**Pontifícia Universidade Católica de Minas Gerais**

**Programa de Pós Graduação em Zoologia de Vertebrados**

# Reprodução do peixe cachorro *Acestrorhynchus lacustris* (Lütken, 1875) em dois trechos do rio São Francisco, a jusante da barragem de Três Marias, Minas Gerais.

**Rafael Zeferino Gomes**

**Belo Horizonte**

**2013**

**RAFAEL ZEFERINO GOMES**

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Dissertação apresentada ao Programa de Pós Graduação em Zoologia de Vertebrados da Pontifícia Universidade Católica de Minas Gerais como requisito parcial para obtenção de título de mestre em zoologia.

**Orientador: Prof. Dr. Nilo Bazzoli**

**Belo Horizonte**

**2013**

FICHA CATALOGRÁFICA
Elaborada pela Biblioteca da Pontifícia Universidade Católica de Minas Gerais

Gomes, Rafael Zeferino
G633r Reprodução do peixe cachorro *Acestrorhynchus lacustris* (Lütken, 1875) em dois trechos do rio São Francisco, a jusante da barragem de Três Marias, Minas Gerais / Rafael Zeferino Gomes. Belo Horizonte, 2013.
35 f. : il.

Orientador: Nilo Bazzoli
Dissertação (Mestrado) – Pontifícia Universidade Católica de Minas Gerais. Programa de Pós-Graduação em Zoologia de Vertebrados.

1. Peixe - Reprodução. 2. Peixe - Ovos. 3. Três Marias, Reservatório de (MG). 4. São Francisco, Rio. I. Bazzoli, Nilo. II. Pontifícia Universidade Católica de Minas Gerais. Programa de Pós-Graduação em Zoologia de Vertebrados. III. Título.



CDU: 597.5

"Reprodução do peixe cachorro *Acestrorhynchus lacustris* (Lütken, 1875) em dois trechos do rio São Francisco, a jusante da barragem de Três Marias, Minas Gerais."

**Rafael Zeferino Gomes**

**Defesa de Dissertação em 22/03/2010**

**Resultado:**

**Banca examinadora:**

**Profa. Dra. Patrícia Massara Martinelli (UFMG)**

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**Prof. Dr. Ralph Gruppi Thomé (UFJF)**

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**Prof. Dr. Nilo Bazzoli (Orientador-PUC Minas)**

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Dissertação apresentada ao Programa de Pós Graduação em Zoologia de Vertebrados da PUC Minas, desenvolvida sob orientação do professor Dr. Nilo Bazzoli e com o apoio das seguintes instituições:

- Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES);

- Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq);

- Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG);

- Companhia de Desenvolvimento dos Vales do São Francisco e do Parnaíba (CODEVASF).

**AGRADECIMENTOS**

- Aos meus pais, Sônia e Fernando pelo total suporte na vida, a quem dedico a conquista;

-Ao meu irmão Rodrigo e sua família, Júnia, Pedro e César, sempre presentes;

- A Elisa pelo companheirismo;

-A toda minha família;

- Aos amigos de Itabira;

-Professor Dr. Nilo Bazzoli pela orientação e formação profissional,

-Aos amigos e parceiros de projeto: André Weber e Diego Nunes;

-Kleber Biana na organização das coletas;

-Aos pescadores Antônio Vanderlei e Antônio Luzimar;

-Aos amigos Ictiólogos: Felipe Talin, Yuri Simões, Fábio Arantes, Alessandro, Rafael Melo, Violeta Perini, Marcella Lourenço, Daniel Melo, Paulo Brant, Vanderlei, Thiago Abdo, Bianco Vizzioli, Lourenço Savassi;

-Aos amigos Biólogos: Nathália C, Gabriela Nunes, Rafael Cerqueira, Matheus Rocha, Luiz Bento, Vinícius Orsini, Bianca Rezende, Areta, Alex;

-Aos amigos da turma do mestrado;

-Rogério e Cledma, sempre dando força;

