Conselho Estadual de Pesca do Estado de Mato Grosso CEPESCA

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RELATÓRIO SIMPLIFICADO DO ESTUDO TÉCNICO DESENVOLVIDO PARA SUBSIDIAR O CEPESCA NA DEFINIÇÃO DO PERIODO DE PROIBIÇÃO DA PESCA VISANDO A PROTEÇÃO DO PERÍODO REPRODUTIVO DOS DE PEIXES DE INTERESSE PESQUEIRO NO ESTADO DE MATO GROSSO.

INTRODUÇÃO

Estudos sobre a reprodução de peixes são ferramentas importantes para compreensão de mecanismos adaptativos e, portanto, fundamentais para elaboração de ações de conservação e manejo, pois a reprodução representa um dos aspectos mais importantes da biologia de uma espécie (Cavalcanti, 1994), sendo que de seu sucesso dependem o recrutamento e a manutenção de populações viáveis (Suzuki & Agostinho, 1997). Neste contexto, a determinação da extensão do período reprodutivo das espécies de interesse pesqueiro é imprescindível para o estabelecimento de medidas protetivas que garantam a renovação dos estoques.

Uma das medidas protecionistas de manejo largamente adotadas no Brasil é a proibição da pesca no período de reprodução das espécies alvo. Em teoria quanto maior o estoque desovante maior seria o recrutamento de indivíduos para a população. Entretanto, o fechamento da pesca traz uma série de consequências econômicas para aquelas atividades que dela dependem. Assim, a definição do período de proibição deve buscar o equilíbrio entre a máxima proteção dos estoques com o mínimo de prejuízo aos usuários do recurso. Desse modo, a definição do período reprodutivo das espécies de interesse pesqueiro e a quantificação desta atividade em cada mês, bem como a estimativa da incerteza associada, é uma informação essencial para embasar a tomada de decisões acerca do período de proibição da pesca visando a manutenção e renovação dos estoques pesqueiros.

O objetivo deste trabalho foi de coletar informações sobre o período reprodutivo dos peixes de interesse comercial nos principais rios das três bacias de MT onde a pesca se concentra. A pergunta que queremos responder é: Qual a probabilidade da espécie de peixe (ou um conjunto de espécies) estar em maturação ou matura em um determinado mês?

AREA DE ESTUDO

A região Centro-Oeste é conhecida como "o grande dispersor de águas" do país, pois deste local, partem rios para quase todas as principais bacias hidrográficas brasileiras, (Souza, 1984). Só no estado do Mato Grosso está localizado as nascentes de três grandes bacias: a Amazônica, Araguaia/Tocantins e a da Platina. Assim, consideramos os seguintes rios para análise:

• Bacia Amazônica: Rios Teles Pires/Arinos e alguns de seus afluentes

- Bacia Araguaia: Rios Araguaia/Garças
- Bacia Alto Paraguai: Rios Cuiabá e Paraguai/Sepotuba

MATERIAL E MÉTODOS

Coleta de dados

Os dados de estádio de maturação gonadal utilizados foram compilados de diversas publicações e relatórios técnicos, além de bancos de dados disponibilizados por diversos pesquisadores de instituições do estado de mato grosso. Dados de amostragens realizadas em 2016 (janeiro a março), coletados por parceiros ligados ao CEPESCA (tabela 1), também foram incluídos nas análises.

Fonte	Referencia
Artigo publicado	COSTA, R.; MATEUS, L. A. F Reproductive biology
	of pacu Piaractus mesopotamicus (Holmberg, 1887)
	(Teleostei: Characidae) in the Cuiabá River Basin, Mato
	Grosso, Brazil. Neotropical Ichthyology (Impresso), v. 7,
	p. 447-458, 2009.
Artigo publicado	BARZOTTO, E.; OLIVEIRA, M.; MATEUS, LAF.
	Reproductive biology of Pseudoplatystoma corruscans
	(Spix and Agassiz, 1829) and Pseudoplatystoma
	reticulatum (Eigenmann and Eigenmann, 1889), two
	species of fisheries importance in the Cuiabá River Basin,
	Brazil. Journal of Applied Ichthyology, online first, 2016.
Artigo publicado	MARTINS-QUEIROZ, M. ; MATEUS, L A
	F.; GARUTTI, V.; VENERE, P. C. Reproductive
	biology of Triportheus trifurcatus (Castelnau, 1855)
	(Characiformes: Characidae) in the middle river
	Araguaia, MT, Brazil. Neotropical Ichthyology
	(Impresso), v. 6, p. 231-236, 2008
Artigo aceito para publicação	BARZOTTO, E. AND MATEUS, L. Reproductive
	biology of the migratory freshwater fish Salminus
	brasiliensis (Cuvier, 1816) in the Cuiabá River basin,
	Brazil. Journal of Applied Ichthyology. 2017 (impress).
Relatório técnico	MONITORAMENTO DA REPRODUÇÃO DE PEIXES
Dr. Claumir Muniz - UNEMAT	DE INTERESSE PESQUEIRO NA BACIA DO ALTO
	PARAGUAI, ESTADO DE MATO GROSSO
Relatório técnico	RELATÓRIO TÉCNICO Nº 04/2016/CFRP -
Neusa Arenhart, Patrícia Toledo Resende	MONITORAMENTO REPRODUTIVO DE ESPÉCIES
Balster de Castilho e Valéria Milani –	DE PEIXES DA BACIA AMAZÔNICA
SEMA/MT	
Banco de dados	2004 a 2012

Tabela 1: Fonte dos dados utilizados como base para as análises.

Dr. Claumir Muniz – UNEMAT	Rio Paraguai
SEMA	
Banco de dados	Out/2010 a mar/2011; nov/2011 a abr/2012 – Rio Araguaia
Dr. Paulo Vênere - UFMT	
Banco de dados Biólogo Francisco de	Abr 2016
Assis	Rio tapirapé (Bacia do rio Araguaia)
Banco de dados	Out 2007 a mar 2008 Rios Arinos e Claro
Neusa Arenhart	Out 2008 a mar 2009 Rio Teles Pires
SEMA – MT	Out 2009 a fev 2010 Rio Teles Pires
	Out 2011 a mar 2012 Rio Teles Pires
	Set a dez 2013 Rio Teles Pires
	Bacia Amazônica (MT)
Banco de dados	Fev, abr, jun, ago e dez 2013
Dra. Solange Arrolho - UNEMAT	Mar, jun, ago, set, out, nov dez 2014
	Abr, jul, set, out, nov 2015
	Mar 2016
	Rio Teles Pires e afluentes
	Bacia Amazônica (MT)

As fontes de informação utilizadas forneceram dados dos os estádios de desenvolvimento gonadal dos indivíduos coletados mensalmente em diversos anos no período compreendido entre 2004 a 2016. Os estádios de maturação definidos foram: Imaturo (IM, jovens); Em maturação (EM); Maduro (MA); Esgotado (ES) e Repouso (RE), (Vazzoler, 1996; Bazzoli, 2003).

Análise dos dados

Considerando que um indivíduo só pode estar maduro ou não, a probabilidade de termos x sucessos em um determinado número de indivíduos n segue uma distribuição binomial:

$$p(x) = \frac{n!}{x!(n-x)!} \theta^{x} (1-\theta)^{n-x}$$

Onde:

n= tamanho do grupo (número de indivíduos amostrados)

x = número de sucessos (em maturação e maturos somados)

 Θ = probabilidade de sucesso (neste caso estar em maturação ou maturo).

Assim, se tivermos n indivíduos amostrados em um determinado mês destes x estiverem maduros podemos estima a probabilidade de estar maduro (teta).

Para estimar teta e quantificar a sua incerteza, usamos a inferência bayesiana que deriva a probabilidade posterior como consequência de dois antecedentes, uma probabilidade anterior e uma "função de verossimilhança" derivado de um modelo de probabilidade para os dados a serem observados.

A inferência bayesiana descreve as incertezas de forma probabilística. As incertezas são modificadas periodicamente após observações de novos dados ou resultados. Este tipo de análise nos permite atribuir distribuições de probabilidade para as informações anteriores (prioris) e combiná-las com a probabilidade de dados para produzir uma distribuição de probabilidade posterior representando nossos resultados atualizadas (Habib et al. 2008)

A inferência bayesiana calcula a probabilidade posterior de acordo com a regra de Bayes (Elison, 2004):

$$P(H \mid E) = \frac{P(E \mid H) \cdot P(H)}{P(E)}$$

Onde:

H- Hipótese

E- Dados

Assim, podemos estimar a distribuição posteriori de teta como:

$$P(\boldsymbol{\theta} \mid \mathbf{X}) = \frac{P(\mathbf{X} \mid \boldsymbol{\theta}) \cdot P(\boldsymbol{\theta})}{P(\mathbf{X})}$$

O modelo que utilizamos para tal atribuiu uma distribuição beta para a priori, uma vez que a probabilidade de estar maduro ou em maturação varia de 0 a 1, e uma distribuição binomial para o parâmetro associado a verossimilhança, dado que temos uma variável binomial.

Estimamos os parâmetros via simulação monte carlo com cadeia de Markov e com prioris não informativas. As análises foram conduzidas no Programa R (R Core Team 2014 JAGS via rjags (Plummer, 2014). Os modelos foram executados com três cadeias com 50 000 iterações após um corte de 5000 iterações. Convergência de cada modelo foi avaliada por meio de gráficos de diagnóstico de convergência Gelman-Rubin e autocorrelação em rjags. Assim, foi possível estimar a probabilidade média de estar em maturação ou maturo em cada mês, bem como o seu intervalo de credibilidade a 95%. A principal vantagem do modelo é que ele pode ser constantemente alimentado, a medida que os dados sejam disponibilizados e novas estimativas podem ser geradas.

RESULTADOS

O modelo foi executado com os dados disponíveis agrupados mensalmente. Na tabela 2 está o número total de indivíduos analisados por bacia e por mês.

Mês	Alto Paraguai	Araguaia	Amazônica (MT)
Janeiro	453	192	149
Fevereiro	281	150	226
Março	245	80	496
Abril	185	37	440
Maio	175	-	-
Junho	160	-	496
Julho	176	-	168
Agosto	171	-	22
Setembro	151	-	458
Outubro	299	91	152
Novembro	379	103	415
Dezembro	384	213	414
Total	3059	866	3436

Tabela 2: Número de indivíduos analisados por mês nas três bacias do estado de Mato Grosso.

A tabela 3 apresenta a médias e os intervalos de credibilidade por mês e bacias. Nos meses de janeiro, outubro, novembro e dezembro as probabilidades de os peixes estarem em processo reprodutivo foram sempre maiores do que 0,4, ou seja, 40 % dos indivíduos analisados estavam em maturação ou maturos. Isto indica que nestes meses a atividade reprodutiva dos peixes é intensa nas diferentes bacias de MT chegando a níveis superiores a 90 % . Entre os meses de fevereiro e agosto, as chances de os peixes estarem em processo de reprodução diminuem em todas as bacias, chegando a valores inferiores a 20 %.

Tabela 3. Média, desvio padrão (dp) e intervalo de credibilidade a 95% da probabilidade
dos peixes estarem em maturação ou maturos nas bacias hidrográficas do Estado de Mato
Grosso.

Local	Meses	Média	dp	Quartil 0.025	Quartil 0.97
B. Paraguai	Jan	0.384577	0.022606	0.369541	0.429377
B. Paraguai	Fev	0.105569	0.017956	0.093034	0.143597
B. Paraguai	Mar	0.036372	0.011966	0.027733	0.062875
B. Paraguai	Abr	0.021241	0.010449	0.013577	0.04601
B. Paraguai	Mai	0.02302	0.011568	0.014609	0.050211
B. Paraguai	Jun	0.012264	0.008449	0.005949	0.033232
B. Paraguai	Jul	0.067129	0.018794	0.053832	0.108514
B. Paraguai	Ago	0.156017	0.027352	0.136656	0.214394
B. Paraguai	Set	0.261538	0.034625	0.237516	0.331071
B. Paraguai	Out	0.614333	0.02832	0.594975	0.669622
B. Paraguai	Nov	0.735035	0.022623	0.720374	0.777452
B. Paraguai	Dez	0.616431	0.024762	0.59961	0.664116
B. Amazônica	Jan	0.51661	0.040268	0.4895	0.593859
B. Amazônica	Fev	0.399608	0.032106	0.378133	0.463027
B. Amazônica	Mar	0.556187	0.022032	0.541445	0.599711
B. Amazônica	Abr	0.043109	0.009622	0.036311	0.063757
B. Amazônica	Jun	0.581962	0.022089	0.567223	0.624691
B. Amazônica	Jul	0.782144	0.031725	0.761748	0.838933
B. Amazônica	Ago	0.540911	0.100285	0.471598	0.727645
B. Amazônica	Set	0.660798	0.021847	0.645946	0.702464
B. Amazônica	Out	0.778931	0.03317	0.757151	0.841158
B. Amazônica	Nov	0.815366	0.018828	0.803069	0.850694
B. Amazônica	Dez	0.838423	0.017926	0.826671	0.871997
B. Araguaia	Jan	0.28897	0.031101	0.267049	0.350359
B. Araguaia	Fev	0.07451	0.017039	0.062327	0.110354
B. Araguaia	Mar	0.058685	0.025516	0.039853	0.117175
B. Araguaia	Abr	0.011579	0.01174	0.003284	0.043604
B. Araguaia	Out	0.910448	0.023347	0.895583	0.950537
B. Araguaia	Nov	0.874983	0.026046	0.858255	0.921282
B. Araguaia	Dez	0.821984	0.02441	0.805607	0.866915

No mês de setembro a estimativa da probabilidade de atividade reprodutiva dos peixes, mostrou tendências distintas entre as Bacias do Alto Paraguai e Amazônica. Na Bacia do Alto Paraguai, a probabilidade média é inferior a 30 % (no intervalo de 23 a 33 %) e na Amazônica é 66 % (no intervalo de 64 a 70%). Para a Bacia Araguaia-Tocantins, não foram encontrados dados para análise neste mês (Tabela 3; figura 1).



