach is important because growth in two phases can be an indication that the energy required for reproduction reflects growth pattern, and therefore, helps to understand and estimate the size at first maturity (Fontoura et al. 2010).

Additionally, LWR has an important application in fishery biology, allowing us to observe the welfare of fish, as temporal variations of the condition factor have been

widely used in the interpretation of changes in biological parameters, such as nutrition, fat accumulation, and gonadal development in fish (Le Cren et al. 1951; Froese 2006; Camara et al. 2011). In this study, the condition factor (k) indicated that both males and females differ seasonally, with lower values during the rainy/dry season for both sexes. On the other hand, the variation in gonadosomatic index (GSI) values and the variation in maturation stage frequencies showed greater activity during the Rainy, Rainy/Dry, and Dry seasons for males and females.

This pattern can be explained by the fact that values of k may be related to periods of increased gonadal activity, when many species reduce food intake and use much of their energy reserves (Lizama & Ambrósio 2002). This is indicated by a decrease in values of k during maturation and spawning periods, and represents a useful additional parameter for the study of seasonal patterns in the reproductive process of fish (Lima-junior et al. 2002; Braun & Fontoura 2004; Gomiero et al. 2007).

Most Curimatidae have a long reproductive period (Romagosa et al. 1984; Carvalho 1984; Hartz & Barbieri, 1994; Barbieri 1995; Schifino et al. 1998; Holzbach et al. 2005; Ribeiro et al. 2007) and multiple spawning events with peaks related to high temperatures and rainy seasons (Barbieri 1995; Ribeiro et al. 2007). In our study, there were mature *C. abramoides* in all sampling periods, but GSI variation and the frequency of occurrence of gonadal maturation stages evidenced two higher peaks of reproductive activity during the rainy/dry and rainy seasons, which is in consonance with most findings for the Curimatidae family. Other species of the *Cyphocharax* genus showed a pattern similar to *C. abramoides*, namely, *C. modestus*, with an annual reproductive cycle, greater intensity during the summer (December-January), and fractional spawning (Barbieri 1995), and *C. voga*, with the same behavior in Emboaba Lagoon, in southeastern Brazil (Hartz & Barbieri 1994).

Species with a long spawning season and multiple spawning events have an advantage over fish with total spawning, as they allow for the minimization of interspecific competition between spawning adult females and among larvae, resulting in greater ability to adapt to the aquatic environment (Nikolsky 1963; Vazzoler 1996). However, other studies with Curimatidae species indicated that they can be total spawners (Alvarenga et al. 2006; Carmassi et al. 2008; Peressin et al. 2012); nonetheless, all authors agree that spawning peaks occur during the rainiest seasons.

The size of sexual maturity in fish populations has been an important parameter to establish control and preservation measures of fish stocks (Cruz et al. 2000; Barbieri et al. 2004). In this study, males reached sexual maturity with a smaller body size than females, which has been reported for other species of the genus, such as *C. nagelli*, with 10.2 cm for males and 10.6 for females; *C. modestus*, with 8.1 cm for males and 8.3 cm for females in Lobo dam (southeastern Brazil) (Barbieri 1995) and 10.8 for males and 11.2 for females in Morgado River (southeastern Brazil (Nomura & Hayashi 1980)); *C. voga*, with 12.1 cm for males and 12.8 cm for females in Custódias Lagoon (Schifino et al. 1998) and other neotropical fishes (Barbieri et al. 2004; Nunes et al. 2004; Gomiero et al. 2007; Fontoura et al. 2010; Freitas et al. 2011).

It usually takes longer for females to enter the breeding season than it does for males, and this fact is associated to an evolutionary reproduction advantage that may be related to the need for greater energy investment in their reproductive activities (Bromley 2003). This is because a larger body size represents a greater coelomic cavity, an increase in fertility rate, and higher accumulation of fat for gonadal development (Parker 1991; Bisazza & Pilastro 1997; Vazzoler 1996; Gomiero & Braga 2006)

In general, the results of this study highlighted the relationship between rainfall periods and the reproductive aspects of *C. abramoides* in the Lower Anapu River, leading

to an increase in GSI values and a decreased condition factor during reproductive peaks. This shows the importance of local precipitation in Amazon lake-like environments and provides important data for the understanding of the reproductive biology of one of the most abundant families in the Neotropical region.

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## **CAPÍTULO II**

### **COMPARING THREE METHODS TO ESTIMATE THE AVERAGE SIZE AT FIRST MATURITY ( $L_{50}$ ): A STUDY CASE ON CURIMATIDAE EXHIBITING POLYPHASIC GROWTH**

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**Comparing three methods to estimate the average size at first maturity ( $L_{50}$ ): A case study on Curimatidae exhibiting polyphasic growth**

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**Abstract:** The size at first maturity ( $L_{50}$ ) is widely used in both ecological and management studies, and because of its importance on the maintenance of fish stocks, mainly of commercial interest, several techniques have been developed to enhance the estimates. Therefore, seeking to balance the quantity and the quality of information available and the easiness to obtain new data, we chose three different methods to estimate the  $L_{50}$  aiming to identify which one is the best with the amount of data available. Considering a classical approach, a logistic model using the length according to a proportion of mature fishes was used (1) by determining the gonadal stage

macroscopically and (2) by using the GSI as proxy of sexual maturity; and finally (3) by using the Length-Weight Relationship (LWR) in a theoretical approach proposed by Fontoura et al. (2010). The proposed methods were applied using the data of a detritivorous fish (*C. abramoides*) monthly sampled using gill nets (knot-to-knot meshes of 15, 20, 25, 30, 35 and 40 mm). Captured individuals were measured to obtain total length (TL) to the nearest 0.1cm and weighed to obtain the total weight (TW) to the nearest 0.1g. The individuals were sexed and the gonadal development was classified macroscopically according to Vazzoler (1996). A total of 872 specimens were captured, with 459 females and 413 males with total length ranging from 9 to 22.5 cm. The estimated  $L_{50}$  using the macroscopic identification (female=13.70; male=13.50), the GSI approach (female=15.04; male=14.74) and the LWR (female = 13.85; male=13.06) were tested and only the GSI values were significantly different for both males ( $t = -14.03$ ;  $p < 0.001$ ) and females ( $t = -9.111$ ;  $p < 0.001$ ). Between the three different techniques performed, and considering the different types of data available and the accuracy of the method, we concluded that the analysis of the LWR in fishes with polyphasic growth is more effective since it only needs length and weight data to be performed and estimate a  $L_{50}$  within the range of the classical logistic model using macroscopic identification and the GSI approach.

**Key-words:** Size at first maturity; Gonadosomatic Index; Length-Weight Relationship; Polyphasic growth

## Introduction

Studies on the reproductive biology of fish species provide important information for both basic fish biology and applied fisheries management (Barbieri, 1995; Marques et al., 2007), and despite the growing need to establish management measures, many of the population and reproductive parameters that would provide useful information for adequate management decisions are still scarce (Favaro et al., 2003; Rosa and Lima, 2008). This occurs mainly because: (1) most available data for many fish species comes from fishery-dependent sources, which usually presents a better description of adult fishes, (2) a general lack of standardized sampling for fish sampling gears and effort, and (3) no monthly samples, which precludes quantifying the reproductive period for many fish species and can bias reproductive parameter analyses (Gavaris, 1980; Safran, 1992; Ticheler et al., 1998; Booth, 2000).