**Resumo**

Ecossistemas aquáticos são impactados em todo o mundo, e dentre impactos, ressaltam-se os barramentos de rios que afetam diretamente a ictiofauna por alterarem as características físico–químicas e vazão da água liberada pelas usinas hidrelétricas. Com objetivo de comparar a reprodução de *A. lacustris* em dois trechos do rio São Francisco, capturaram-se, entre maio de 2011 a junho de 2012, 521 exemplares adultos com auxílio de redes de emalhar de 3 a 8 mm entre nós opostos. O trecho 1 localiza-se logo à jusante da barragem de Três Marias e o trecho 2, na confluência do rio São Francisco com o rio Abaeté. De cada exemplar foram registrados comprimento total (CT), peso corporal (PC) e peso gonadal (PG) para calcular o índice gonadossomático (IGS) e o fator de condição de Fulton (K). Fragmentos de gônadas foram fixados em líquido de Bouin por 8-12 horas e submetidos às técnicas histológicas de rotina. Fragmentos de ovários maduros foram pesados e fixados em solução de Gilson para determinar a fecundidade. O diâmetro dos folículos vitelogênicos (DFV) foi medido em lâminas histológicas sob microscópio de luz acoplado com ocular micrométrica. Os parâmetros físico-químicos da água foram obtidos com sonda Horiba U-10. Peixes capturados no trecho 2 apresentaram maiores valores de CT e PC. Os valores médios de IGS e K de fêmeas e machos não mostraram diferenças estatísticas entre os peixes de cada trecho, bem como os valores médios de DFV e fecundidade em lotes. A presença de folículos pós ovulatórios foi observada nos ovários de fêmeas capturadas nos dois trechos a qual está associada ao longo período de desova. A ocorrência de classes de tamanhos variados de folículos vitelogênicos indicaram que a desova de *A. lacustris* é do tipo parcelado. Os resultados mostraram que *A. lacustris*, diferentemente de outros teleósteos Neotropicais, não tem sua reprodução comprometida pelo impacto da barragem de Três Marias, onde as condições termais e hidrológicas do rio São Francisco estão alteradas pela água do hipolímnio liberada pela usina hidrelétrica.

Palavras chave: teleósteo neotropical, desovas múltiplas, estratégia reprodutiva, longo período reprodutivo.

**Abstract**

Aquatic ecosystems are impacted worldwide, and of these impacts, the rivers damming directly affect fish populations by altering the physico-chemical and discharge of water released by hydroeletric power plants. To compare the reproduction of *A. lacustris* in two differently sections of the river São Francisco, adult specimens were captured between May 2011 to June 2012, 521 adult specimens with the aid of gillnets with 3-8 mm between opposite knots. The first section is located immediately downstream the Three Marias dam, and section 2, located at the confluence of the São Francisco and the Abaeté rivers. Of each specimen were recorded total length (TL), body weight (BW) and gonad weight (GW) to calculate the gonadosomatic index (GSI) and Fulton's condition factor (K). Fragments gonads were fixed in Bouin's fluid for 8-12 hours and subjected to routine histological techniques. Fragments of mature ovaries were weighted and fixed in Gilson fluid in order to determine fecundity. The diameter of vitellogenic follicles (DFV) was measured on histological sections under light microscopy coupled with ocular micrometer. The physico-chemical parameters of water were obtained with probe Horiba U-10. Statistical tests were performed for the variables analyzed. Fish captured in section 2 had higher values of TL and BW. The mean values of GSI and K of females and males showed no statistical differences between fish of each section, as well as the average values of DFV and batch fecundity. The presence of post ovulatory follicles was observed in the ovaries of females captured in the two samples sections which was associated with long spawning period and the occurrence of classes of varying sizes of vitellogenic follicles indicated that spawning of *A. lacustris* is the multiple type. The results showed that *A. lacustris* unlike other Neotropical migratory teleost, has its reproduction not compromised by the impact of the Três Marias dam, where thermal and hydrological conditions of the river São Francisco are altered by water from the hypolimnion released by the hydroelectric power plant.

Key words: dam impact, multiple spawning, reproductive strategy, long breeding season.

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# INTRODUÇÃO

## *Ictiofauna Neotropical*

Entre as regiões zoogeográficas do mundo, a Neotropical, que inclui a América do Sul, abriga o maior número de peixes de água doce do planeta, aproximadamente 4475 espécies (Reis et al., 2003) o que representa cerca de 25 % da ictiofauna mundial (Brito et al., 2007), e desses peixes, a maior parte, cerca de 2587, ocorre em território brasileiro (Buckup et al., 2007). Estes números refletem a grande disponibilidade e variedade de ambientes e a marcante história evolutiva da ictiofauna ao longo do tempo (Brito et al., 2007; Langeani et al., 2009). De modo geral, os ecossistemas aquáticos estão atualmente bastante impactados, incluindo a América do Sul, região Neotropical, e esta condição influi diretamente na sua ictiofauna. Dentre os principais impactos relatados para ecossistemas aquáticos fluviais estão o barramento dos rios, a perda de vegetação, poluição, introdução de espécies exóticas e sobrepesca (Barletta et al., 2010; Welcomme et al., 2006; Abell et al., 2008).

O barramento dos rios causa grande impacto sobre a ictiofauna por constituir separação física entre os organismos e pela transformação de um ambiente lótico em lêntico, com a formação de reservatório e também pela regulação da vazão a jusante (Agostinho et al., 2008; Santos et al., 2012). Dentre os reservatórios, existem aqueles em que ocorre estratificação térmica na coluna d'água com formação de hipolíminio nas camadas mais profundas. Nos reservatórios, a captação de água pode ser realizada no hipolímnio para a geração de energia nas usinas hidrelétricas (UHE´s), e após este processo a água é liberada a jusante das UHE´s com os parâmetros físico-químicos alterados, tais como temperatura, oxigênio dissolvido, turbidez e condutividade elétrica. Esta condição da água é negativa para a biota aquática, especialmente para os peixes, que são ectotérmicos, e tem a temperatura corporal regulada pela temperatura da água (Clarkson & Childs, 2000; Olden & Naiman, 2010).