Figura 1: Probabilidade mensal dos peixes estarem em período reprodutivo nas diferentes bacias hidrográficas do Estado em Mato Grosso.

Em síntese, as estimativas demonstram que a os maiores valores de probabilidade estão nos meses de outubro, novembro e dezembro quando consideradas as três bacias do estado em conjunto (figura 2).



probabilidade de estar em maturação ou maduro

Figura 2: Probabilidade mensal dos peixes estarem em período reprodutivo considerando as bacias hidrográficas do Estado em Mato Grosso em conjunto.

Informações adicionais

- As informações aqui apresentadas estão sendo aprimoradas e adequadamente descritas para a compor um manuscrito que será submetido a um periódico científico qualificado da área de avaliação Biodiversidade (CAPES), visando a sua publicação.
- Os artigos publicados utilizados dos quais os dados foram compilados estão em anexo.
- Em respeito ao direito a propriedade intelectual dos dados fornecidos pelos pesquisadores, os relatórios e bancos de dados brutos utilizados aqui estão depositados no Laboratório de Ecologia e Manejos Recursos Pesqueiros da UFMT.

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Reproductive biology of pacu *Piaractus mesopotamicus* (Holmberg, 1887) (Teleostei: Characidae) in the Cuiabá River Basin, Mato Grosso, Brazil

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The purpose of this study was to analyze the reproductive strategy and life-history traits involved in the reproductive processes of *P. mesopotamicus* in two regions of the Cuiabá River basin, the first of these encompassing its headwaters (Cuiabazinho River - Rosário Oeste) and the other the flood area of the Cuiabá River (Porto Cercado - Poconé). A total of 391 individuals were sampled monthly from August 2006 to July 2007, 159 from the headwaters and 232 from the flood area. The size structure indicated that the females in the headwaters attain longer lengths than the males, while no differences in length were found in the flood area. Females predominated in the headwaters, while males outnumbered females in the flood area. The reproductive period in the headwaters extended from October to March, but was restricted to October and November in the flood area. Feeding activity showed significant differences in both sexes between the stages of gonadal maturation, with the highest values recorded in stages of gonadal inactivity. The length at first maturity was 34.89 cm and the length at which all specimens were ready for reproduction was 44 cm at both sampling sites. The spawning type was total and fecundity was positively correlated to the size of the female.

O presente estudo teve como objetivo analisar a estratégia reprodutiva e os traços de história de vida relacionados aos processos reprodutivos de *P. mesopotamicus* em duas regiões da bacia do rio Cuiabá: uma que representa a região das cabeceiras (rio Cuiabazinho - Rosário Oeste) e outra que abrange a área de inundação do rio Cuiabá (Porto Cercado - Poconé). Indivíduos foram amostrados, mensalmente, no período de agosto de 2006 a julho de 2007. Foram amostrados 391 indivíduos, sendo 159 de ambiente de cabeceira e 232 de área alagada. A estrutura de tamanho mostrou que em ambiente de cabeceira as fêmeas atingem comprimentos maiores que os machos, enquanto que em área alagada não houve diferença nos comprimentos. Ocorreu predomínio de fêmeas em ambiente de cabeceira, e de machos em área alagada. O período reprodutivo para o ambiente de cabeceira e stendeu-se de outubro a março e para a área alagada foi restrito a outubro e novembro. A atividade alimentar apresentou diferenças significativas para ambos os sexos entre os estádios de maturação gonadal com os maiores valores encontrados em estádios de inatividade gonadal. O comprimento de primeira maturação foi de 34,89 cm e o comprimento em que todos estão aptos a reproduzirem foi de 44 cm para ambos os locais. O tipo de desova é total e a fecundidade foi positivamente relacionada ao tamanho da fêmea.

Key words: Reproduction, Size at first maturity, Sexual proportion, Pantanal.

Introduction

Piaractus mesopotamicus (Holmberg, 1887), popularly known as *pacu*, belongs to the family Characidae, subfamily Myleinae (Britski *et al.*, 1999). Geographically, it is widely distributed in the La Plata River basin formed by the Paraguay, Paraná and Uruguay rivers and their tributaries (Reis *et al.*, 2003; Romagosa *et al.*, 1988). This fish lives in lotic environments and spawns after a period of migration upriver (Ferraz de Lima & Chabalin, 1981).

Migration plays a fundamental role in the reproductive success of fishes, because it allows for the search for suitable environments for egg fertilization (the gathering of large numbers of individuals of both sexes), initial development (high oxygenation and food availability), and conditions of low predation rates (Agostinho *et al.*, 2007; Godin, 1997). These regular seasonal changes in population distribution can frequently lead to major implications for commercial fishing (Metcalfe *et al.*, 2002), since migration is a common trait in the life-history of many economically important fish

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species. For example, one of the behavioral traits of large migratory freshwater fish is the temporal and spatial separation of the habitats they use for reproduction, growth and feeding during different stages of life (Agostinho *et al.*, 2007).

The beginning of reproduction is a critical phase in the life of an individual, because the energy heretofore directed at growth and survival is now spent in a potential conflict between the time and resources allocated for reproduction, or for survival and growth (Wootton, 1998). This event is related to growth rate, which in turn depends on environmental conditions and individual characteristics (Nikolsky, 1969).

According to Donald & Winemiller (1989), tropical fishes of seasonal environments can be divided into three major groups: (I) equilibrium strategists - associated with local sedentary populations with few fluctuations, prolonged reproductive seasons and care of offspring; (II) opportunistic strategists - characterized by small fish, rapid maturation, multiple spawning and high capacity to recolonize disturbed habitats; (III) seasonal strategists - which may attain large sizes, have high fecundity and do not show parental care. These last exploit the periodic expansion of the aquatic environment through reproductive migration and total spawning at the onset of the rainy season.

Among the main aspects that comprise the reproductive strategy of fish species are sex ratio, egg size, fecundity and the reproductive period (Nakatani *et al.*, 2001; Hutchings, 2002). Understanding these aspects can be considered the first step in establishing the principal life-history patterns of fish species and in determining recurrent patterns, which normally facilitate the prediction of behavior in a given period and geographic area (Matthews, 1998; Mazzoni & Silva, 2006).

Individuals must reach a given age or size before they are able to reproduce. This size is the mean length at first maturity, defined as the length at which 50% of the individuals of a population are sexually mature (King, 1995). This is an important life-history trait, information that must be known for successful fish management, since it is fundamental for the establishment of measures that avoid the exploitation of juveniles and the resulting depletion of spawning stock (Hilborn & Walters, 1992).

Piaractus mesopotamicus is a highly important species in the Paraguay River basin due to its commercial value, and is one of the species most sought after by amateur and professional fishermen (Catella, 2001; Vaz, 2001). It is the most frequently caught species in Mato Grosso do Sul, representing almost 1/3 of the total production of professional fishing between 1994 and 1999 and corresponding to 2,393.8 tons (30.2%) of the total catch (Catella, 2001). In the Cuiabá River in the State of Mato Grosso, the species represented 13.52 tons (8%) of the commercial catch in 2001 and 2002 in Cuiabá, occupying the fourth position in landings (Mateus et al., 2004). However, statistical fisheries data from the 1980's indicate that the species ranked third in landings from the Cuiabá River (Ferraz de Lima, 1986; Silva, 1986). Moreover, some studies indicate that the pacu fish stock is overexploited in the Cuiabá River (Vaz, 2001) and in the southern reaches of the Pantanal of Mato Grosso (Catella, 2001; Peixer & Petrere, 2007). Despite the species economic importance, more detailed data about its reproductive strategy in natural environments are scanty, making it difficult to take effective management measures to ensure the maintenance of its stock.

Therefore, based on the hypothesis that reproductive processes respond differently to different regions, the present work consisted of a comparative analysis of the reproductive strategy and life-history traits involved in the reproductive processes of P. mesopotamicus in two regions of the Cuiabá River basin: one encompassing the headwaters of the basin (Cuiabazinho River - Rosário Oeste) and the other the flood area of the Cuiabá River (Porto Cercado - Poconé). The reproductive process was evaluated over a one-year period in order to: (i) characterize the population structure in terms of total length and sex ratio; (ii) determine the reproductive period, considering the microscopic analysis of the stages of gonadal maturity and the gonadosomatic index (GSI); (iii) estimate the quantitative indices of the nutritional condition (stomach somatic index - IS) and energy reserves (hepatosomatic index - IH) and their correlation with the reproductive stages; (iv) estimate the length at first maturation (L_{50}) ; and (v) determine the type of spawning and fecundity of the species under study.

Material and Methods

Study Area

The Cuiabá River is one of the tributaries of the Paraguay River, that seasonally overflows and floods. This is due to the fact that during the rainy season, the volume of water exceeds the river's flow capacity, and because the low declivity of the floodplain, flood of extensive areas of the Pantanal Basin (Carvalho, 1986). The Pantanal of Mato Grosso is a floodplain covering 138,183 km² and is part of the basin of the Upper Paraguay River, which begins in the Serra dos Parecis mountain range and runs southwards until it joins the Paraná River. This floodplain lies between the 14° and 22° S parallels of latitude and 53° and 61° W meridians of longitude (Carvalho, 1986). The Upper Paraguay River drains an area of approximately 500,000 km², two thirds of which are located in the Brazilian States of Mato Grosso and Mato Grosso do Sul (Girard, 2002).

The Cuiabá River basin covers an area of approximately 28,732 km² up to the proximities of the municipality of Barão de Melgaço, MT. Its headwaters are located in the municipality of Rosário Oeste in the foothills of the Serra Azul mountain range, and it is formed mainly by the Cuiabá da Larga and Cuiabá do Bonito Rivers. The confluence of these two rivers forms the Cuiabazinho River, which then joins the Manso River, becoming the Cuiabá River. The Cuiabá River basin can be subdivided into the upper Cuiabá, which comprises the uplands region, showing a considerable difference in levels, with various streams and gradually diminishing declivity, and the middle Cuiabá in the plains region, with low declivity up to the Pantanal wetlands (Cavinatto, 1995).

Samplings were carried out in the headwaters (in the region of Rosário Oeste), about 190 km from the city of Cuiabá, and in the flood area (in the region known as Porto Cercado), situated in the northern part of the Pantanal in the municipality of Poconé, about 140 km from Cuiabá, Mato Grosso State (Fig. 1).

Data Collection

Piaractus mesopotamicus specimens were collected monthly from August 2006 to July 2007. The fish were caught using fishing nets with mesh openings varying from 17 to 20 cm, throw nets with mesh openings of 18 to 20 cm, fishing hooks, sweep nets and boulters.

Records were made of each specimen's total length (TL; cm) and total mass (TM; kg). After the biometric measurements, each specimen was subjected to a longitudinal incision along the abdominal surface from the urogenital opening to the head for inspection of the abdominal cavity and identification of the sex.

The gonads, liver and stomach of all the captured specimens were removed and weighed (g). To classify the stages of maturation and confirm the sex, the whole gonads were fixed in 10% formalin for five days, stored in 70% alcohol and subjected to routine histological techniques of paraffin embedding and staining with Hematoxilin and Eosin. The stages of gonadal development were classified as: Immature (IM, juveniles), Maturing (MG), Mature (MA), Spawned (SP),

and Rest (RE) (Vazzoler, 1996; Bazzoli, 2003). To determine the spawning period and type, the diameters of 10 ovarian follicles per female were measured randomly using the MOTIC[®] 3.0 program with 10x magnification.

Fecundity was estimated based on the ovaries of all the mature females, using the gravimetric method (Vazzoler, 1996): the weight of the fixed ovaries was measured and a subsample was removed from each ovary and weighed. The oocytes contained in the subsamples were dissociated in a modified Gilson solution, counted and measured, and the rule of three was applied to estimate the total number of oocytes in the ovaries. All oocytes were counted according to Ivankov's method (1985), which is based on Potential Fecundity (PF), *i.e.*, the initial vitellogenic oocyte reserve, the resource for the achievement of final fecundity by the gradual reabsorption of excess vitellogenic oocytes.

The rainfall and water flow data of the Rosário Oeste and Porto Cercado (Poconé) Station were obtained from the Civil Defense Department of the State of Mato Grosso and from Brazil's National Water Agency (ANA). That information was related to the maturation phase graphically.

Data Analysis

The data on fish length were grouped by site into classes of 5 cm intervals to determine the absolute frequency distribution of juveniles and adults during the sampling



Fig. 1. Geographic location of the sites sampled in the Cuiabá River basin, Mato Grosso State, Brazil.

period. To investigate the possible differences in TL between the sexes and sites, a bifactorial analysis of variance was applied. The hypothesis that the sex ratio differs from the expected 1:1 was tested by the chi-square test (χ^2) to verify the possible significant differences at the sampled sites.

The reproductive period was established by the monthly relative frequency distribution of the gonadal maturation stages identified considering only adult individuals (MG, MA, SP and RE) for the collection period (months), and by the monthly analysis of variation of the values of the gonadosomatic index (GSI = GM x 100/TM, where: GM is gonad mass and TM is total mass). Because the supposition of normality of the GSI data was not reached, a comparison of the months was made by the Kruskal-Wallis test (H-test).