The average size at first maturity ( $L_{50}$ ) describes the individual body size at which 50% of a population is mature, and is one of the most important leading parameters for fisheries management. Size-at-maturity helps calculate the maturity and reproductive schedule of a population as a tradeoff to plastic (i.e., variable) lifetime growth patterns (Lorenzen 2016) and allows for estimates of the maximum length of a species, minimum catch size, and therefore appropriate size-restrictions for fisheries-dependent gears (e.g., mesh size of fishing nets and the appropriate hooks size for fishing lines) (Shephard and Jackson, 2005; Wilberg et al., 2005; Binohlan and Froese, 2009; Kinas and Andrade, 2010; Schill et al., 2010; Stark, 2012) and thus helps ensure the stocks survival. Classical methods for estimating  $L_{50}$  of fishes range from the *probit* method to multivariate and logistic models that use the length or age according to a proportion of mature fishes identified macroscopically or using Gonadosomatic index (GSI) as proxy of sexual

maturity (Trippel and Harvey, 1991; Richards et al., 1990; Chen and Paloheimo, 1994; Fontoura et al., 2009). Both these approaches (macroscopic and GSI) are classically accepted in fisheries management as providing accurate and unbiased estimates of  $L_{50}$  (ICES, 2008); however both of these methods are often lethal for individuals sampled for maturity, or have drastic sublethal effects on individuals (Crim and Glebe, 1990) and cost time in terms of intensity of sampling and technical laboratory work.

A promising nonlethal way to estimate  $L_{50}$  auxiliary to either the macroscopic or GSI sampling approach is to use the length-weight relationship (LWR;  $W = a.L^b$ ) described by Huxley (1924) which considers that the growth rate of an individual is equal throughout its life and that body dimensions (weight and length) increase relative to each other according to an allometric coefficient constant (b). However, fish often go through two or more stages of growth, as at the beginning of its development when abrupt physiological changes occurs (Vergara et al., 2013) or by posterior morphological modifications (Safran, 1992). A fundamental change in somatic growth patterns of most fish species is the change in energy allocation when individuals of a population reach sexual maturity, since there is an investment in gonadal development (Shuter et al., 2005; Quince et al., 2008a, 2008b). Hence, a changepoint in allometry over the lifetime LWR of individuals of a population can correspond to the size at which individuals mature.

Bervian et al. (2006) reviewed the original Huxley (1924) equation using three different approaches to develop a statistical model for accurate estimates of relative growth. As a result, the best fit was obtained by considering a polyphasic model where a growth pattern can be divided into at least two different phases, suggesting that a change in the growth parameters was associated with sexual maturity. Considering this polyphasic growth model, Fontoura et al. (2010) tested the hypothesis that from the LWR analysis of a fish with polyphasic growth it is possible to infer the size at maturity, based

on the premise that the change in energy invested into reproduction is reflected in a change in growth parameters.

Seeking to balance the quantity and the quality of information available and the easiness to obtain new data to estimate the size at first maturity, the objectives of this study were to choose three different methods to compare and contrast estimates of  $L_{50}$  aiming to identify which one is the best with the amount of data available. We first considered the classical approaches; a logistic model using the standard length according to a proportion of mature fishes that were matured in two different ways, (1) by determining the gonadal stage macroscopically and (2) by using the GSI as proxy of sexual maturity. In addition, considering that changes in allometric growth patterns can reflect the reproduction influence on the amount of energy directed for body growth, (3) we aim to test the hypothesis proposed by Fontoura et al. (2010) in order to ascertain whether weight and length data provide adequate information to estimate the average size of first maturity of fish with polyphasic growth. The proposed methods were applied using data of *Cyphocharax abramoides* (Kner, 1858), a detritivorous Amazonian Curimatidae chosen for its abundance, monthly reproduction, and well identifiable gender and maturation status.

## **Methods**

### *Study area*

Individuals of *Cyphocharax abramoides* were collected in the National Forest of Caxiuanã (FLONA of Caxiuanã), located between the municipalities of Melgaço and Portel in Pará State (Figure 14). The FLONA has its basin defined by the Anapu River with discrete flood pulse, with annual water level variations around 1.2 m (Costa et al.

1997) and 30cm daily. According to Montag and Barthem (2006) the FLONA is perhaps one of the only regions in the Amazon with characteristics of a true lake and could provide a comparison with natural reservoirs of the Amazon Basin.

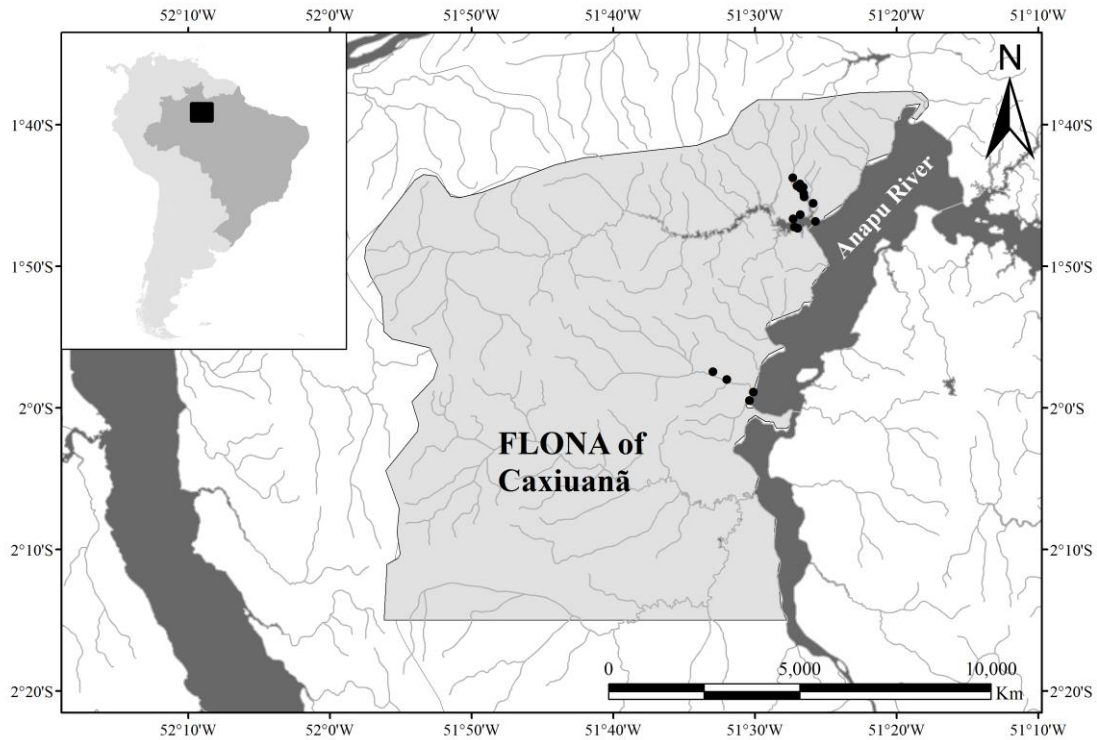


Figure 14 - Study area and data location inside the FLONA of Caxiuanã, in Pará State, Brazil (South America).

### *Specimens data*

Monthly samples of *C. abramoides* were taken from May 2012 to April 2013 in rivers and streams of the FLONA of Caxiuanã using gill nets (knot-to-knot meshes of 15, 20, 25, 30, 35 and 40 mm and total length of approximately 200 meters) submersed between 16 and 22 o' clock. Captured individuals were measured to obtain total length (TL) to the nearest 0.1cm and weighted to obtain the total weight (TW) to the nearest 0.1g. One ventral-longitudinal incision was made to observe and remove the gonads. The



individuals were sexed and the gonadal development was classified macroscopically according to Vazzoler, (1996) in: - **immature (A)** - the ovaries are threadlike, translucent, very small sizes, and placed along the dorsal wall requiring less than 1/3 of the coelomic cavity, without vascularization signals and no observed oocytes to the naked eye. The testicles are small, threadlike and translucent, with position similar to the ovaries; **at maturity (B)** - the ovaries occupy about 1/3 to 2/3 of the coelomic cavity showing intense vascular network; the naked eye can observe small and medium opaque granules (oocytes). The testicles take up little more than 1/3 of the abdominal cavity, present a lobed shape and its membrane ruptures under a certain pressure, eliminating milky, viscous sperm; **mature (C)** - the ovaries occupy almost the entire coelomic cavity, and present big, opaque and / or translucent oocytes visible to naked eye, whose frequency varies with the progress of maturation; the testicles are presented whitish, occupying much of the coelomic cavity; with low pressure its membrane breaks up, flowing less viscous sperm than in the previous stage; **spawned (D)** - ovaries presented in different degrees of tenderness, with membranes stretched and hemorrhagic aspect, occupying again less than 1/3 of the coelomic cavity. The testicles are flaccid with hemorrhagic aspect and the membrane does not break under pressure.