To quantitatively analyze feeding activity during the reproductive and sampling period, a calculation was made of the stomachsomatic index (IS) and the hepatosomatic index (IH), which represent the percent of the organ's mass in relation to the total mass (TM) of the fish. Since the indices did not show a normal distribution, the Kruskal-Wallis test was applied to test differences in the values of these indices by gonadal maturation stage separately for females and males.

To estimate the mean length at first maturity (L_{50}) and the length at which all the individuals were ready to participate actively in the reproductive cycle (L_{100}), distributions were built of the proportion of adults in each total length class at both sites, including all the adult specimens (which were in the gonadal maturation stages of MG, MA, SP and RE). The resulting curve was fitted to the following expression:

$$p = \frac{1}{1 + e^{\alpha(\text{Lso-TL})}}$$

where, p = Proportion (number of adult individuals/total number of individuals (juveniles and adults); α = Intercept of the ratio; L₅₀ = Length at first maturity; and TL = Total length. The α and L₅₀ parameters were estimated by nonlinear regression (King, 1995).

An analysis was made of the relative frequency distribution of the ovarian follicle diameter grouped into classes of a 100 mm intervals. The relative frequency of the class of ovarian follicle diameter was calculated for each period and site to identify the time of gonadal maturation, to identify the type of spawning and to determine possible variations in the oocyte development phase between the sites. The relative frequency analysis of ovarian follicle diameter indicates the phase of gonadal development.

To ascertain possible fecundity (F) relationships between the TL, total fish mass and total gonad mass, by sampling site, a simple linear regression was applied, estimating coefficients a and b after log-transforming the values of the variables. To check whether there was a difference in gonad mass between the sites, an analysis of covariance (ANCOVA) was made.

All tests were performed using the statistical package Systat version 12 (Wilkinson, 2007). A confidence level of 95% ($\alpha = 0.05$) was used in all the tests.

Results

A total of 391 individuals were sampled. Seven juveniles and 152 adults were captured in the headwaters. Juveniles were found in the months of September, April, May, June and July (Fig. 2a). The specimens analyzed from the flood area comprised 32 juveniles and 200 adults. Juveniles were found in all months except October, November and December (Fig. 2b). The analysis of the 159 individuals from the headwaters showed a mean TL of 47.34 cm (SD = 9.58), where the minimum was 34 cm, and maximum 75 cm. TL varied from 34 to 75 cm in females (n = 105), and from 34 to 74 cm in males (n = 54). The 232 individuals from the flood area showed a mean TL of 41.51 cm (SD = 4.90), with a minimum TL of 28 cm, and maximum of 58 cm. The TL varied from 29 to 55 cm in females (n = 89), and from 28 to 58 cm in males (n = 143). There was an interaction between the sex and site factors ($F_{1.397} = 10.26$; p = 0.0014), with the females in the headwaters, on average, larger than the males, but no difference in length was found between females and males in the region of Porto Cercado (Fig. 2c-d).

In the headwaters there was a significant difference in the sex ratio ($\chi^2 = 16.35$; p = 0.000, n = 159), with a predominance of females (105) in relation to males (54) in almost all the TL classes. A significant difference in the sex ratio ($\chi^2 = 12.56$; p = 0.000, n = 232) was also found in the flood area, but here males predominated (143) over females (89) in most TL classes.

With regard to gonadal maturation stages, 178 females were analyzed, 101 from the headwaters and 77 from the flood area. In the Cuiabazinho in October and July, there was a high frequency of maturing (MG) females. Mature (MA) females were found in October, January, February and March, with the highest frequency occurring in January and the lowest in March, indicating that the reproductive period occurred from October to March. A low frequency of spawned (SP) specimens was found in February. Individuals at rest (RE) occurred practically throughout the entire year, with a low frequency in October (Fig. 3a).

In the flood area, a high frequency of maturing (MG) specimens was found in August, September, March and July. October and November were the months of high frequency of the mature (MA) stage, indicating the greater reproductive activity in those months. No individuals in the spawned (SP) stage were found. Specimens at rest (RE) were found in every month except October and November (Fig. 3b).

The GSI showed a significant difference among the sampling periods for females (H = 39,997; d.f. = 9; p < 0.001); (H= 39,230; d.f. = 11; p < 0.001) and males (H = 17,783; d.f. = 7; p = 0.013); (H = 83,353; d.f. = 11; p < 0.001) in the headwaters and flood areas, respectively. We found that, in the headwaters, the females displayed a higher variation in GSI in October, January, February and March, and the males in September and October (Fig. 4a-b). In the flood area, the GSI of the females showed higher variations in October and November, while that of males showed variations in September, October, November, December, January and February (Fig. 4c-d).



Fig. 2. Absolute frequency of young (\blacksquare) and adult (\square) individuals in the headwaters - Rosário Oeste, MT (a), and in the flood area - Poconé, MT (b), and frequency distribution by class of TL (cm) of *P. mesopotamicus* females (\blacksquare) and males (\blacksquare) in the headwaters (c) and the flood area (d) between August 2006 and July 2007.



Fig. 3. Relative frequency of *P. mesopotamicus* females in the headwaters - Rosário Oeste, MT (a) and in the flood area - Poconé, MT (b) according to the stages of gonadal maturation (■ MG; ■ MA; ■ SP and ■ RE) between August 2006 and July 2007.

Local rains and rising water levels are related to the maturation phase of the pacu. In the headwaters, a positive relationship was found between the rainfall index and water level, with the reproductive period of *P. mesopotamicus* males peaking in February. In the flood area, the rainfall index and water level increased in October, coinciding with the peak of the female reproductive period in October and November (Fig. 5a-d).

The IS and IH of the females showed significant differences between the maturation stages (IS: H = 12.14; p = 0.017; IH: H = 16.29; p = 0.003), with the females at rest showing a higher IS and the immature females a higher IH. The males displayed significant differences only in the IH (H = 15.11; p = 0.004), with males with spawned gonads showing a higher IH relative to the other stages of maturity (Fig. 6a-d).

In the headwaters, the estimated L_{50} was 34.82 cm, with a confidence interval between 32.77 and 36.88 cm ($\alpha = 0.55$; $r^2 = 0.72$), and the estimated L_{100} was 40 cm. The estimated L_{50} in the flood area was 35.14 cm, with a confidence interval between 34.10 and 36.17 cm ($\alpha = 0.41$; $r^2 = 0.95$), and the estimated L_{100} was 44 cm. Because these ratios did not show significant differences considering the overlaping of the confidence intervals of the L_{50} , the data were grouped, resulting in a single expression representative of the species under study, corresponding to the total number of fish sampled in the two regions. The mean total length at first



Fig. 4. Monthly variation of the gonadosomatic index (GSI) of *P. mesopotamicus* females and males in the headwaters (a,b) and the flood area (c,d), respectively, between August 2006 and July 2007.

maturity (L_{50}) was estimated to be 34.89 cm, with a confidence interval between 32.57 and 37.21 cm ($\alpha = 0.397$; $r^2 = 0.713$). The estimated mean total length at which all the individuals were ready to reproduce (L_{100}) was 44 cm.

For the headwaters, 1050 ovarian follicles from 105 females (juveniles and adults) were measured, which varied from 9.76 μ m to 1023 μ m. The mean follicle diameter was 156.07 μ m (SD = 191.81 μ m). In all the months, the relative frequency distribution of follicle diameters was concentrated in smaller diameters. In October this distribution shifted to intermediate diameters. No female specimens were caught in November. In October, January, February and March, the relative frequency of follicle diameter was distributed in larger diameters, displaying two modes that represent the peak of spawning in the headwaters (Fig. 7a).

In the flood area, 890 ovarian follicles from 89 females were measured, varying from 9.76 μ m to 1016 μ m. The mean follicle diameter was 165.79 μ m (SD = 196.67 μ m). In all months, the relative frequency distributions of follicle diameter were concentrated in smaller diameters. However, in October and November the relative frequency of follicle diameters was distributed in larger diameters, displaying two modes and indicating that spawning was total; hence, the smaller mode represented the reserve stock while the higher one corresponded to mature oocytes (Fig. 7b).

Fifteen mature females were analyzed, 7 from the headwaters and 8 from the flood area. The values of absolute fecundity, expressed as numbers of oocytes to be possibly eliminated during spawning, showed a variation of 236,147 to 1,960,970 oocytes, with a mean of 887,674 (SD = 601,040) in the headwaters, and a variation of 64,179 to 589,309 oocytes, with a mean of 258,099 (SD = 178,523) in the flood area.

In the headwaters, total length of the females analyzed varied from 41 to 73 cm, fish mass varied from 1.75 to 9.75 kg, and gonad mass varied from 55 to 775 g. Total length in the flood area varied from 38 to 52 cm, fish mass varied from 1.05 to 2.70 kg, and gonad mass varied from 34 to 352 g. Fecundity was positively correlated with total length, total mass and gonad mass at both sampling sites (Fig. 8), *i.e.*, fecundity was found to increase with body size. Gonad mass did not differ between sites ($F_{1:13} = 0.722$; p = 0.410); thus, there was no difference between the headwaters and flood area with respect to fecundity rate.

Discussion

Piaractus mesopotamicus females attain longer lengths than males and occur in higher numbers in larger-size classes in the headwaters. Few individuals of larger size classes were found in the flood area and the males outnumbered the females in the intermediate classes. A larger growth attained by the females of some species may be a tactic dictated by their reproductive behavior (Veregue & Orsi 2003). The larger size of females than of males in fish species of total spawning and external fecundation may be related with sexual dimorphism (differences in body shape and size) (Chech & Moyle, 2004),



Fig. 5. Relative frequency of the stage of gonadal maturation (MA) by collection period for *P. mesopotamicus* females (\blacksquare) and males (\blacksquare) with the monthly variations in rainfall ($___$) and water level ($___$) in the headwaters (a, b) and the flood area (c, d), respectively, between August 2006 and July 2007.



Fig. 6. Variation in the stomachsomatic index (IS) of *P. mesopotamicus* females and males (a, b) and hepatosomatic index (IH) of females and males(c, d), respectively, according to the stages of gonadal maturation between August 2006 and July 2007.

revealing a strategy for the production of larger numbers of oocytes, which would ensure the survival of the species and the maintenance of the population, aimed at overcoming the adversities imposed by the environment on ovuliparous species (Santos, 1980; Lowe-McConnell, 1999).

According to Fisher's theory (1930), natural selection is manifested equally in the production of males and females, and the expected proportion in most fish species is 1:1 (Vazzoler, 1996). However, the *P. mesopotamicus* population studied here showed significant differences in its sex ratio. This ratio may show significant differences even in analyses of different populations of the same species or at different times (Nikolsky, 1969). In the Itaipu reservoir, female *Leporinus friderici* outnumber males, while in the Corumbá reservoir (both of them in the Paraná River basin), the sex ratio is equal (Lopes *et al.*, 2000). Differences in sex ratio may reflect differential mortality or birthrates between the sexes, reproductive periods, food availability and predatory activity, or even fish shoal behavior (Araújo & Gurgel, 2002).

In the *P. mesopotamicus* population of the headwaters and flood area, the differences in the proportion of sexes may be explained by the hypothesis of the evolutionary development of reproductive strategies, which is closely linked with the environment and the selective forces in action during its history (Agostinho *et al.*, 2007). This is a large migratory species that requires wide free reaches of the basin, which enable it to roam over great distances (Ferraz de Lima, 1986).

Although the most important migrations are reproductive ones, there are also migrations of a thermal or seasonal, trophic or nutritional, and ontogenetic or growth-related nature, most of them associated with the hydrological regime.

The annual reproductive cycles are synchronized with the seasons of the year in response to the environmental conditions, whose rhythms involve endogenous and exogenous factors. Synchronization is achieved through the



Fig. 7. Relative frequency distribution of the ovarian follicle diameter (μ m) of *P. mesopotamicus* in the headwaters (a) and flood area (b) between August 2006 and July 2007.

interaction between the neuroendocrinal system and the environmental factors (Harshman & Zera, 2006; Sumpter, 1997). The most favorable time of the year for fish reproduction is that in which the environment offers the minimal conditions necessary for the development of the juvenile phases, ensuring adequate food availability, protection against a variety of predators, and favorable abiotic conditions.

The changes occurring in the river's water levels and the sequences of dry and rainy seasons represent the principal controlling event for fish reproduction in low-latitude environments. Due to the slight variations in the temperature and photoperiod of these sites, the reproductive period of fishes in tropical regions coincides with the time of year when rainfall is at its highest (Wootton, 1998).

The reproductive period of *P. mesopotamicus* was spatially differentiated in terms of duration. In the headwaters, this period comprises the months of October, January, February and March, while in the flood area it occurs in

October and November. Note that the reproductive period in the headwaters extends over two additional months. This may be due to the ovarian activity, which is reduced and remains in syntony and adjusted to the environmental conditions, thus ensuring that the release of oocytes coincides with the period when the environmental characteristics are the most favorable for the offspring's highest possible survival rate. This phase is known as the "period of dormancy", whose duration varies from one species to another and which, in Brazilian fishes, lasts from a few weeks up to several months (Zaniboni Filho & Nuñer, 2004). The reproductive periods found in this study can be attributed to the period of dormancy and the difference in local climatic and/or ecological factors, such as the rainfall and river water level that are characteristic of the sampled regions. Reproduction of mature P. mesopotamicus females takes place not only in the region of the headwaters but can also occur in the floodplain, although no spawned females were found.