Voucher specimens were deposited in the ichthyology collection of the Museu Paraense Emílio Goeldi - MPEG (Belém, Pará, Brazil) under MPEG 30477 and MPEG 30478 codes.

#### *Statistical analysis*

The  $L_{50}$  was determined using two different methods, both based on the number of mature individuals per length class, according to the formula

$$P = \frac{P_{max}}{1 + e^{-\ln(19)\frac{TL-L_{50}}{\delta}}} \quad (\text{eq. 1})$$

where P is the proportion of mature individuals,  $P_{max}$  is the asymptote (presumed 1.0 in a maturity model), TL is the total length (cm),  $L_{50}$  is the average length (cm) when 50% of the population is sexually mature, and  $\delta$  is the average increment (cm) after  $L_{50}$  to reach 95% proportion mature (as dictated by the log of the odds ratio  $\ln(19)$ ). In the macroscopic approach, only males and females in stages B, C, or D were considered mature and all other individuals were considered immature. In the second method, the reproductive maturity was established using the average values of the Gonadosomatic Index (GSI) for males and females (Santos 1978) according to the formula  $GSI = (Gonad\ Weight/Total\ Weight)*100$ , with 5% cut proposed by Marques et al. (2007) and Fontoura et al. (2009). The 5% cut considers as mature an individual with a minimum of 5% of the maximum gonad weight. The proportion of mature fish per centimeter group (rounded to the nearest half centimeter; ranging from 8–22.5 cm) were used as the data in eq. 1 and we estimated the parameters  $L_{50}$  and  $\delta$  by minimizing the negative log likelihood using a binomial probability density function, where the number of trials are the number of fishes in each centimeter group, and the number of successes are the number of mature fishes in each centimeter group. Model fitting was done using the function *mle* in R version 3.2.3 (R Core Team 2015) with starting values of 15 and 10 for  $L_{50}$  and  $\delta$ , respectively. Asymptotically normal standard errors for the  $L_{50}$  parameter were derived by taking the square roots of the diagonal elements of the inverse of the Hessian matrix from the maximum likelihood solution, and 95% confidence intervals for  $L_{50}$  were approximated by taking the maximum likelihood estimate for  $L_{50}$  +/- twice the standard error of the parameter.

The potential for polyphasic allometric growth in *C. abramoides* was evaluated from the length-weight relationship (LWR) in Huxley (1924). We used the formula:

$$TW = aTL^b \quad (\text{eq. 2})$$

where TW is the total weight (g),  $a$  is the coefficient of proportionality, TL is the total length (cm), and  $b$  is the allometric shape coefficient. After the construction of the model, the proportional residuals ((Observed weight – expected weight) / Observed weight) were plotted as a function of TL to identify possible trends in the data looking for a potential shift in allometric growth associated with maturity. The proportional residues were used in place of regular residuals (Observed weight – expected weight) in order to minimize heteroscedasticity present in the data. Preliminary estimates of the parameters  $a$  (coefficients of proportionality) and  $b$  (allometric shape parameter) from equation 2 were estimated by minimizing the sums of squares in the proportional residuals using the *Solver* routine (Microsoft Excel®) fitted to observed length and weight data. Analyses of the residual graphs from the fitted eq. 2 showed that the proportional residuals were not randomly distributed (around 0 on the x axis) along the y-axis. The break in the residual pattern indicated a point of change in the growth pattern (Fontoura et al. 2010), meaning that the LWR was not suitable for a regular potential equation, suggesting polyphasic growth. To confirm the existence of residuals trends along the subjects' lengths we used a linear regression. The growth in different phases represents a change point in allometric growth for *C. abramoides* individuals associated with the investment into reproduction. According to Bervian et al. (2006), such phases can be described by different power equations that can be switched on and off at a change point defined as a stanza changing length.

Unlike Bervian et al. (2006), which defined the stanza changing length (SCL) with a logistic equation with two shape parameters that created the switch function between the juvenile and adult phases, we estimated the SCL parameter directly as a change point

between two different length–weight relationships associated with the juvenile phase and mature phase. Hence, the following:

$$TW = \begin{cases} a_1 TL^{b_1}; & TL < SCL \\ a_2 TL^{b_2}; & TL \geq SCL \end{cases} \quad (\text{eq. 3})$$

where  $a_1$  and  $b_1$  represent the coefficients of proportionality and allometric scalar for the juvenile phase, respectively,  $a_2$  and  $b_2$  represent the coefficients of proportionality and allometric scalar for the adult phase, respectively, and  $SCL$  (Stanza Changing Length) is the size that represents the average length (in cm) where the second growth phase occurs. Suitable starting values for  $SCL$  were determined by taking the logarithm of eq. 3 and fitting a continuous piecewise linear regression using the *segmented* package in R (Muggeo 2008). Using the initial starting values provided by the piecewise-linear model, we then fitted length and weight data to eq. 3 and estimated the parameters  $a_1$ ,  $b_1$ ,  $a_2$ ,  $b_2$ , and  $SCL$  (all five parameters were constrained positive) by minimizing the sums of squares of the proportional residuals using the function *mle* in R version 3.2.3. Similar to above, asymptotically normal standard errors for the  $SCL$  parameter were derived by taking the square roots of the diagonal elements of the inverse of the Hessian matrix from the minimized sums of squares solution, and 95% confidence intervals for  $SCL$  were approximated by taking the minimized sum of square residuals estimate for  $SCL$  +/- twice the standard error of the parameter. We then verify whether the stanza changing length is effective in estimating the first mature size by contrasting the mean and 95% CI for the  $L_{50}$  (macroscopic and GSI based) to the mean and 95% CI for the  $SCL$  from the LWR approach.

## Results

A total of 872 *C. abramoides* specimens were captured, with 459 females and 413 males identified macroscopically with total length ranging from 9 to 22.5 cm. Females presented a mean length of 17.4 cm ( $\pm$ SD 3.8) and average weight of 84 g ( $\pm$ SD 30.5), while males showed mean length of 16.4 cm ( $\pm$  SD 1.9) and weight of 63g ( $\pm$  SD 21.3). Both females and males presented a high number of specimens on reproduction period (females: 80.44%; males: 44.7. The species presented monthly spawning and mature and immature individuals were capture every month (Figure 15).

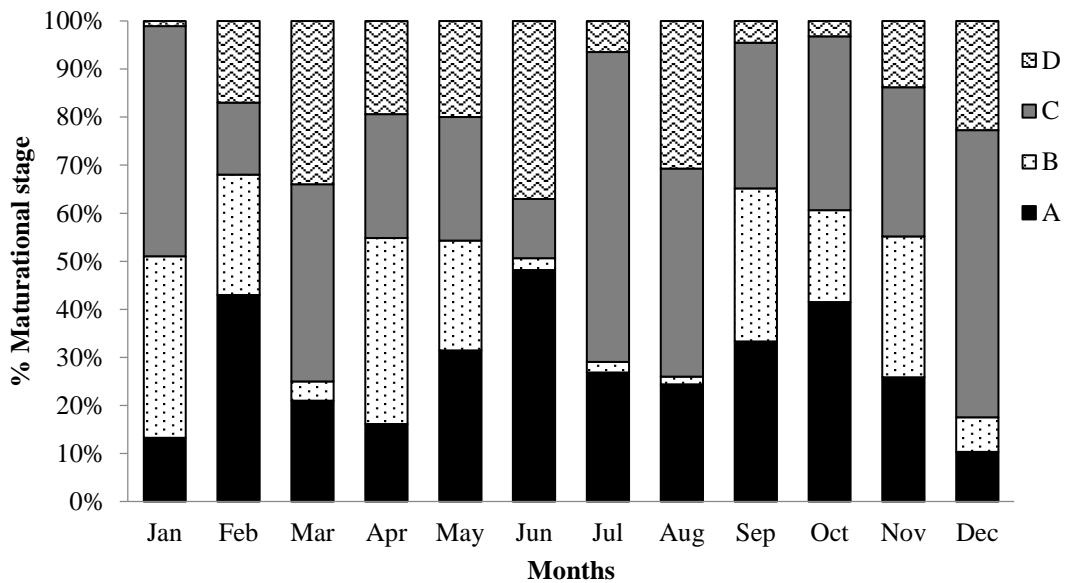


Figure 15 - Maturational stages for both male and female of *Cyphocharax abramoides* collected in FLONA of Caxiuanã, Pará - Brazil

### *Logistic Model: Macroscopic gonadal identification and GSI approach*

In both analyses, females presented a slightly higher  $L_{50}$  than males, but the estimates using the GSI were higher for both genders considering the two approaches (Table 2). All males larger than 18 cm (Macroscopic) and 19.5 cm (GSI approach), and

all females larger than 16 cm (Macroscopic) and 18.5 cm (GSI approach) were classified as adults (Figure 16).

Table 2 - Estimated size at first maturity ( $L_{50}$ ) for male and female *C. abramoides* using two different approaches in a Logistic Model.

Species	Sex	N	Method	L50
<i>Cyphocharax abramoides</i>	Male	425	GSI	14.27 (13.98 – 14.57)
	Female	460	GSI	14.47 (14.15 – 14.8)
	Male	363	Macroscopic	13.48 (12.95 – 14.01)
	Female	409	Macroscopic	14.01 (13.58 – 14.43)

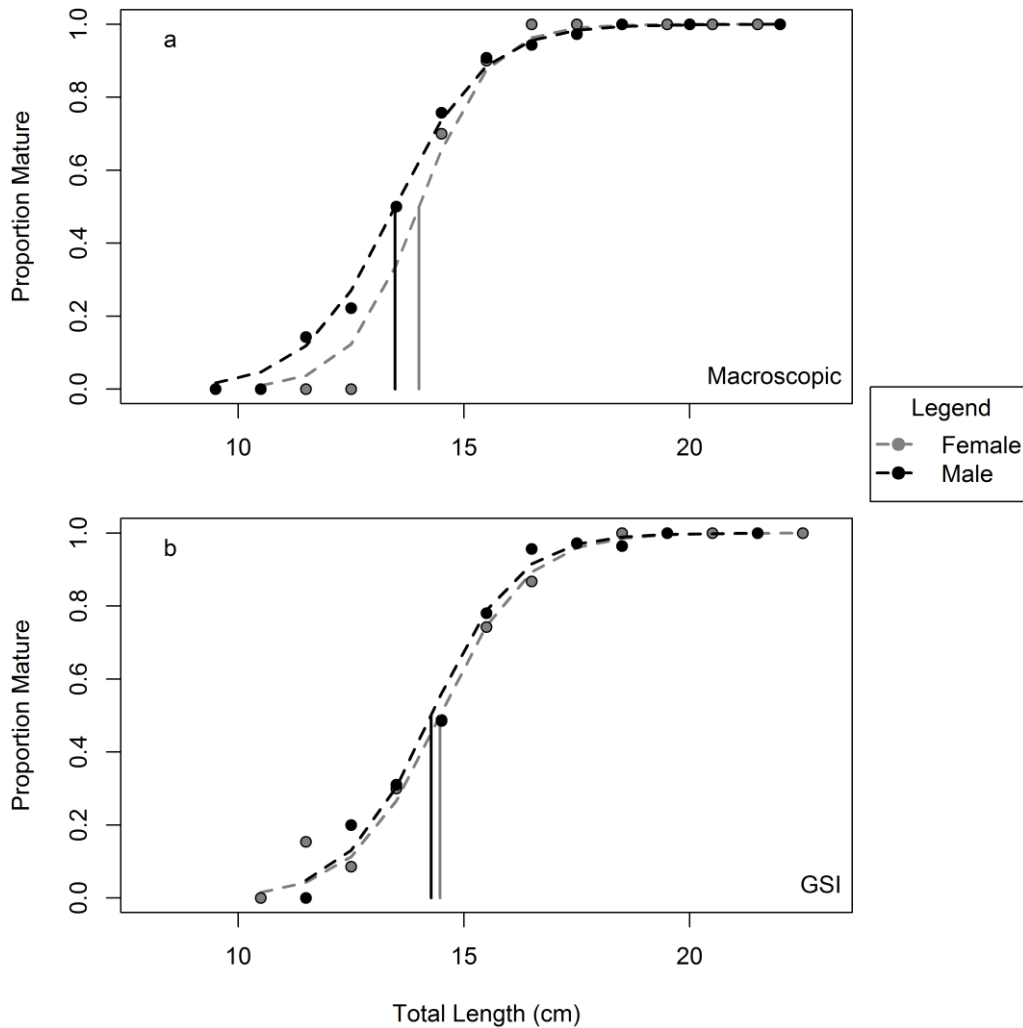
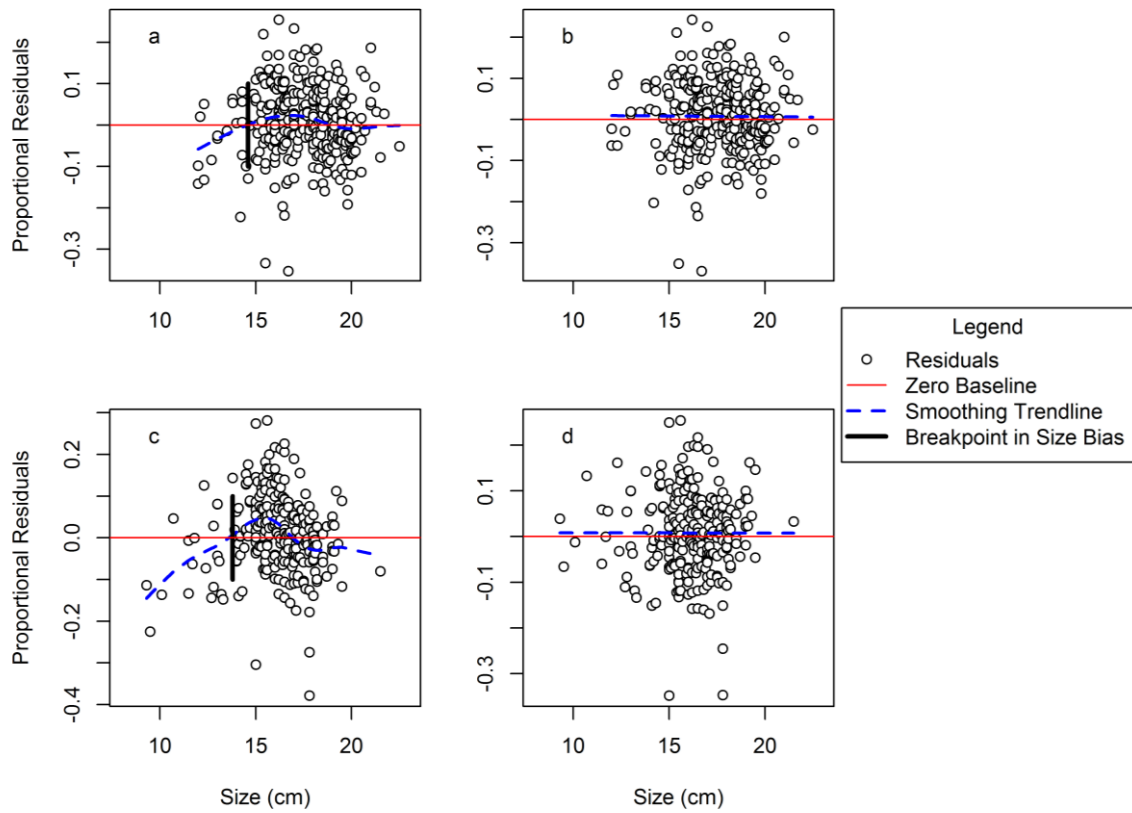


Figure 16 - Proportion of sexually mature male and female *Cyphocharax abramoides* by body size (total length) using a macroscopic identification and GSI approach.