Fig. 8. Relationship between fecundity (number of oocytes) and the TL, TM and GM of *P. mesopotamicus* in the headwaters (1a, 2a and 3a) and flood area (1b, 2b and 3b), between August 2006 and July 2007.

Commercially exploited tropical fish species habitually reproduce synchronously with the rising waters, since these species need to migrate to their reproduction grounds, most of them spawning at the onset of the first rains while others delay their arrival at the spawning sites Thus, large shoals migrate upriver to spawn at sites where there is greater availability and better quality of food, as well as adequate shelter for protection against predators, thus ensuring the safety of their offspring (Junk *et al.*, 1997; Vazzoler *et al.*, 1997; Saint-Paul & Villacorta-Correa, 1999; Welcomme, 2001). Therefore, the beginning of migration appears to coincide with the first rains, which occur at the end of the dry season (Agostinho *et al.*, 2003).

Flooding plays an important role in the recruitment of species with other reproductive strategies, by influencing successful spawning and through the effects on the survival of juveniles (Bailly *et al.*, 2008). Rainfall and temperature are

generally considered the determining factors that trigger the reproductive cycle (Menezes & Vazzoler, 1992). Some environments may sometimes not show limiting environmental factors over a given period of time, enabling reproduction to extend for several months.

Life-history strategies can be seen as mechanisms to maintain equilibrium in the amount of energy expended on reproduction, growth and metabolism (Chech & Moyle, 2004). Considering that both spawning and the processes it involves, such a gonadal maturation and migratory displacement, require the spenditure of energy, an increase in energy allocated to one of these traits should result in a decrease of energy for another (Stearns, 1992). Hence, the greater feeding activity observed in pacu in the phase of gonadal inactivity may indicate that during this period the females, especially, grow tissues for later investment in reproduction (Vazzoler, 1996).

The length at first maturity is an important life-history trait that needs to be determined for successful fish management, since it is fundamental for the establishment of measures to avoid overexploitation of juveniles and consequent reduction of the spawning stock (Wootton, 1998; Mateus et al., 2004). The determination of the size at first maturity serves not only to underpin management measures but also to clarify important factors of population dynamics. These factors include the genetic representativeness in future generations, provided by reproduction efficiency, which is directly correlated with the size at which the species enters reproductive maturity and with the conditions that establish this process (Begon & Mortimer, 1990). This trait is closely associated with the interaction between genotype and environment, and hence, with growth, showing spatial and temporal intraspecific variations related to the prevalent biotic and abiotic environmental conditions (Vazzoler et al., 1997).

As indicated by the results of this study, the mean length at first maturity, L_{50} , was estimated to be 34.89 cm and the L_{100} was 44 cm in the headwaters and flood area. Ferraz de Lima *et al.* (1984) estimated the L_{50} of *P. mesopotamicus* females at 34 cm and L_{100} at 42 cm in the flood area, in Mato Grosso. The L_{50} value estimated in the present study is consistent with that estimated by Ferraz de Lima *et al.* (1984), *i.e.*, 34 cm. The estimated L_{100} is very close to the minimum capture size of 45 cm established for *P. mesopotamicus* by State Law N° 7155 of October 21, 1999 and CONSEMA Resolution N° 01 of March 16, 2000.

In situations of overfishing, Gulland (1988) recommends reducing mortality by fishing and increasing the permitted age of first capture. However, increasing the age of first capture would directly affect larger individuals. Thus, due to the fact that fishing activities are highly selective, not only for reasons of yield but also because of legal restrictions (minimum capture size), fishing has led to artificial selection in populations. The law forbids the capture of juveniles, which are extremely valuable because they ensure the recruitment abundance of new cohorts in subsequent years, but the lack of control over the capture of large matrices seems damaging to the viability of the stocks (Agostinho *et al.*, 2007).

The type of spawning of fishes is the way in which the females release oocytes within a reproductive period. In tropical environments, total spawning is generally observed in large migratory species. Fishes that spawn totally are characterized by the synchronous development of the oocytes (Rizzo *et al.*, 1996) and by the low frequency of partially spawned females (Godinho & Tavares, 1994). Histologically, the ovaries of *P. mesopotamicus* show characteristics of "group-synchronous" oocyte development. The type of spawning can be determined through analyses of the frequency distribution of oocyte diameter as a function of the stage of gonadal maturation. This evidence refers to the occurrence of the mature (MA) stage and to the highest gonadosomatic index (GSI) values in a short period and to

the bimodal distribution of the oocytes. In the other stages of the reproductive cycle, the frequency distribution of oocyte diameter displays a single mode.

Fecundity is a life-history trait that can be estimated by the number of oocytes that complete their development and are released in each reproductive period, *i.e.*, reproductive investment. This phenomenon depends, first, on the total volume of the coelomic cavity available to house the ovaries, and second, on the volume of oocytes. It is a measure of the reproductive potential of fishes (Menezes & Vazoller, 1992; Chech & Moyle, 2004).

The relationship between fecundity and the variables of length, body mass and gonad mass were linearly positive for the *P. mesopotamicus* sampled in this study. The highest correlation was found between fecundity and gonad mass in the headwaters and between fecundity and body length in the flood area. Several studies have identified a positive relationship between the number of oocytes and fish size (Adite *et al.*, 2006; Brickle *et al.*, 2005; Magalhães *et al.*, 2003; Mazzoni & Silva, 2006; Moffett *et al.*, 2006; Sato *et al.*, 2006; Tarkan, 2006; Martins-Queiroz *et al.*, 2008).

Older individuals of some fish species produce larvae that have a substantially greater potential for survival (Bobko & Berkeley, 2004). These new conclusions extend the knowledge that large individuals usually have exponentially high fecundity. This is important, since large fish are frequently the target of commercial and sport fishing. Older adult fish produce larvae with greater vitality; thus, older fish presumably have greater metabolic reserves, investing more energy into each progeny (Berkeley *et al.*, 2004; Bobko & Berkeley, 2004).

Fecundity may show adaptive variations within the same population or in different populations, which reflect changes in life style (Niklosky, 1969). Ultimately, this variation may be due to food supply, time of the onset of sexual maturation, temperature, latitude, type of spawning and the number of times the fish has spawned, as well as the existence or absence of mechanisms of progeny protection (Niklosky, 1969). However, in this study, we found no spatial differences in fecundity, possibly because it involved a single stock (Calcagnotto, 1998) that roams widely through the basin (Ferraz de Lima, 1986).

In summary, the aspects that determine the reproductive strategy of *P. mesopotamicus* in the Cuiabá River basin reflects expressions of traits closely related to fitness, such as the reproductive period, maturation size, spawning type and fecundity in the life of the individual, according to the lifehistory, the trade-off between the adopted strategy and the optimal period of life ensures the maintenance of the species. These parameters are fundamentally important in the evaluation and management of the stock. Therefore, measures that ensure the stock's protection during the period of reproduction from October to March, and the size at first maturity, are crucial for the maintenance of the population in this environment, as well as for fish preservation, conservation and management.

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ORIGINAL ARTICLE

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Reproductive biology of *Pseudoplatystoma corruscans* (Spix and Agassiz, 1829) and *Pseudoplatystoma reticulatum* (Eigenmann and Eigenmann, 1889), two species of fisheries importance in the Cuiabá River Basin, Brazil

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Summary

The aim of this study was to compare the reproductive processes of two large catfishes, Pseudoplatystoma corruscans and Pseudoplatystoma reticulatum, from the Cuiabá River Basin. The reproductive process were assessed to: (i) characterizing the population structure and sex ratio; (ii) determining the reproductive period; (iii) estimating and quantify the nutritional status and energy reserves and their relationships with reproductive stages; and (iv) estimating the body length at first gonad maturation (TL_{50}) . Individuals were sampled monthly from July 2006 to February 2008 using gill nets, seine nets, hooks, casting nets and trotlines. In addition, 294 individuals were obtained from the artisanal fishery in the city of Cuiabá, Mato Grosso state, Brazil. Total lengths were greater for females for both species, with no significant difference in the sex ratio; growth was considered isometric, except for P. corruscans males. Peak reproductive activity was in December and January. Differences in the hepatosomatic index (HSI) in P. corruscans depended on the gender. In P. reticulatum the HSI did not differ between sexes or among developmental stages. For P. corruscans, the estimated mean total length at first maturity (TL_{50}) was 62.92 cm, and for P. reticulatum 57.84 cm. Action to ensure the protection of the fish stocks should consider their reproduction processes, which are important for the equilibrium of these populations in the Cuiabá River, as well as for fisheries management.

1 | INTRODUCTION

In the Cuiabá River, the genus *Pseudoplatystoma* (Bleeker, 1862) is represented by the *Pseudoplatystoma corruscans* (Spix and Agassiz, 1829) and *Pseudoplatystoma reticulatum* (Eigenmann and Eigenmann, 1889). *Pseudoplatystoma corruscans* also occur in the São Francisco and Paraná rivers (Argentina, Brazil and Uruguay), while *P. reticulatum* occur in the Paraguay and Amazon rivers (Buitrago-Suárez & Burr, 2007). They are large migratory fishes and phylogenetically-related species (Godinho, Kynard, & Godinho, 2007; Pereira, Foresti, & Oliveira, 2009) with a seasonal reproductive cycle linked to the fluviometric levels (Agostinho, Gomes, & Pelicice, 2007; Lowe-McConnell, 1975; Vazzoler, 1996). These large catfishes have an important ecological role in top-down

regulation (Agudelo-Córdoba et al., 2000; Barthem & Goulding, 1997). Furthermore, they are of high commercial value, with a potential for artisanal fishing and fish farming mainly due to their excellent flesh quality (Frascá-Scorvo, Baccarin, Vidotti, Scorvo Filho, & Ayroza, 2008; Inoue, Ceccarelli, & Senhorini, 2003), making them quality products with strong export opportunities (Crepaldi et al., 2006). It is therefore not surprising that these species are subject to intense commercial and sport fishing in all regions where they occur (Mateus, Penha, & Petrere, 2004). Studies have demonstrated that *P. reticulatum* is in imminent threat of being overfished in the Pantanal of Mato Grosso, Brazil, and action to reduce the fishing impact is urgently needed (Mateus & Penha, 2007). Although *P. corruscans*, stocks are not overexploited (Mateus & Penha, 2004, 2007; Mateus et al., 2004), they are -WILEY-

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Despite the importance of these two species and the recognition that biological data are essential to support management decisions, there are few studies regarding their reproductive biology in the natural environment. Information is available in Brazil for the Miranda (Rezende et al., 1995) and San Francisco rivers, but there are no similar studies for the Cuiabá River, where *P. corruscans* and *P. reticulatum* together represented more than 40% of the catch by artisanal fisheries in the early 2000s (Mateus et al., 2004). Thus, our focus was to compare and analyze life history traits and relationships regarding their reproduction in the Cuiabá River Basin. The reproductive process was assessed as to: (i) characterization of the population structure according to individual total length and sex ratio; (ii) determine the reproductive productive process with the reproductive stages; and (iv) estimate body lengths at first gonad maturation (TL_{50}).

2 | MATERIALS AND METHODS

2.1 | Study area

The Cuiabá River Basin is located between the geographical coordinates 14°18′–17°00′S; 54°40′–56°55′W, comprising approximately 28700 km². Its headwaters are located in the municipality of Rosário Oeste, and are the major sources of the Cuiabá da Larga and Cuiabá do Bonito rivers; downstream, the confluence of these two rivers is called the Cuiabazinho River, which then becomes the Cuiabá River after the Cuiabazinho joins the Manso River (Figueiredo & Salomão, 2009). Its main tributary rivers are the Coxipó, Aricá-Mirim, Itiquira, Aricá-Açu and São Lourenço.

The river basin is composed of different geomorphological regions, each with well-defined and intrinsic biotic and abiotic characteristics that correspond to areas of plateau and surrounding mountain ranges (upper Cuiabá River) and lowland (middle Cuiabá River), with a depression leading to the Pantanal wetland (lower Cuiabá River) (Cavinatto, 1995). The region has a tropical climate, with a winter dry season and a rainy summer (AwKoppen type: Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). The hydrological regime is characterized by the seasonal river level variation and the annual cycle of flooding, which generally occurs between December and June (Fantin-Cruz, Loverde-Oliveira, Bonecker, Girad, & Motta-Marques, 2011).

2.2 | Data survey

Individuals were sampled monthly from July 2006 to February 2008 along one sector of the Cuiabá River (14°18′–17°00′S; 54°40′– 56°55′W), between the Manso River, upper Cuiabá River (14°52′16″S; 55°47′8″W) and Porto Cercado, Lower Cuiabá River (16°15′24″S; 56°37′22″W). Samples were taken over an extensive area (approx. 300 km) of river to obtain the most representative sampling of the populations studied, since there is often a strong divergence among geographically proximate populations of the large catfish *P. corruscans* (Swarça, Fenocchio, Cestari, & Dias, 2005), and because such large catfish often undergo extensive spawning migrations (Godinho et al., 2007; Pereira et al., 2009).

Sampling was for one week per month, using a combination of methods in order to capture the maximum number of each species. Sampling was done with gill nets (mesh sizes of 17–20 cm), seine nets (mesh sizes of 18–20 cm), hooks, casting nets and trotlines in the upper and low Cuiabá River. In addition, 294 individuals were obtained from the artisanal fishery in the city of Cuiabá, Mato Grosso state, Brazil (middle Cuiabá River).

Based on their morphology, any individuals that appeared to be hybrids were excluded from the study. However, some hybrids could have had a strong morphological resemblance to one of the parents, and thus remained undetected; the possibility of hybrids in the data set, although slim, thus cannot be ruled out. Fluviometric data was obtained from the Brazilian National Water Agency (ANA, 2015).