#### *Length-Weight Relationship (LWR)*

According to the analysis of proportional residues of the Huxley LWR, the monophasic allometric growth model was deemed inadequate for both females ( $r^2 = 0.006$ ;  $p = 0.043$ ) and males ( $r^2 = 0.007$ ;  $p = 0.046$ ) and a trend in biased residuals across total length (Figure 17). This bias concentrated with negative residuals (and consequently overestimation of the weight by the monophasic equation) until about 14

cm where an apparent shift in allometry occurred suggesting potential for polyphasic growth among *C. abramoides* individuals (Figure 17



).



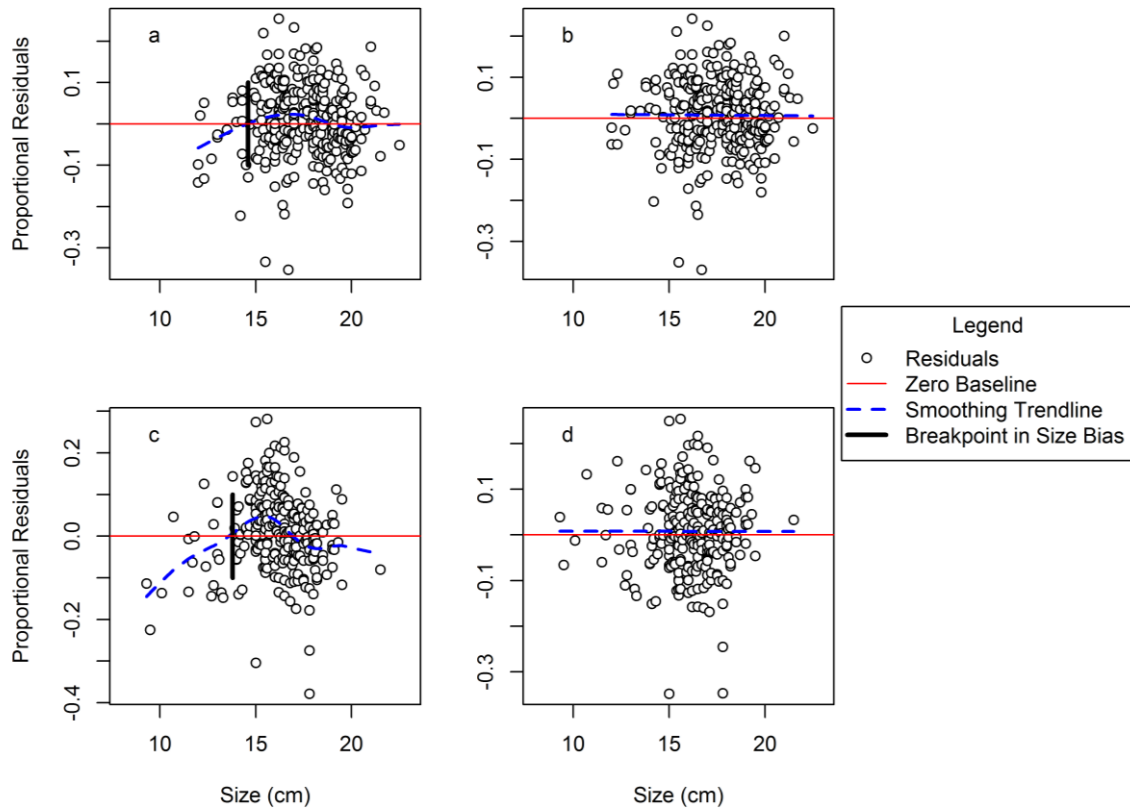


Figure 17 - Trends in the proportional residuals distribution across size for male (lower panels) and female (upper panels) individuals presuming monophasic (left panels) and polyphasic (right panels) growth in *Cyphocharax abramoides* collected in FLONA of Caxiuanã, Pará - Brazil.

The polyphasic growth model helped account for the bias across sizes and improved LWR estimation while detecting the apparent shift in allometry occurring around 14cm (Figure 17). The estimated parameters for the polyphasic growth females showed a change in growth rate at 15.82 cm with positive allometric growth in the juvenile phase and negative allometric growth in the adult phase. For male *C. abramoides*, a change in the allometry was observed at 15.48 cm with positive allometric growth in the juvenile phase and negative allometric growth in the adult phase (Table 3).

Table 3 - Estimated stanza changing length (SCL) and length-weight parameters for the polyphasic growth pattern in male and female *Cyphocharax abramoides* collected in FLONA of Caxiuanã, Pará – Brazil.

<b>Sex</b>	<b>Phase</b>	<b>Coefficient of proportionality (a)</b>	<b>Coefficient of allometry (b)</b>	<b>Stanza Changing SCL (cm)</b>	<b>SCL Confidence Interval</b>
<b>Female</b>	Phase 1	0.007	3.30	15.82	13.34 – 18.3
	Phase 2	0.025	2.85		
<b>Male</b>	Phase 1	0.005	3.43	15.48	13.41 – 17.55
	Phase 2	0.046	2.60		

The less-lethal, noninvasive, less expensive, and relatively easier LWR method provided an accurate indicator of the size at first maturity that was not significantly different from the two other techniques. Although the LWR method tended to be ~ 1.0 cm larger than the GSI approach and was less precise (the 95% CI were wider in the LWR method than in macroscopic or GSI), the 95% confidence intervals of the LWR method covered the  $L_{50}$  estimates from both the macroscopic identification and GSI approaches for both genders (Table 4).

1 Table 4 - Comparison of three different methods for estimating the size at first maturity (total length in cm) for male and female *C. abramoides*  
 2 with associated sample size (N) and maximum likelihood estimates (with asymptotically normal 95% confidence intervals in parantheses) of the  
 3  $L_{50}$  from logistic regression or stanza changing length (SCL) and the relative pros and cons of each method

Method	Sex	N	L50 or SCL (cm)	Pros	Cons
GSI	Male	425	14.27 (13.98 – 14.57)	Good precision/accuracy Not biased by individual perception	High financial costs Medium sampling and processing time High fish mortality
	Female	460	14.47 (14.15 – 14.8)		
Macroscopic	Male	363	13.48 (12.95 – 14.01)	Good precision/accuracy	High financial costs Needs qualified personnel Biased by individual perception High sampling and processing time High fish mortality
	Female	409	14.01 (13.58 – 14.43)		
LWR	Male	261	15.48 (13.41 – 17.55)	Low financial costs Not biased by individual perception Low fish mortality Low processing time	Can be a biased or imprecise indicator of maturity
	Female	292	15.82 (13.34 – 18.3)		

## Discussion

The choice of the best technique to determine the size at first maturity ( $L_{50}$ ) in fishes will depend on the project needs, available data sources, and available resources. However, what data give results that are more accurate in this estimate? This will depend mainly on three factors: (1) the accuracy in measures of length and weight of fish and gonads is the most important thing to consider, as length classes are defined from length data, and  $L_{50}$  can be estimated through LWR and GSI; (2) the ability to identify the gonads macroscopically, because there is no qualified personnel to process the samples and students with different skills levels do most of the work (Fontoura et al., 2009); and (3) the type of growth of the species, since the  $L_{50}$  of fishes with two growth phases can be reflected in the Stanza Changing Length (SCL) (Fontoura et al., 2010).

In this study, considering the above-mentioned items, we estimate the  $L_{50}$  of the detritivorous species *Cyphocharax abramoides* using three different methods – a macroscopic maturational stage classification, the Gonadosomatic Index (GSI) approach and the Length Weight relationship for the Stanza Changing Length (SCL) – hoping to identify the best technique and data source given plausible limitations in resources and sampling time. As a result, we found a good precision/accuracy using the macroscopic identification method and the GSI approach; however, both analysis presented several cons that highlighted the LWR as a good predictor that presented cons that may surpass its less precise estimation.

Considering the large amount of sampled and identified individuals per class length in this study, we considered that a macroscopic gonadal classification is a good estimate of reality, and the  $L_{50}$  value estimated for males and females accurate. Logistic models use the length or age according to a proportion of mature fish, and the more

precise identification of these stages by length class, the more accurate the estimate. Therefore, it is expected that maturational stages identified microscopically to be more effective, but microscopic analysis takes time, expenses, and several laboratory supplies, and is not always a viable option. However, the macroscopic definition of the maturational stages can be performed in the field or laboratory and, with the exception of the difficulty of identifying immature individuals of some species and the lack of qualified personnel to process the samples, tends to be effective in its purpose.

The GSI analysis considers a proportion of the gonadal and total body weight and is generally used to determine the breeding season of a species (Oliveira et al., 2011). However, it can also be used as proxy of sexual maturity and therefore another way to determine maturational stages by length class (Fontoura et al., 2009). In this study, the GSI approach presented a good estimate with the lowest 95% confidence intervals indicating the efficiency of the model. One must take into consideration that this analysis is more indicated to monthly spawning species, such as *C. abramoides*, since seasonal spawning will introduce non-reproductive specimens into the sampled population leading to an artificial low  $GSI_{max}$ , inducing an important error in the estimate of the  $L_{50}$  (Marques et al., 2007).

Finally, when considering the SCL as a predictor of  $L_{50}$ , as proposed by Fontoura et al. (2010), we found that the obtained  $L_{50}$  were higher and less precise, but with 95% confidence intervals that covered the  $L_{50}$  estimates from both macroscopic identification and GSI approach for both genders. This method also highlights another use for the allometric factor obtained in the LWR relationship. Allometry is a common feature during development, and the changes in the growth rates of body parts, total length and total mass are well known for fish and starts at the beginning of the life cycle, during the prelarval and larval stages (Osse and Boogaart, 1995; Gisbert, 1999) and continues until

adulthood with allometric changes that can reflect energy intake and energy directed for expenditure on metabolism, such as reproduction (Quince et al. 2008a; Boukal et al., 2014).

In this study, the positive allometry in the first phase followed by a negative allometry in the second phase for both genders, with higher values for females in the second stanza, help establish the different growth rates before and after the estimated SCL. Similar results were also found in the Fontoura et al. (2010) studies for both analyzed characid species. Despite the few studies about allometric changes in fish after the larval stage to compare, these results may indicate that the species needs to become larger faster prior to maturity, and that females gain more weight than males after maturity due to higher energy demands for egg development. Also, fish that occupy a low position in the food chain do not need to grow as fast after reaching majority as large predators, that may require to be sufficiently larger than their prey (Pawar et al., 2012).

The results confirm the potential for LWR to estimate size at first maturity in fishes exhibiting polyphasic growth, and highlights the potential of the LWR for the study of fish stocks and provides a potential route for a more practical  $L_{50}$  assessment, since it only uses length and weight information of the individuals. Still, more studies are necessary to evaluate the effectiveness of the tool in biologically relate it to sexual maturity. Between the three different techniques performed in this study, and considering the different types of data available to most fisheries and the accuracy of the method, we concluded that the LWR in fishes with polyphasic growth and the stanza changing length is an effective, affordable, and lesslethal manner to estimate the size at maturity presenting a viable alternative to classical logistic models using more invasive and lethal sampling of maturity identification.

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## **CAPÍTULO III**

### **ESTIMATING THE AVERAGE SIZE AT FIRST MATURITY ( $L_{50}$ ) IN FISH BY BAYESIAN METHOD**

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## Estimating the average size at first maturity ( $L_{50}$ ) in fish by bayesian method

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**Abstract:** A Bayesian model was constructed to estimate the length size at first maturity ( $L_{50}$ ) of fishes using three species of different orders. The script is based on a proportion of mature individuals per length class using simple data as (i) length classes defined according to each fish species, (ii) total number of specimens for each length class and (iii) mature specimens (gonadal stage B, C and D) for each length class. A Bayesian approach is a good choice for the task because it provides a theoretically justified means with simple data that can be easily replicated to other fish species, family or order. In the study, the  $L_{50}$  for all three species were estimated: *C. abramoides* (Females = 13.8cm; Males = 12.9cm), *A. ucayalensis* (Females = 12.8cm; Males = 10.9cm) and *A. anableps*

(Females = 11.9cm) and replicated a thousand times, confirming the effectiveness of the method.

**Key words:** Sexual maturation, Fishes, Bayesian inference.

## **Introduction**

The average length of first sexual maturity ( $L_{50}$ ) describes the point at which 50% of the population is mature. That estimate is an important biological aspect of fish populations, allows defining the average adulthood size (Castro 1999). The determination of this parameter provides basis for the establishment of management strategies that ensure the sustainable exploitation, since the knowledge of the  $L_{50}$  can help define the minimum catch size and the maximum length of a species, the mesh size of fishing nets and the size of appropriate hooks for fishing lines (Shephard & Jackson 2005; Wilberg et al. 2005; Binohlan & Froese 2009; Kinas & Andrade 2010; Schill et al. 2010; Stark 2012).

Different methods have been proposed to estimate the  $L_{50}$  based on non-reproductive and reproductive individuals (Fontoura et al. 2009), and the classification of gonadal stages (Gerritsen et al. 2003). Although several methods for estimating the  $L_{50}$  can be found in the literature, most are based on logistic equations using classical statistic inferences (Dadebo et al. 2003; Lewis & Fontoura 2005; Fontoura et al. 2009) and require a very large sample size. However, actually different inference approaches are highlighted, mainly due to the advent of computational facility and an improvement of theoretical-practice statistical framework (Speed 1985; Garfield & Burrill 1996; Memória 2004). In classical statistic the inference results in point estimates with 95% confidence intervals that are based on hypothetical repetitions and do not make a direct statement about the true parameter, while the bayesian inference allows a direct probability of the

parameter of interest, using random parameters and fixed data (Doll e Lauer 2013) and permit good prediction accuracy even with small sample sizes (Kontkanen et al. 1997).

These characteristics lead to an increase in the use of the Bayesian inference over the past decades, especially in ecological studies (Reckhow 1990; Meyer & Millar 1999; Borsuk et al. 2004; McCarthy 2007; Rivot et al. 2008; Royle & Dorazio 2008; Kéry 2010) and also in fisheries management (Mäntyniemi et al. 2005; Wilberg et al. 2005; He & Bence 2007; Chen et al. 2013; Doll & Lauer 2013).

Given the difficulty in acquiring a large sample of a certain fish population, this paper aims to determine the effectiveness of the Bayesian method in the size at first sexual maturity estimation using a small sample size. For this,  $L_{50}$  of each species will first be estimated using a large sample size, which will generate not only the average size at first maturity but also an estimated sample size able to generate the same result, indicated by the a stabilization of the estimates.

## **Methods**

The average size at first maturity ( $L_{50}$ ) was determined considering a proportion of mature individuals per length class. For this, all individuals were measured in total length and total weight (0.1cm and 0.1g of accuracy). To establish the maturation stage of species, specimens were open to observe, remove and weight the gonads (0.1g), which were later sexed and the gonadal development was classified macroscopically in four stages in (A) immature, (B) maturing, (C) mature and (D) spawned females or spent males, in accordance with the maturity key of Nunez & Duponchelle, (2008). Only individuals in stages B, C and D where used in this analysis.

Voucher specimens were deposited in the ichthyology collection of the Museu Paraense Emílio Goeldi - MPEG (Belém, Pará, Brazil): *C. abramoides* = MPEG 30477,



MPEG 30478; *A. anableps* = MPEG 16581, MPEG 16582, MPEG 16583, MPEG 16584, MPEG 16585, MPEG 16586, MPEG 16751 e MPEG 16752.

*Key criteria and target species*

To estimate the  $L_{50}$  with accuracy and show the effectiveness of the method, two criteria were taken into account: (1) a proportion of mature individuals more or less evenly distributed along the length classes and (2) to show that the script is able to run the analysis for any fish species, three Actinopterygii species of different orders and reproductive characteristics were chosen, as follows:

*Cyphocharax abramoides* (Kner, 1858) (Characiformes: Curimatidae).