2.3 | Biometrics

For each specimen the total length (TL, cm) and total weight (TW; kg) were recorded. Once these measurements were obtained, each sampled specimen had a longitudinal incision made along the abdominal surface, whereby the gonads and livers were removed and weighed on a precision scale (0.01 g).

2.4 | Gonad processing

Analyses of the developmental stages were first made macroscopically, based on color, degree of turgidity, peripheral blood flow, presence of semen (for males), and diameter of visible oocytes (for females) (following Vazzoler, 1996). To confirm the maturity stages, the gonads were then fixed in 10% formalin and subjected to conventional histology techniques with paraffin immersion. Gonadal development stages were defined as: Immature (I); Maturing (MT); Mature (M); Spawned (S) and Resting (R) (Vazzoler, 1996; see Tables 2 and 3 in Brown-Peterson, Wyanski, Saborido-Rey, Macewicz, & Lowerre-Barbieri, 2011).

2.5 | Data analysis

Data on individual lengths were grouped into 10 cm size classes to obtain absolute frequency distribution for males and females during the sampled period. Sex ratio was tested with a Chi-square test (χ^2). To assess the possible differences in TL between sexes and species, we used a factorial analysis of variance. A *t*-test was used for each species to investigate whether there were differences between sexes.

The reproductive period was assessed using the relative monthly frequency of gonad maturation stages in females, plus analyses of monthly gonadosomatic index values for males and females (GSI = GW/TW, where: GW is the gonad weight, TW is the total weight). As the assumption of normality for GSI data was not achieved, the between-months comparison was made with a permutational analysis of variance, performed separately for the factors sex and species.

To evaluate the relationship between the energy supplies during the reproductive stages, we calculated a hepatosomatic index (HSI), representing the percentage of each organ weight in relation to an individual fish body weight. To assess the differences in this index by gender and maturational stages, we used a permutational factorial ANOVA (Anderson & Braak, 2003) considering the presence of outliers, with the gender and maturity stage as the explanatory variables, and the index as the response variable. For index and species, a permutational ANOVA was performed, with a paired Tukey test a posteriori when the existence of significant differences was detected (Higgins, 2004). The tests were performed using 10,000 permutations.

Estimated average length at first maturity (TL_{50}) , was obtained via the relationship between the proportion of adults in each length class (including all individuals located in the maturation stages MT, M, S and R) and total length. The following equation was used:

$$p = \frac{1}{1 + e^{a(\mathsf{TL}_{50} - \mathsf{TL})}}$$

where: p = ratio (number of adults/total number of individuals; α = intercept of the relation; TL₅₀ = length at first maturation, TL = total length. Parameters α and TL₅₀ were estimated by nonlinear regression (King, 1995).

Statistical analyzes were performed with R software. A significance level of p = .05 was used in all cases. Data normality was tested with a Shapiro-Wilk's test, and data were expressed as a mean ± standard deviation.

3 RESULTS

A total of 470 individuals were sampled: 153 males and 121 females for P. corruscans, and 106 males and 90 females for P. reticulatum. There was no significant difference in the sex ratio for either species (P. corruscans: 1: 1.3; χ^2 = 3.50; df = 1; p = .06; P. reticulatum: 1:1.2; χ^2 = 1,148; *df* = 1; *p* = .284). Total length (TL) varied from 51 to 163 cm in P. corruscans (91.75 ± 20.04), and from 39 to 115 cm in P. reticulatum (77.96 ± 12:33). Interaction between sex and species was significant ($F_{1:461}$ = 51.26; p < .001): P. corruscans females were larger on average than males (103.35 \pm 19.85 and 82.07 \pm 14:52, respectively) and there was a significant difference between sexes in relation to length (t = 9.88, df = 212.9, p < .05) (Fig. 1a). P. corruscans females dominated size classes exceeding 100 cm.

For P. reticulatum there was no difference between sexes in overall length (t = 1:39; df = 168.4, p = 00:16) or size [79.09 ± 13.79 and 76.59 ± 10.91 , respectively] (Fig. 1b), with females achieving greater lengths - a predominance which began at 90 cm.

Gonads from 198 adult females (116 P. corruscans, 82 P. reticulatum) were analyzed. Higher proportions of adult females were present throughout the sampling period for both species, especially at the Rest (R) stage.



40

an, 00, 10, 10, 10, 10,

Length class (cm) □Female ■Male

Sex

53.5 1435

(a)

Number of individuals

(b)

Number of individuals

50

30

20

10

£3.

35

3° 1° 2°

FIGURE 1 Absolute frequency of females (white) and males (black) for total length classes (10 cm) and boxplot of total length relative to sex of Pseudoplatystoma corruscans (a) and Pseudoplatystoma reticulatum (b), Cuiabá River, July 2006 to February 2008. Boxplot: horizontal line within box = median; boundaries of box = first and third quartiles (interquartile range IQR); bars = denote lower (first quartile – $1.5 \times IQR$) and upper (third quartile + $1.5 \times IQR$) inner fences: circles = outliers

January and December were exceptional months for male (M) P. corruscans, and maturing females (MT) P. corruscans in December and February, and P. reticulatum in October and November. For P. corruscans, females with depleted gonads occurred from January to April, and from February to April for P. reticulatum. Immature individuals were found in April, May and August for P. corruscans, and in April, July, September and October for P. reticulatum (Fig. 2).

Monthly gonadosomatic index values (GSI) showed significant differences in both species, for females (*P. corruscans*, $F_{11:107}$ = 11.96; p < .001 and P. reticulatum, F_{11:77} = 3.72; p = .03) and for males (P. corruscans, $F_{11:135} = 9.25$; p < .001 and P. reticulatum, $F_{11:94} = 5.24$; p < .001). Males and females of both species had higher GSI values in January and December (permutational Tukey test a posteriori: p < .05for paired comparisons; Fig. 3).

We observed a relationship between the fluviometric level and peak of the breeding season for both P. corruscans and P. reticulatum females and males, which were more frequent in catches in the months when the Cuiabá River had a higher water level (Fig. 4).

Differences in the hepatosomatic index (HSI) varied between sexes for P. corruscans (interaction for sex/stage: F4:269 = 3.49; p = .02; Fig. 5a). The HSI for mature males was lower when compared to maturing males (p = .04). However, the HSI for females showed no significant differences between maturation stages. For P. reticulatum,



FIGURE 2 Relative frequency of females: (a) *Pseudoplatystoma corruscans* and (b) *Pseudoplatystoma reticulatum*, according to gonadal maturation stages, Cuiabá River, July 2006 to February 2008. Maturity stages: (I) Immature; (MT) Maturing; (M) Mature; (S) Spawned and (R) Resting

the HSI did not differ between sexes (F4:184 = 3.75; p = .13), or stages (F4:184 = 0.08; p = 0.93), and there was also no interaction between these factors (F4:184 = 0.37; p = .70) (Fig. 5b).

The length wherein at least 50% of individuals have already reproduced (TL_{50}), when estimated separately for sex, indicated that length at first maturation differs only between male and female *P. corruscans*, as here there was no overlap in the confidence intervals estimates (CI). Female *P. corruscans* had a TL_{50} of 58.65 cm (CI: 53.97-63.33 cm), while this was 64.19 cm (CI: 64.10-65.35 cm) for males. *P. corruscans* females also achieved maturity at shorter lengths than males.

For *P. reticulatum*, females had a TL₅₀ of 53.59 cm (CI: 51.63– 55.55 cm), and males of 60.54 cm (CI: 54.90–66.18 cm). In *P. corruscans*, with both males and females combined, the TL₅₀ was estimated at 62.92 cm (CI: 62.45–63.39 cm), and the length in which all individuals had already reproduced (TL₁₀₀) was approx. 85 cm (Fig. 6a). For *P. reticulatum*, the TL₅₀ was 57.84 cm (CI: 55.47–60.22 cm) and TL₁₀₀ approx. 75 cm (Fig. 6b). While there was no overlap of CIs, *P. corruscans* reached maturity at significantly longer lengths than did *P. reticulatum*.

4 | DISCUSSION

As expected, the Cuiabá River Basin populations of *P. corruscans* and *P. reticulatum* generally synchronize their reproductive period with the river flooding. Reproduction in large Neotropical migratory fish is highly seasonal in order to enhance the chance for reproductive success.

Sex ratios may be different in various age-cohorts, depending on particular factors that act differentially on the composition. However, in our study the sex ratios did not differ significantly from 1:1 for either species. Overall, more males were captured in the intermediate size classes. Such reproductive strategies can be advantageous, as males could fertilize a higher number of oocytes, increasing chances of perpetuation of the species (Wootton, Evans, & Mills, 1978). However, the largest *P. corruscans* were females, which can also be a reproductive strategy to increase fertility (Agostinho & Julio, 1999).

The reproductive period is closely linked to the rainy period because this occurs when the stream and marginal lentic ecosystems have a combination of food availability, protection from predators, and favorable abiotic conditions that maximize the chances of juvenile survival (Lowe-McConnell, 1999; Nakatani et al., 2001).

Throughout the reproductive cycle of a fish species, marked changes can be observed in the gonads (Nikolski, 1963). We noted that the gonadal index peaked in months during which maturing and mature individuals were present, making it clear that the gonadal development occurs in line with the increase in gonads, indicating that the IGS might be considered one of the best indicators of the reproductive season (Vazzoler, 1996).

The literature suggests that the use of metrics such as the hepatosomatic index are useful in indicating how fish use environmental resources to generate energy to meet the costs of reproduction (Vazzoler, 1996). However, we found such indices were influenced by sex only for *P. corruscans*, where a higher hepatosomatic index was found in mature males. This could be explained by the role of the liver in the mobilization of energy reserves necessary for gametogenesis (Querol, Querol, & Gomes, 2002).

The mean length at maturation is defined as the length at which 50% of the population is sexually mature and is, therefore, the smallest size at which the species begins to reproduce under a specific suite



FIGURE 3 Boxplot of monthly variation in gonadosomatic index (GSI): *Pseudoplatystoma corruscans* (a) females and (b) males; *Pseudoplatystoma reticulatum* (c) females and (d) males, Cuiabá River, July 2006 to February 2008. Horizontal line within box = median; boundaries of box = first and third quartiles (*interquartile range IQR*); bars = denote lower (first quartile – $1.5 \times IQR$) and upper (third quartile + $1.5 \times IQR$) inner fences; circles = outliers

of environmental conditions (Agostinho et al., 2007; King, 1995). This is an important life history characteristic, and is essential information if a management plan for a particular fish species is to be successful (Hilborn & Walters, 1992). The mean length at first maturity LT_{50} was estimated at 62.92 cm (LT_{100} = 85 cm) for *P. corruscans* and 57.84 cm (LT_{100} = 75 cm) for *P. reticulatum*. During the sampling period in Cuiabá River, the minimum length for which fishing was legally permitted was 85 cm and 80 cm, respectively, for *P. corruscans* and *P. reticulatum* (Law N°. 8.515 of 30 June 2006). The minimum legal catch size is closest to the TL_{100} value estimated in our study.

Possible hybrids between the two catfish species may have been captured in the rivers of the Paraguay basin (Vaini, Grisolia, Prado, & Porto-Foresti, 2014). Natural hybridization, albeit rare, can happen (Genovart, 2009). Another possibility is that some fish farms present in the river basin are performing artificial hybridization using female *P. corruscans* (Vaini et al., 2014). In the Cuiabá River Basin where the two species occur in sympatry, recorded a hybrid *P. reticulatum* × *P. corruscans* (Veríssimo, Pavanelli, Britski & Moreira 2005). However, it also

takes an effort to identify hybrids in the natural environment using molecular or genetic techniques, combined with the visual methods (Hashimoto, Prado, Senhorini, Foresti, & Porto-Foresti, 2013).

Despite the limited nature of the data, it appears that reproduction of *P. corruscans* and *P. reticulatum* in the Cuiabá River Basin are timed to coincide with favorable conditions for the development of eggs and larvae, in terms of the greatest availability of food and shelter (Nakatani et al., 2001). The coincidence of the reproductive period of migratory species with floods should be a guarantee, both qualitatively and quantitatively, of maximum food availability during early developmental stages, thus promoting swift growth and hence a rapid passage through those stages most vulnerable to predation (Welcomme, 1979). Changes in abiotic conditions and overfishing are some of the factors that can cause alterations in reproductive strategies (Lowe-McConnell, 1999). Hence, migratory species, which use different habitats during their life cycle, are highly dependent on the integrity of large areas of a river basin. Accordingly, they have the potential to serve as a target species for a scientifically based





FIGURE 4 Proportion of mature individuals relative to total number (bars) and mean water level by month (line), Cuiabá River, July 2006 to February 2008, female and male (a) *Pseudoplatystoma corruscans* and (b) *Pseudoplatystoma reticulatum*. Numerator = Number of mature females (white) and males (black) per month; Denominator = Total number of females (white) and males (black) per month

FIGURE 5 Hepatosomatic index (HSI) of (a) *Pseudoplatystoma corruscans* and (b) *Pseudoplatystoma reticulatum*, relative to maturation stage, Cuiabá River, July 2006 to February 2008. Maturity stages: (I) Immature; (MT) Maturing; (M) Mature; (S) Spawned; (R) Resting. Mean values (column) and error bars (bars) derived to permutational ANOVA



conservation strategy (Agostinho, Thomaz, & Gomes, 2005). Studies to provide data-supporting measures to ensure stock protection via reproductive processes are of fundamental importance for a population balance of these species as well as for their effective conservation and for fisheries management.