The Curimatidae family is distributed in Central and South America and are highly specialized for consumption debris (Bowen 1983). They present small size, no teeth and live grouped near the bottom of water bodies (Fink & Fink 1978). Most Curimatidae have a long reproductive period (Romagosa et al. 1984; Carvalho 1984; Hartz & Barbieri 1994; Barbieri 1995; Schifino et al. 1998; Holzbach et al. 2005; Ribeiro et al. 2007) and multiple spawning (Barbieri 1995; Ribeiro et al. 2007). The  $L_{50}$  of several Curimatidae species show that females usually take longer than males to reach sexual maturity, in other words, the  $L_{50}$  in males is smaller than in females (Nomura & Hayashi 1980; Schifino et al. 1998; Barbieri 1995).

*Ageneiosus ucayalensis* Castelnau, 1855 (Siluriformes: Auchenipteridae)

The Auchenipteridae family group is known as catfish floating logs, comprising catfish with small to medium sizes endemic of the Neotropical region (Ferraris 2003). The *Ageneiosus* La Cépède, 1803 specimens are exclusive and widely distributed

throughout lowland waters of the Neotropical region in Central and South America (Ferraris 2007) and little is known about this fish feeding and reproductive ecology.

*Anableps anableps* (Linnaeus, 1758) (Cyprinodontiformes:Anablepidae)

The genus *Anableps* includes three species and are among the largest viviparous fishes of the order Cyprinodontiformes (Burns & Flores 1981). The *Anableps* genus reproduce throughout the year (Miller 1979; Burns & Flores 1981; Nascimento & Assunção 2008), with reports of reproductive peaks (Nascimento & Assunção 2008).

*Estimating the average size at first maturity of fish ( $L_{50}$ )*

The analysis is divided in two stages: (1) the  $L_{50}$  estimate and (2) observing the stabilization of the estimation with sample size.

$$(1) \quad \begin{aligned} xp_i &= \text{Binomial}(p_i, n_i) \\ \text{Logit}(p_i) &\sim \alpha + \beta x_i \\ \alpha &\sim \text{Normal}(0, 1.0E^4) \\ \beta &\sim \text{Normal}(0, 1.0E^4) \end{aligned}$$

Where  $xp_i$  is the proportion of mature individuals at class  $i$ ;  $n_i$  is the total number of individuals at  $i$ , and  $x_i$  is the length class at  $i$ . (2) Then, a  $y$  sample size was taken from the sample, wherein the probability of an individual sample was  $ri/n$ , where  $r$  is the number of individuals at the class  $i$ . At every random sampling, the proposed model made  $L_{50}$  estimates and the sample with  $y$  sizes were repeated 1000 times to estimate the credibility intervals for 95%. The size  $y$  was increased in intervals of 10 individuals, ranging from 10 to 500 sampled individuals from the population observed in the sample.

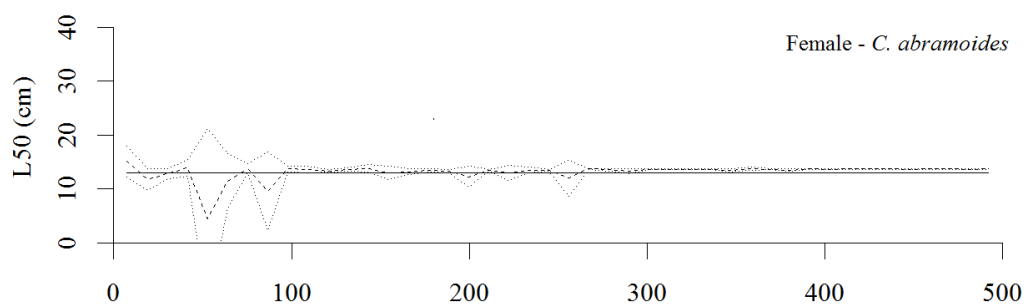
## **Results and Discussion**

The advantages of Bayesian modeling include flexibility in model structure, in the application of different data sources and it provides a theoretically justified means with simple data that can be easily replicated (Juntunen et al. 2012). In this study, we aimed to

determine the effectiveness of the Bayesian method in the size at first sexual maturity estimation using a small sample size. A total of 402 females and 363 males of *Cyphocharax abramoides*, 600 females and 230 males of *Ageneiosus ucayalensis* and 316 females of *Anableps anableps* were used to estimate the  $L_{50}$  (Table 5). Both females and males of *C. abramoides* presented a good fit with few peaks during the repetitions of the estimate, and although other species presented several peaks, an adjustment to the centerline that corresponds to  $L_{50}$  estimated by the model for all species can be observed. After repetitions, a stabilization of the estimates suggests a minimum sample size for obtaining similar  $L_{50}$  results (Figure 18).

Table 5 - Length-Weight Relationship for three fish species obtained from the Bayesian inference.

Specie	Sex	N	$L_{50}$ using total sample size (cm)
<i>Cyphocharax abramoides</i>	Female	402	13.884 ( $\pm 1.066$ )
	Male	363	12.931 ( $\pm 0.239$ )
<i>Ageneiosus ucayalensis</i>	Female	600	12.577 ( $\pm 0.446$ )
	Male	230	10.996 ( $\pm 0.835$ )
<i>Anableps anableps</i>	Female	316	11.912 ( $\pm 1.483$ )