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Reproductive biology of *Triportheus trifurcatus* (Castelnau, 1855) (Characiformes: Characidae) in the middle rio Araguaia, MT, Brazil

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The study of the reproductive biology of *Triportheus trifurcatus* of the middle rio Araguaia was carried out using 275 specimens obtained in seven collections conducted in the period between November 2003 and January 2005. Females prevailed among the classes of intermediate length, as well as in the total number of samples. On the other hand, males prevailed in the smaller classes. The average length of females was larger than that of males. Four stages of gonadal maturation were macro- and microscopically identified: B - maturation, C - mature, D - spent and E - resting. Stage A (immature) was not found in the habitats sampled. The smallest-length male was 110 mm in standard length, and the smallest female, 119 mm. The spawning period occurred from November to January, with reproductive peak in December/January, coinciding with the highest water levels. The absolute fecundity is considered low, and there is a positive correlation between fecundity and gonad weight, body weight and standard length. Food ingestion during the reproductive period did not suffer any interference, and it is suggested complete spawning for this species.

A biologia reprodutiva de *Triportheus trifurcatus*, do médio rio Araguaia, é estudada com base em 275 exemplares obtidos em sete coletas, realizadas no período de Novembro/2003 a Janeiro/2005. Nas classes de comprimento intermediário e na amostragem geral, as fêmeas predominam, mas nas classes menores prevalecem os machos. As fêmeas se apresentam, na média, maiores que os machos. Quatro estádios de maturação gonadal são identificados, macro e microscopicamente: B- maturação, C-maduro, D-esgotado/ espermiado e E- repouso. O estádio A (imaturo) não foi registrado nos biótopos amostrados. O menor exemplar macho maduro apresenta 110 mm e a menor fêmea 119 mm de comprimento padrão. O período de desova ocorre de Novembro a Janeiro, com pico reprodutivo em Dezembro/Janeiro coincidindo com os níveis mais elevados das águas. A fecundidade absoluta é considerada baixa e há correlação positiva entre fecundidade e peso das gônadas, peso corporal e comprimento padrão. A ingestão de alimento não sofre interferência durante o período reprodutivo e é sugerida desova total para a espécie em estudo.

Keys words: Fecundity, Sex ratio, Reproductive cycle, Gonadal maturation.

Introduction

The Neotropical ichthyofauna is the most diversified in the world, and is estimated to contain as many as 8,000 species (Schaefer, 1998). In contrast to its huge richness, basic biological knowledge is minimal for most of these species. The ichthyofauna of the Tocantins-Araguaia drainage is no exception. For example, systematized data about reproduction in a great number of species are not completely reliable. This has serious implications, such as contradictions in the assessment of the fishing resources. The species of the genus *Triportheus* are among the fewest known so far. According to Soares & Junk (2000), and Batista & Petrere Jr. (2003), *Triportheus* spp. appeared to be a prominent species unloaded at the fish market of Manaus (AM), in the period of 1994-96. Lowe-McConnell (1999) also mentions that *Triportheus* represented one of the main genusfoods in the Manaus market, consumed especially when bigger fish were scarce. Although lacking official data, the species of *Triportheus* are broadly consumed, especially by the riverine communities of the Tocantins-Araguaia basin (based on personal observations as well as those of fisherman colonies).

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Information on the reproductive cycle are important for knowledge of the role and life history of the fish and are essential for the study of population dynamics, besides addressing conservation and management policies (Hartz *et al.*, 1997). The purpose of the present work was to study the reproductive biology of *Triportheus trifurcatus* (Castelnau, 1855) in a sector of the middle rio Araguaia, with regard to their sex ratio, size of first sexual maturation, population structure, macro- and microscopic classification of the reproductive cycle stages, time and type of spawning, and fecundity. Food ingestion during the reproductive cycle was also investigated.

Material and Methods

Study area. The Tocantins-Araguaia hydrographic basin covers an area of 967,059 km² and extends throughout the states of Goiás, Tocantins, Pará, Maranhão, Mato Grosso, and the Brazilian Federal District. In this system, the flood season lasts from October to April, with its peak in February, in the upper course, and March in the middle and lower courses.

During flooding, Araguaia and its main affluents, the rio das Mortes and rio Cristalino, form an enormous flood plain and reduce their water volume between May and September, with drought peaks in September.

Data collection and analysis. Samples of *T. trifurcatus* (according to Malabarba, 2004) were captured with fishhooks and gillnets, with mesh sizes varying from 20 to 70mm. The collections were made in the middle rio Araguaia, in the area called Travessão do Ouro Fino $(15^{\circ}50'15"S - 51^{\circ}58'43"W)$ (Fig. 1) in the main channel and at the mouth of two small tributaries, Ouro Fino and Jacutinga streams, at intervals from 60 to 80 days, from November 2003 to January 2005. Voucher specimens are deposited in the fish collection of the Laboratory of Ichthyology of Instituto Universitário do



Fig. 1. Geographic location of the collection site of *T. trifurcatus*, in Travessão do Ouro Fino, middle rio Araguaia, MT.

Araguaia, Universidade Federal de Mato Grosso, Brazil (Series ICLMA065; ICLMA109, ICLMA172).

Water level measurements were obtained from the Defesa Civil de Cuiabá, MT, which in turn received the information from the Agência Nacional de Águas (ANA).

Information on standard length (SL; expressed in mm), sex, gonad and body weights (WG, WB), and stomach weight (WS) as well (all weights expressed in g) of each fish specimen was obtained. Sex determination and the classification of the reproductive cycle stage were based on macro- and microscopic analysis of the gonads, according to Vazzoler (1996) and Narahara et al. (1988). Fragments of gonads were fixed in Bouin's solution for 8 to12 h, submitted to routine histological processing and stained with hematoxylin-eosin (HE). Gonads were then classified according to the following stages: B - maturation, C - mature, D-spent and E - resting. The sex ratio was determined by grouping the data obtained from 2003 to 2005, in length classes. The chi-square test (X²) was applied, assuming the expected proportion of 1:1 between males and females. Differences in the average length of males and females were tested using Student's t test.

Reproductive activity was evaluated according to the frequency of occurrence of the gonad maturation stages and temporary analysis of the gonadosomatic index (GSI = WG x 100/WB). Food ingestion during the stages of gonad maturation was analyzed according to the stomach weight index (SSI = WS x100/WB). Differences between the average values of GSI among periods, and SSI among maturation stages, were tested for males and females separately using analysis of variance (ANOVA). Data were transformed into natural logarithms so as to meet the assumption of normality. When significant differences were found, the Sheffé post hoc test was applied.

In each maturation stage, the oocytes were measured with the aid of ocular micrometric methods (100 times magnification), for the analysis of the size frequency distribution and determination of the spawning type. Ovaries of 10 females in stage C (mature) were put into Gilson solution for dissociation of the oocytes for subsequent counting. Fecundity was estimated according to Vazzoler (1996), with slight adaptations. Absolute fecundity (F) was obtained as follows: F = (Vn)/v, where F = total number of oocytes, n =average number of oocytes obtained from the subsamples, V = total solution volume, and v= subsample volume. The relationships between fecundity and length, fecundity and body weight, and fecundity and gonad weight were analyzed using simple linear regression. The statistical analyses were accomplished assuming a significance level of 0.05.

Results

A total of 275 specimens of *T. trifurcatus* were collected in seven collections carried out in the middle rio Araguaia, of which 158 (57%) were females and 117 (43%) males. In January 2004, males prevailed (X^2 =6.533; p=0.0106), while in April 2004, females prevailed (X^2 =9.846; p=0.0017). Regarding the general

sex ratio during the period between November 2003 and January 2005, the females prevailed. Females' length varied from 100 to 186 mm (137.2 \pm 11.5) and males' length, from 105 to 160 mm (126.7 \pm 10.0). Males prevailed in the smaller classes, while females prevailed in the intermediate classes. On average, females were larger than the males (t = 7.93; p <0.01; degrees of freedom = 273). The smallest mature female was 119 mm, and the smallest male, 110 mm (Table 1).

Males and females were observed in stage B (maturation), C (mature), D (spent), and E (resting). Individuals in the stage A (immature) were not recorded during the study. Mature males were more frequent during the months of November 2003, January 2004, November 2004 and January 2005. Mature females were found more frequently in the month of November of 2003 and 2004. There were high frequencies of exhausted females during the month of January of 2004 and 2005. The temporary variation of the maturation stages showed a relationship with the change in the river water level. With the initial rains and flooding season, a relatively high number of mature fish could be observed already in November. Higher mature sampling frequencies occurred during flooding and during the dry season, where the majority of the individuals are resting. GSI varied significantly among the sampling periods for females ($F_{6;148}$ =107.217 and p <0.000), and males ($F_{6};_{97}$ =25.520 and p <0.000) (Table 2). The highest values occurred during the month of November of 2003 and 2004 for females, and November 2003 and January 2004 for males (Fig. 2). The SSI values did not differ significantly between the maturation stages, for males ($F_{3;97}$ =2.161 and p=0.097) or for females ($F_{3:148}$ =1.769 and p=0.155).

The distribution of oocyte size frequency appears to be of single modal type in stages B, D, and E and bimodal for C, and provides evidence for the displacement of modals (Fig. 3). Larger oocytes were found in C and smaller in E, B and D, respectively (Table 3). The smallest type in stage C represents the storage stock and the largest, mature oocytes.

Absolute fecundity varied from 3,533 to 13,833 oocytes, with an average of 7,920 (s=3,472; n=10) and there was a positive relationship between this and standard length (a=-348.54; b=309.96; r²=0.58; p=0.01; n = 10), body weight (a=-6,421.8; b=266.95; r²=0.62; p=0.006; n = 10), and gonad weight (a=-898.19; b=2,548.6; r²=0.78; p <0.001; n = 10) (Fig. 4). The relative fecundity was 35.4 oocytes per mm in individuals of 125 mm, and of 83.5 oocytes/mm in specimens with 145mm in length.

Table 1. Absolute frequency of standard length classes (SL) of *T. trifurcatus* from the middle rio Araguaia, from November 2003 to January 2005 (*p < 0.05, **p < 0.01).

SL (mm)	Males	Females	X^2	Р
100-115	10	1	7.36	0.0067**
115-130	67	35	10.04	0.0015**
130-145	37	79	15.21	0.0001**
145-160	2	37	31.41	0.0000**
160-175	1	5	2.67	0.1025
175-190	0	1	1.00	0.3173
Σ	117	158	6.11	0.0134*



Fig. 2. Gonadosomatic index (GSI) for collections of females (A) and males (B) of *T. trifurcatus* of the middle rio Araguaia, from November 2003 to January 2005.

Table 2. Number of individuals (N), mean values and standard deviation of the gonadosomatic index (GSI) in each period for females and males of *T. trifurcatus* from the middle rio Araguaia, from November 2003 to January 2005. In the same column, different superscript letters indicate statistically significant differences (p<0.05).

	Fema	iles	Male	8
Period	Ν	GSI	Ν	GSI
Nov/03	13	4.848±1.716 ^a	10	0.605 ± 0.299^{a}
Jan/04	8	1.164±0.209°	22	0.975±0.410°
Apr/04	68	0.303 ± 0.259^{b}	36	0.156±0.169 ^b
Jun/04	7	0.220 ± 0.086^{b}	15	$0.114{\pm}0.078^{b}$
Sep/04	23	0.289 ± 0.291^{b}	15	0.057 ± 0.146^{ab}
Nov/04	22	5.427±2.295 ^{ac}	11	0.529±0.168 ^{ac}
Jan/05	17	1.509±0.980 ^b	8	0.309±0.123 ^{ab}



Table 3. Means and standard deviation (X \pm SD), minimum (Min.) and maximum (Max.) for size of oocytes per gonad maturation stage (GMS) in females of *T. trifurcatus* from the middle rio Araguaia.

GMS	$\overline{X} \pm SD (\mu m)$	Min. (µm)	Max. (µm)
В	87.50±24.74	50.00	131.25
С	391.21±85.93	250.00	499.00
D	89.25±24.64	31.25	131.25
Е	69.60±27.20	25.00	125.00







Fig. 3. Relative frequency (%) of oocyte diameter classes (μm) of *T. trifurcatus* females of the middle rio Araguaia, at maturation stages B, C, D and E.

Fig. 4. Relationship between fecundity and standard length (A), body weight (B), and gonadal weight (C) for females of *T. trifurcatus* from the middle rio Araguaia.

Discussion

The population of T. trifurcatus of the middle rio Araguaia showed a sex ratio of 1.3 female for each male of two females for each male. On only one occasion was the number of males higher than that of females, a period in which, apparently, spawning would have already happened (January) or would had happened recently. Wootton (1989) points out that some fish species with external fecundation, for example, T. *trifurcatus*, have a larger proportion of males during the peak of the reproductive cycle, when females show high fecundity indices. According to this author, such behavior could be advantageous considering that the males could fecundate a larger number of oocytes, thereby increasing the probability of perpetuation of the species. The sex ratio can also be influenced by environmental pressure factors (Veregue & Orsi, 2003), as well as by behavioral aspects, due to a partial segregation of individuals of the two sexes (Hartz et al., 1997). In T. trifurcatus of the middle rio Araguaia, there are still differences regarding the frequency of males and females in length classes, which may indicate differentiated growth rates between sexes (Wootton, 1989).

The structure in length of T. trifurcatus shows that females reach a length larger than that of males, which is a common feature encountered among other teleost species [e. g., Schizodon fasciatus Spix & Agassiz, 1829, Rhytiodus argenteofuscus Kner, 1858, and R. microlepis Kner, 1858 (Santos, 1980); Pygocentrus piraya (Cuvier, 1819) (Cruz et al., 1996); Poecilia viviparous Bloch & Schneider, 1801, Phalloptychus januarius (Hensel, 1868), and Jenynsia lineata (Jenyns, 1842)(Aranha & Caramaschi, 1999); Colossoma macropomum (Cuvier, 1818) (Villacorta-Corrêa & Saint-Paul, 1999); Leporinus friderici (Bloch, 1794) (Lopes et al., 2000)]. This type of dimorphism may be advantageous to the species. A larger asymptotic size in females may indicate a greater fecundity, since the number of oocytes is related to the increase in weight (Lowe-McConnell, 1999; Santos, 1980) or size, which is demonstrated in T. trifurcatus.

The size of the smallest female and male sample captured in the mature stage can be an indicative of the size of the first maturation (Bazzoli *et al.*, 2003). The values found for *T. trifurcatus* in the middle rio Araguaia are smaller than the ones mentioned for the Imperatriz region (rio Tocantins), by Braga, (1990) (122 mm) and for the lower rio Tocantins, by Santos *et al.* (1984) (about 150 mm).

The reproductive activity of *T. trifurcatus* is remarkably seasonal: peak occurrence of mature females coinciding with the highest water levels; spent females, with the decrease in water levels; and females in resting, when the waters reach their lowest levels. Surprisingly, immature specimens (A) (Vazzoler, 1996 and Narahara *et al.*, 1988), were not collected in the sampled biotypes, indicating that they either occur in diverse biotypes or that the equipment used was not efficient for the capture of samples in this stage. We set out from the hypothesis that immature individuals occupy the flooded areas, especially temporary ponds, a fact common to numerous species of symptotic characiform fishes [e. g., Prochilodus nigricans Agassiz, 1829; Psectrogaster amazonica Eigenmann & Eigenmann, 1889, Steindachnerina amazonica (Steindachner, 1911); Hemiodus unimaculatus (Bloch, 1794), Anodus orinocencis (Steindachner, 1887); Astyanax argyrimarginatus Garutti, 1999, A. elachylepis Bertaco & Lucinda, 2005, Serrasalmus spilopleura Kner, 1858, Cynopotamus tocantinensis Menezes, 1987, Galeocharax gulo Cope, 1870, and Acestrorhynchus spp., among others]. The higher average GSI values occur during the flooding months. Thus, the reproductive activity peak of the species in question coincides with the raining season with high water levels, i.e., from November to January. These findings corroborate studies conducted in other areas, either for this or for any other similar species [Santos et al. (1984) in the lower rio Tocantins; Goulding (1979), in the rio Madeira; Braga (1990) in the Imperatriz-Estreito, rio Tocantins; Höfling et al. (2000), Salto Grande reservoir, rio Paranapanema].

According to Vazzoler & Menezes (1992), the reproductive cycles may be synchronized with the seasons in response to the environmental conditions, whose rhythms involve endogenous and exogenous factors. In accordance with these authors, unlike food availability, photoperiod and temperature should not be limiting factors in reproduction of tropical fish. The food amount increases with the increment of the water level, when floods enlarge the environment and provide for shelter and food appropriate for the initial phases of development (Vazzoler & Menezes, 1992, Lowe-McConnell, 1999). In T. trifurcatus, the data presented here demonstrate that the higher offer is not reflected in higher food ingestion by the adult samples. Neither a possible decrease in feeding rhythm nor an interruption of ingestion during reproduction or particularly at the reproductive peak was corroborated. Goulding (1980) reported that T. angulatus (Spix & Agassiz, 1829) and T. elongatus [T. culter (Cope, 1872)] do not feed when migrating in the rio Madeira.

The mature gonad stage and the higher GSI values occur in a short period of the year. In the mature stage, the distribution of oocytes is still bimodal, while in the other stages of the reproductive cycle the distribution of oocytes sizes is unimodal. This finding suggests that this species shows complete spawning in this environment.

Fecundity and the variables gonad weight, standard length and body weight show a positive correlation in *T. trifurcatus* of the middle rio Araguaia. The greatest correlation is seen between fecundity and gonad weight. As previously mentioned, the relationship fecundity/length found in *T. trifurcatus* indicates that larger females produce more oocytes, but when compared to the other migrating Characiformes, their total fecundity is considered low [e.g., *Curimatus gilbert* (*=Cyphocharax gilberti*) (Quoy & Gaimard, 1824): 12,749 to 75,734 (Romagosa *et al.*, 1984); *C. modestus* (Fernández-Yepes, 1948): 9,760 to 93,280 Barbieri & Hartz, 1995); *Semaprochilodus brama* (Valenciennes, 1850): 136,922 to 48,154 and *Anodus* sp.: 63,162 to 144,600).

The present study on T. trifurcatus shows a positive

relationship between rainfall and water levels, with the peak of the reproductive period coinciding with the rainy season. Therefore, measures that guarantee the protection of the stock in the process of reproduction during the period from November to January are of fundamental importance for population balance in this environment, as well as for the preservation and the increment in current fish stocks.

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ORIGINAL ARTICLE

Reproductive biology of the migratory freshwater fish Salminus brasiliensis (Cuvier, 1816) in the Cuiabá River basin, Brazil

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Summary

This study provides information on the reproductive biology of the dorado Salminus brasiliensis from the Cuiabá River basin, through determinating life history trajectories (length at first maturity, fecundity and spawning type) and the reproductive period, thus establishing the relationships between biotic factors and rainfall and river levels that might act as potential triggers for maturation and spawning. Salminus brasiliensis is a migratory species of great commercial and ecological importance. Individuals were collected monthly from August 2013 to July 2014 in the Cuiabá River. Analyses were performed based on biometric data and the gonads. Females attained greater lengths than males in the Cuiabá River basin, and with a significant difference in the sex ratio in favor of females. Peak reproductive activity occurred in November and December and was related to rainfall. Reproduction was seasonal, with spawning and reproductive potential correlating positively with the length and mass of the gonads of mature females. Average length at first maturity for females was estimated at 55 cm. A regular (annual) monitoring programme of essential biological variables to support a trend analysis over years (not just a snapshot study, as this one) is strongly recommended in order to give advice for the proper fishing policies in the region, as without regular basic data on the population dynamics, management is not possible.

1 | INTRODUCTION

39 Salminus brasiliensis Cuvier, 1816, commonly known as dorado, is found mainly in the Prata Basin (Barbieri, Salles, & Cestarolli, 2001; Froese & 40 Pauly, 2015; Lima et al., 2003). Known to fishermen as 'the king of the 41 42 river', it is one of the most popular species among pro onal and 2 sport fishermen in South America (Carosfeld et al., 2003 43 tial for aquaculture, S. brasiliensis is also highly appreciated in gastron-ΔΔ omy (Fracalossi, Zaniboni Filho, & Meurer, 2002). The dorado is a large, 45 carnivorous and migratory species that carrel over 400 km during 46 3 its reproductive period (Hahn et al., 2011) 47 tions have even been recorded as reaching 1,000 km (Petrere, 1985). 48 49 Salminus brasiliensis populations are vulnerable to an indiscriminate fishing concentration effort, as well as also threatened by a range of 50 51 environmental changes, either natural or arising from multiple human 52

uses of the river systems they inhabit. There has been a marked reduction in the overall population size in recent years (Agostinho, Gomes, & Pelicice, 2007) and considered vulnerable to extinction in many major river basins within Brazil, including the Grande and Tietê rivers (Rosa & Lima, 2008), the Uruguay River basin (Marques et al., 2002; Rio Grande do Sul. Decree n. 41.672, 11 June 2002), and the Paraná, Paranapanema, and Iguaçu rivers and their tributaries (Agostinho et al., 1999; Reis, Kullander, & Ferraris, 2003; Abilhoa & Duboc, 2004; Agostinho et al., 2007; Paraná. Portaria IAP n. 211, 19 October 2012). Fishing for dorado was recently banned indefinitely in the Upper Paraguay basin in the area covered by the state of Mato Grosso, Brazil (Mato Grosso. Law n. 9.893, 1 March 2013). Prior to the harvest moratorium (ca. February 2013), a minimum capture size of 65 cm was in place for the species (Mato Grosso. Law n. 9.096, 16 January 2009).

In neotropical rivers, migratory fish reproduction usually occurs within the upper reaches between October and March, being the period of greatest flooding and highest temperatures (Agostinho et al., 2008; Bailly, Agostinho & Suzuki, 2008; Carolsfeld et al., 2003; 4 Godinho and Kynard, 2009 coler, 1996). Reproductive seasonality **S**

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 is the result of the interaction of endogenous and abiotic factors (temperature, photoperiod, rainfall and water level), which act as triggers
 to stimulate gonad maturation (Barbieri et al., 2000; Carolsfeld et al., 2003; Suzuki, Vazzoler, Marques, Lizama, & Inada, 2004).
 Therefore it is of great importance to review the mechanisms that

6 regulate the populations of those important species for fishing in the 7 Cuiabá River region, especially with regard to declining populations, 8 since the conservation of a given aquatic biological resource requires 9 knowledge of its biological, behavioral and environmental variables that are important in determining how a given population survive 10 ۳d 11 7 reproduces in different environments (Danzmann et al., 1991). Studies of fish reproduction based on gonadal maturation analyses 12 13 and quantitative indicators (length at first sexual maturity) have often 14 been used to understand the ecological role of a species within a given environment (Hutchings, 2002; Vazzoler, 1996; Vincentini & Araújo, 15 16 8 2003). Biological knowledge is essential for the conservation and management of the species, thus a monitoring program is necessary to 17 18 measure biological variables to aid in the conservation of fish stocks 19 and to support the fisheries policy of the region. Hence, the aim of this study was to analyse the reproductive biology of S. brasiliensis from 21 the northern part of the Pantanal, through the determination of life 22 history trajectories (length at first maturity, fecundity and spawning 23 type) and the reproductive period, and thus establish the relationships 24 between biotic and abiotic factors that might act as potential triggers 25 for maturation and spawning.

2 | METHODS

2.1 | Study area

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31 The Cuiabá River basin, one of the most important in formation of the basin of the Upper Paraguay (BUP), covers an area of approximately 28,732 km², stretching 828 km from its source in the municipality of Rosário Oeste to the vicinity of the city of Barão de Melgaço (Pantanal 35 Norte, Mato Grosso). Main tributaries are the Cuiabá da Larga and Cuiabá do Bonito rivers. After the confluence of these rivers, the river 37 is then known as the Cuiabazinho, which flows into the Manso River, and then is known as the Cuiabá (Severi, 1999). Its main tributaries are 39 the Coxipó, Aricá-Mirim, Itiquira, Aricá-Açu, São Lourenço and Manso 40 rivers, the latter being the location of the Manso hydroelectric power plant. The Cuiabá divides into the Upper Cuiabá (which traverses the 41 plateau region and undergoes a considerable altitude change, creating 42 43 several rapids before gradually decreasing in declivity), and the Middle Cuiabá, a post-plateau section that flows across a shallow plain into $\Delta \Delta$ the Pantanal (Cavinatto, 1995). 45

2.2 | Sampling

Monthly surveys were conducted on the Cuiabá River (14°18′17°00′S and 54°40′-56°55′W), between August 2013 and August
2014 with fishhook-type circle hooks for greater efficiency in capture. For each specimen, standard length (SL, cm) and body mass
(M, kg) were recorded. Total length was not applied, as it was not a

reliable metric, plus the caudal fins of many individuals were damaged. The river level and rainfall data were provided by the National Water Agency (ANA) and the Institute for Space Research (INPE), respectively.

2.3 | Gonad processing

After biometrics, the gonads of all specimens were removed and weighed on a precision balance (0.01 g). Developmental stage determinations were prepared using conventional histological techniques; the material was then embedded in resin according to the methodology of Hayat (1993). The gonads were removed and individualized in plastic Eppendorf tubes containing the identical fixative solution for 48 hr. After the fixation procedure the gonads were washed with water, dehydrated in 70% alcohol for 24 hr, 95% alcohol for 4 hr, and 95% alcohol added to plastic resin type glycol methacrylate at a ratio of 1:1 for 8 hr. Finally, they were infiltrated in this plastic resin for 24 hr without a catalyst and included in the same resin added catalyst. The blocks were cut with glass knives to 3 mm thickness; these slices were then collected sequentially with approx. 30 mm space between each slice so that each blade contained 3–5 slices; 1–3 blades per individual were produced and stained by toluidine blue-borax.

Male and female gonadal development stages were defined as: Immature (I, juveniles) individuals that had never spawned; Developing (D) ovaries; Spawning Capable (SC) mature individuals ready to spawn; Regressing (RGS) after the spawning phase; and Regenerating (RGT) sexually mature individuals, but reproductively inactive when captured (see tables 2 and 3 in Brown Peterson, Wyanski, Saborido Rey, 30 Macewicz, & Lowerre Barbieri, 2011).

2.4 | Data analysis

Size class data was grouped into 5 cm class bins to obtain a length frequency distribution during the sampling period. Student's *t*-test was used to assess differences in SL between the sexes. Differences in sex ratios were tested with a chi-squared test (χ^2).

Reproductive periodicity was established via monthly identification of maturation stage relative frequency, with only adults considered (D, SC, RGS and RGT), and an analysis of the monthly gonadosomatic index values (GSI = $GM \times 100/M$, where: GM = gonad mass; M = body mass (de Vlaming, Grossman, & Chapman, 1982). A non-parametric analysis (Kruskal-Wallis *H*-Test) was used for comparisons between months for the GSI when considering the presence of outliers. The relationships among adults (D, SC, RGS and RGT) and abiotic data (river level and rain) were examined graphically using the monthly relative frequency.