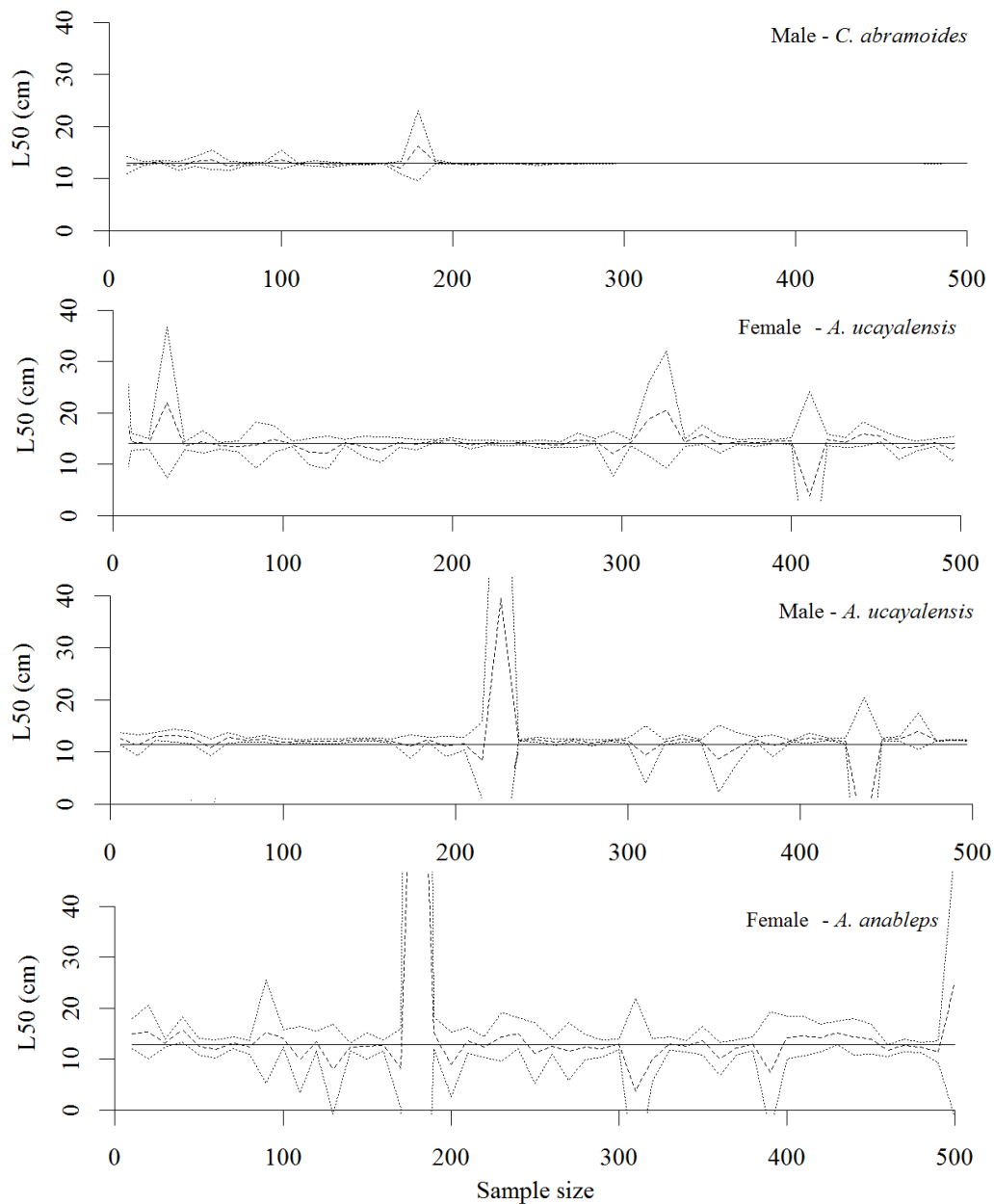


Figure 18 – Average size at first sexual maturity ( $L_{50}$ ) for three fish species (*C. abramoides*, *A. ucayalensis* and *A. anableps*) using a Bayesian approach. The central line corresponds to the  $L_{50}$  estimated by the model, the dashed lines corresponds to the actual, minimum and maximum values of  $L_{50}$  estimated among 1000 repetitions, and presents an adjustment of the intervals with the stabilization of values, suggesting a minimum sample size for obtaining the same  $L_{50}$  result.

The thousand repetitions of  $L_{50}$  estimates for each species showed that for *C. abramoides* and *A. ucayalensis* the adjustment was more effective. However, for *A. anableps*, estimates did not easily adjusted to the model, which may have happened because in a viviparous specie the internal fertilization and energy investment that females need to reallocate to reproductive activity, embryonic development and maintenance of the embryos until the time of spawning is different from oviparous fish (Gunderson 1997).

In this study we observed that bayesian estimates are effective in estimating the  $L_{50}$ , corroborating with a study by Doll & Lauer, (2013) where the traditional methods of statistical inference was compared with a bayesian approach, resulting in a good estimate using a large sample size, but in more precise estimates by Bayesian inference when a small sample size was used. This is an important finding for fish populations and management studies, as fewer data is necessary to estimate the parameter accurately using a single script for any fish order.

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### **Appendix: Bayesian model**

```
x<-c(1,2,3,4,5...) # Length Classes
y<-c(1,2,3,4,5...) # Mature individuals per length class
z<-c(18,78,37,16) # Total number of individuals per length class
```

```
analyse.glm<-function(x,y,z){
fishspecie<-cbind(y,z-y)
xc<-x-mean(x)
fishspecie.glm<-glm(fishspecie~xc,family=binomial(link=logit))
se<-sqrt(sum(fishspecie.glm$residuals^2)/(length(x)-2))
est<-coef(fishspecie.glm)
v.beta<-summary(fishspecie.glm)$cov.unscaled
m<-500
prec<-rgamma(m,(length(x)-2)/2,se^2*(length(x)-2)/2)
sig2<-1/prec
library(MASS)
beta.conj<-numeric()
for(i in 1:m){
beta.conj<-rbind(beta.conj,mvnorm(1,est,sig2[i]*v.beta))
}
l50<- -beta.conj[,1]/beta.conj[,2]+mean(x)
return(mean(l50))
}
```

```
function.final<-function(number,aleat){
classes<-c(1:4) # Number of length classes
results<-numeric()
for(i in 1:aleat){
```

```

sample<-table(sample(classes,number,replace=T,prob=z/sum(z)))
rbinom(4,as.numeric(sample),y/z) # Number of length classes
final<-rbind(rbinom(4,as.numeric(sample),y/z),as.numeric(sample)) # Number of length classes
results [i]<-analyse.glm(x,final[1,],final[2,])}
#final<-
c(as.numeric(quantile(results,0.025)),mean(results),as.numeric(quantile(results,0.975)))
#return(final)
erro.pad<-sd(results)/sqrt(aleat)
mean<-mean(results)
return(c(qt(0.025,aleat)*erro.pad+mean,mean,qt(0.975,aleat)*erro.pad+mean))
}
function.final(110,1000)

results.final<-matrix(0,ncol=4,nrow=50)
for(i in 1:50){
results.final[i,]<-c(i*10,function.final(i*10,1000))}
results.final

par(family="serif")
plot(results.final[,1],results.final[,3],type="l",ylim=c(1,45),xlab="Sample size",
      ylab="L50 (cm)",axes=0,lty=2)
lines(results.final[,1],results.final[,2],lty=3)
lines(results.final[,1],results.final[,4],lty=3)
lines(results.final[,1],rep(16.5,49)) # Add here the L50 for the study specie
axis(1,pos=0)
axis(2,pos=0)
arrows(170,5,170,10,angle=15)

```

## CONSIDERAÇÕES FINAIS

A maioria das espécies de peixes que ocorrem em planícies de inundação possuem seu período reprodutivo delimitado pelos períodos de cheia e seca do rio, que controlam os fatores físico-químicos da água, bem como a disponibilização de alimentos e ambientes para proteção da nova prole. Em planícies inundadas com características lacustres, além do efeito do pulso de inundação, a precipitação local pode ser a principal fonte reguladora do início do período de reprodução e desova das espécies. No Baixo Rio Anapu, que apresenta estas características, a análise dos principais parâmetros reprodutivos do curimatídeo detritívoro *Cyphocharax abramoides* mostrou que a espécie apresenta um período reprodutivo longo, com desova múltipla ao longo do ano e com dois picos de desova relacionados com os períodos pluviométricos, corroborando com a hipótese de que as chuvas influenciam a reprodução da espécie.

Por ser uma espécie detritívora abundante na região, o *C. abramoides* parece ser um bom indicador de como a precipitação local pode influenciar os peixes que compõem as camadas mais baixas da cadeia alimentar, que são mais rapidamente afetados pela entrada de material orgânico carregado pelas chuvas das florestas adjacentes para o meio aquático.

Além dos parâmetros reprodutivos que auxiliam na determinação dos períodos de reprodução e desova das espécies, o tamanho médio de primeira maturidade ( $L_{50}$ ) é amplamente utilizado em estudos de manejo, e desenvolver e testar novas técnicas para estimá-lo é de fundamental importância para ajudar a garantir a sobrevivência de estoques pesqueiros, principalmente aqueles de importância comercial. Dentre as técnicas utilizadas para estimar o  $L_{50}$ , a utilização da relação peso-comprimento se mostrou bastante eficaz e com resultados acurados, necessitando somente de dados de peso e comprimento dos peixes em diferentes classes de comprimento, ocasionando um menor

custo operacional, laboratorial e em uma menor mortalidade de peixes durante a coleta. Portanto, apesar de o uso de informações da observação macroscópica das gônadas apresentar um resultado preciso, a relação peso-comprimento para peixes com crescimento polifásico se mostra uma ferramenta bastante útil na estimativa. Porém, considerando a necessidade de diminuir a quantidade de dados necessária para estimar o  $L_{50}$  de peixes e assim a mortalidade de peixes e o esforço amostral, a inferência bayesiana também se mostrou eficaz na estimativa do  $L_{50}$ , podendo ser utilizada para diversas ordens de peixes e mesmo com um baixo número amostral.

Portanto, o presente trabalho apresenta informações importantes para o entendimento das relações entre períodos pluviométricos e o comportamento reprodutivo de espécies de peixe detritívoras, além de fornecer opções de métodos estatísticos robustos e eficazes para a estimativa do tamanho de primeira maturação sexual que podem ser aplicados a outras ordens de peixes, e que podem ser selecionados de acordo com as necessidades do estudo a ser desenvolvido.