To determine spawning type, the diameters of 10 oocytes from females were measured using LAS 2.4 software (Leica Application Suite) and frequency distributions performed relative to oocyte diameter within the sampling periods. This allowed how females release mature oocytes during the spawning season to be established.

A gravimetric method (Hunter, Lo, & Leong, 1985; Kjesbu & Holm, 10 1994) was used to estimate fecundity using the 10 mature ovaries with 1

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the highest gonad development stage. A sub-sample was removed from each ovary and weighed. The oocytes contained within the subsamples were dissociated in Gilson solution, counted, measured, and the total number of oocytes in the ovary estimated. Oocytes were counted according to the method of Ivankov (1985). Relationships among fecundity, standard length, body weight and gonad weight were tested with linear regressions.

To estimate average length at first maturation (L_{50}), adult distribution ratios were constructed for each standard length class including all adult specimens (D, SC, RGS and RGT), and the data then adjusted to the logistic model (King, 2007):

$$p = \frac{1}{1 + e^{\alpha(\text{SL50-SL})}}$$

where: p = ratio (number of adults/total number of individuals); α = relation intercept; SL₅₀ = length at first maturity; SL = standard length. Parameters α and SL₅₀ were estimated by nonlinear regression (King, 2007). To estimate the length at first maturity (L₅₀) represented by the total length (TL) of individuals, a linear regression was performed between SL and TL and the model used to estimate TL predicted.

Statistical analyses used R software (R Core Team, 2014). Significance was determined at α = 0.05 and used in all analyses. The assumptions of parametric tests were evaluated by graphical analyses of waste and the data expressed as a mean ± standard deviation.

3 | RESULTS

A total of 121 specimens were analysed. Of these, 50 were males and 71 females, with a significant difference in the sex ratio (1:1.4, $\chi^2 = 3.64$, df = 1, p = .05). Standard length (SL) ranged from 31 to 67 cm (51.6 ± 9.00). Females ranged from 31 to 67 cm, and males from 31.5 to 65 cm. There was a significant difference (t = 3.19, df = 118.7, p < .01) between the mean length of females (53.61 ± 9.53) and males (48.79 ± 7.04) (Figure 1).

Sixty-three adult and eight juvenile (immature) females, plus 46
adult and four immature males were analysed. Adults were more prevelant than juveniles during the entire sampling period. For females,
those at the regenerating gonadal stage (RGT) were most common,
while mature male individuals (SC) were more common.

42 Mature females (SC) were present from August to December, 43 while individuals with spent gonads were present between January 44 and April, and immature individuals were captured in September and 45 October [Figure 2a,b]. Mature males were present from August to 46 January, while individuals with regenerating gonads (RGT) were only 47 captured from May to July [Figure 2c,d].

There was a relationship between the river water level and maturity stages (D, SC, RGS and RGT) of females and males (Figure 2a,c, respectively). The highest river level (March) coincided with females in regeneration and males in regression (RGS). The highest frequencies of females and mature males coincided with high rainfall levels (October, November, December; Figure 2b,d). From March to August, females in



FIGURE 1 Absolute frequency of females (white) and males (black) by standard length (SL) (5 cm), and boxplot (insert) of the standard length in relation to sex for *Salminus brasiliesis*, Cuiabá River, August 2013 to July 2014. Boxplot: horizontal line within box = median; boundaries of the box = first and third quartiles (*interquartile range IQR*); bars = denote lower and upper inner fences

reproductive pause were predominant (RGT), while males in this condition were common only between May and July.

Monthly gonadosomatic index (GSI) values were significantly different for females (M = 31.32, df = 11, p < .05) and males (M = 24.03, df = 8, p < .05); both sexes showed a greater variation in the GSI between October and January [Figure 3a,b].

Absolute fecundity values as the number of oocytes potentially expellable at spawning showed variations between 257,653-816,023 ($457,747 \pm 194,060$; N = 10). Standard length of analysed females varied between 51.5 and 63 cm, and body weight from 3.35 to 6.70 kg. Gonad weight ranged from 121.8 to 300.8 g. Fecundity was positively related to standard length, body weight and gonads mass (Figure 4), demonstrating an increase in fecundity in proportion to body size.

Combining adult and juvenile individuals, 993 oocytes from 71 females ranged from 62.4 to 1215.3 μ m (342.6 ± 258.4). For most months the relative frequency distribution of the oocyte diameter was concentrated in the smaller sizes, most notably from March to July. In August, this distribution shifted to intermediate widths. From September to January, the relative frequency distribution in the oocyte diameter was most common in larger sizes, exhibiting two modes and indicating that spawning was complete, with the distribution of smaller oocytes representing the stock of reserves, while larger sizes were mature oocytes (Figure 5). Histologically, the ovaries had oocyte development characteristics typical of the 'synchronous in two groups,' type—i.e. simultaneous signs of two batches of oocytes in the ovary, the definition of 'reserve stock oocytes' being those that will mature synchronously and are eliminated upon spawning.

The first maturation length at which at least 50% of individuals already reproduced (SL_{50}), indicated no difference between males and females, with overlapping confidence intervals (Cl). Females had a SL_{50} of 38 cm (Cl: 37.9–38.3 cm) and SL_{100} 44 cm [Figure 6a]. For males this was 37 cm (Cl: 36–38 cm) SL_{100} and 45 cm [Figure 6b]. Combined male and female SL_{50} was estimated at 37 cm (Cl: 36.7–37.7 cm), and



FIGURE 2 Relative monthly frequency of maturation stages in (a) females (b) males [(c and d)] in relation to changes in water level [(a and c)] and precipitation level [(b) and (d)], Cuiabá River, August 2013 to July 2014. Gonadal development stages: Immature (I); Developing (D); Spawning Capable (SC); Regressing (RGS) and Regenerating (RGT)

for all individuals that had already reproduced (SL₁₀₀) this was approx. 48 cm [Figure 6c].

The relationship between the total length (TL) and standard length (SL) was linear (Female: a = 3.69, b = 1.08, $r^2 = 0.95$, p < .05; Male: a = 3.64, b = 1.06, $r^2 = 0.95$, p < .05, both sexes: a = 3.04, b = 1.09, $r^2 = 0.95$; p < .05). By linear regression analysis, the equivalent values for the average size at first maturity for total length (TL₅₀) were (females): TL₅₀ = 45 cm (Cl: 40-49.7 cm); TL₁₀₀ = 52 cm; (males): TL₅₀ = 43 cm (Cl: 37.6-48 cm); TL₁₀₀ = 52; and (both sexes): TL₅₀ = 43.5 cm (Cl: 40-46.5 cm); TL₁₀₀ = 55.5 cm.

4 | DISCUSSION

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Aspects that describe the reproductive strategy of *S. brasiliensis* in the Cuiabá River basin are mainly related to rainfall, as can be seen by the synchronization between floods and major life cycle components, such as the time of maturation and spawning. The species displayed seasonal reproduction in timing of spawning, and the reproductive potential correlated positively with the length and mass in the gonads of mature females.

51 Females were larger and more common, resulting in a significant 52 bias in sex ratio. This sexual dimorphism in relation to variation in 53 II body size between sexes is common in fish species (Nikolsky, 1969) with females usually dominating the larger size classes. This is possibly related to a tactic linked to reproduction, since fertility increases with the length of the individual tinho and Júlio, 1999; Wootton, **12 1991**), increasing reproductive success (Vazzoler, 1996). For the sex ratio, several factors may impact the sexes differently, including mortality, growth and behavior (Mazzoni & Caramaschi, 1995; Vazzoler, 1996). Alternatively, when the species suffers intensive fishing, such variations can occur between populations of the same species, as well as in different periods within a population (Nikolsky, 1969).

Salminus brasiliensis has a seasonally defined reproductive period from August to January. The early maturation and increased gonadosomatic index (GSI) values coincide with the first rains. In their study in the Pantanal floodplain, Bailly et al. (2008) observed that regardless of the reproductive strategy, the spawning peaks precede the flooding peaks, showing that the association of reproduction with the onset of an increase in the water level is a standard common to most members of the Pantanal ichthyofauna. However, the duration of spawning responds to interannual variations in the water cycle, varying across groups with different reproductive strategies (Bailly et al., 2008; Vazzoler, 1996; Winemiller & Rose, 1992). The effect of the flood pulse triggers the connection between stream and marginal lentic ecosystems (Junk, Bayley, & Sparks, 1989), providing the conditions necessary for juvenile stage survival, by ensuring food availability, protection from predators, as well as favorable abiotic conditions 1

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FIGURE 3 Monthly changes in gonadosomatic index (IGS) for *Salminus brasiliesis* (a) females and (b) males, Cuiabá River, August 2013 to July 2014. Horizontal line within the box = median; boundaries of the box = first and third quartiles (*interquartile range IQR*); bars = denote lower (first quartile – 1.5 × *IQR*) and upper (third quartile + 1.5 × *IQR*) inner fences; circles = outliers

30 13 (Lowe McConnel, 1999; Mazzoni & Iglesisas Rio, 2002; Nakatani
 31 14 et al., 2001).

The increase in oocyte production ensures the survival of the species 33 as well as a balanced population structure able to overcome environmental adversities (Lowe-McConnel, 1999). The way in which females 34 released oocytes within the analysed spawning season shows that the dorado has a seasonal reproductive period, characterised by the expul-37 sion of a single batch of mature oocytes in a relatively short time inter-38 val, while maintaining a reserve batch that will mature later for use in 39 the next reproductive period (Vazzoler, 1996; Winemiller & Rose, 1992). 40 Species with this type of reproduction invest more in their total repro-41 ductive effort than in the offspring themselves, as they have the advan-42 tage of being able to migrate to the areas most favorable to spawning 43 and, by releasing abundant oocytes (Godinho, Lamas, & Godinho, ΔΔ 2009), can enhance the juvenile survival rates (Winemiller, Agostinho, 45 & Caramaschi, 2008). However, this strategy becomes a major drawback when rivers are dammed, since such species require geographically dis-46 47 tinct habitats to complete their life cycle (Agostinho et al., 2007).

Sexual maturation in fish occurs along a trajectory of size and age that is dependent on the demographic conditions, which themselves are determined by genes and the environment (Stearns & Crandall, 1984). TL₅₀ is the length at which 50% of the population is sexually mature and, therefore, is the smallest size at which the species begins to replace itself (Agostinho et al., 2007). Considering



FIGURE 4 Relationship by maturation stage between fertility (number of oocytes) and (a) Standard length (cm); (b) Body weight (g); (c) Gonad mass (g) for *Salminus brasiliesis*, Cuiabá River, August 2013 to July 2014. Black points = individuals

that the current study estimates the TL_{100} to be 55.5 cm, legally captured fish with a minimum landing size of 65 cm should have already reproduced at least once. Barbieri et al. (2004), comparing **15** the reproductive strategies of *Salminus maxillosus* between 1946 and 1996 to 1997 in Mogi Guaçu in Sao Paulo, concluded that there were changes in some reproductive tactics, such as reducing the size (Lpm) and age (lpm) of first gonadal maturation, reduction in the average maximum length of specimens (L ∞) and minimum size of adult specimens captured (Lmc) as a result of intense overfishing and abiotic adversities.

Regulations based on adopting only minimum size requirements for capture have the effect of removing either larger and older fish, or those that are genetically predisposed to rapid growth and late maturation (Berkeley, Hixon, Larson, & Love, 2004). This results in selection pressure within populations that favor the survival of those fish that mature at an earlier age and invest more in their reproduction at a smaller size (Berkeley et al., 2004). However, minimum and maximum



FIGURE 6 Distribution of the proportion of adult *Salminus brasiliensis* (a) adult females (b) adult males and (c) both sexes by default L_s length class, Cuiabá River, August 2013–July 2014. SL₅₀ = length wherein at least 50% of individuals already reproduced; SL₁₀₀ = length at which all individuals already reproduced

limits can also favor the survival of a greater number of large specimens, and thus maintain genetic variability.

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Overall, data from this study made it possible to investigate some of the reproductive abilities of *S. brasiliensis* in the Cuiabá River with a view to promoting the species' survival. The data may be applied to future models of inventory evaluation, and cab also be used in the guidance for laws, administrative measures, management and preservation. Due to the lack of scientific studies on fish stocks before implementation of the dorado fishing ban in Mato Grosso, it is not possible to state whether the previous fishing effort concentration on schools of dorado was indiscriminate, yet this is one of the principal factors that can cause changes in reproductive strategies (Lowe-McConnel, 1999). However, the reproductive strategies of this species can also be influenced by various natural environmental changes, or by one or more of a suite of multiple uses to which the Cuiabá River basin systems are subjected. 2

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In this context, it is essential that there is regular (preferably annual) monitoring of these key biological variables, so that the stock status, reproductive capacity and recruitment can be assessed regularly, enabling regional fisheries policy decisions to be based on scientifically supported evidence and not on arbitrary guesswork. Knowledge of the population status is essential for effective conservation and fisheries management of any species. A set of factors in the dorado, including its popularity in professional and sport fishing, high commercial value, ecological importance (top-down regulation) and the degradation of the aquatic environment entails an urgency, whereby it is essential that the knowledge gaps concering the life cycle and ecology of S. brasiliensis are identified. This will ensure that future conservation initiatives are based on scientifically analysed biological data, not on mere speculative assumptions.

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