## *Área de estudo*

A bacia do rio São Francisco é a terceira maior da América do Sul, drenando 645.067 km² e abrangendo vários estados brasileiros. O rio São Francisco nasce na serra da Canastra em Minas Gerais e percorre 3.160 km até a sua foz localizada entre os estados de Alagoas e Sergipe (Godinho & Godinho, 2003; Langeani et al., 2009). A barragem da usina hidrelétrica de Três Marias, localizada no alto rio São Francisco (18°12'51.37"S; 45°15'42.45"W) possui

reservatório com capacidade de armazenar $21\times10^9 m^3$ de água, e inunda uma área de aproximadamente 100.000 ha. Na estação chuvosa (novembro a fevereiro), na qual ocorre estratificação térmica na coluna d´água no reservatório de Três Marias com formação de hipolíminio, que possui como características menor temperatura e menor oxigênio dissolvido nesta camada da coluna d´água. Como a água do hipolíminio é captada para utilização das turbinas da usina hidrelétrica, a água liberada a jusante possui temperatura 2-3° C mais fria do que a água normal do curso do rio (Esteves et al.,, 1985; Santos et al., 2012).

A jusante a barragem de Três Marias, o primeiro tributário de grande porte é o rio Abaeté na margem esquerda, e alguns estudos (Sato et al., 2005, Arantes et al., 2010; Domingos et al., 2012; Thomé et al., 2013) indicam que na região de confluência entre estes rios, há melhores condições para a reprodução de teleósteos migradores, sendo estas condições relacionadas á maior temperatura, oxigênio dissolvido e turbidez da água do rio Abaeté.

***Ciclo reprodutivo, estádios de maturação gonadal e tipo de desenvolvimento ovariano, tipo de desova e espermatogênese***

A reprodução dos peixes é cíclica, correspondendo a um período de repouso intercalado por períodos de atividade sexual os quais finalizam com o surgimento de nova prole. No período de repouso, as gônadas estão com tamanho reduzido, contendo apenas células gametogênicas em fases iniciais de desenvolvimento. Com o progresso do ciclo, elas acumulam ovócitos vitelogênicos ou espermatozoides que serão liberados no momento da reprodução (Godinho, 2007; Schulz et al., 2010). O ciclo reprodutivo dos peixes está vinculado às variações de temperatura e ao regime de chuvas (Bazzoli, 2003). Na região tropical o verão é quente e chuvoso, coincidindo com a estação reprodutiva da maioria dos peixes brasileiros. Assim, a reprodução dos peixes pode ser caracterizada por sazonalidade (Lowe-McConnell, 1987). A duração do período reprodutivo variável entre as espécies, e é relacionada ao tipo de estratégia reprodutiva.

As fases do ciclo reprodutivo podem ser classificadas em estádios de maturação gonadal, baseando-se nas características macroscópicas e microscópicas das gônadas e nas variações do índice gonadossomático durante o ciclo. Para fazer o diagnóstico preciso dos estádios é necessário utilizar a análise histológica como ferramenta, pois a técnica permite identificar os tipos celulares presentes nos ovários e testículos (Bazzoli 2003; Núñez & Duponchelle, 2009).

Existem três tipos principais de desenvolvimento dos ovários: *sincrônico* em que há amadurecimento de todos os seus ovócitos em um único lote; *grupo-sincrônico*, no qual pelo menos dois tipos de ovócitos em fases diferentes de desenvolvimento estão presentes no ovário durante a estação reprodutiva, e *assincrônico*, no qual existem ovócitos em todas as fases de desenvolvimento, sem um tipo predominante (Lowe-McConnell, 1987; Andrade et al., 2001; Godinho, 2007; Núñez & Duponchelle, 2009; Lubzens et al., 2010).

O tipo de desova pode ser total, quando a maior parte dos ovócitos vitelogênicos é ovulada em um único evento ou em um curto período de tempo, ou do tipo parcelado, quando os ovócitos vitelogênicos são recrutados em lotes para a ovulação (Lowe-McConnell, 1987; Andrade et al., 2001; Godinho, 2007; Núñez & Duponchelle, 2009; Lubzens et al., 2010).

De acordo com Schulz et al. (2010) a espermatogênese é um processo complexo, que consiste na proliferação e diferenciação de uma espermatogônia diploide em espermatozóides maduros, com motilidade, este processo pode ser influenciado pela temperatura da água. O túbulo seminífero dos teleósteos é constituído por uma membrana basal e células mióides que abrigam o epitélio germinativo, formado por células de Sertoli e células germinativas. A espermatogênese dos teleósteos é cística, pois as células de Sertoli que circundam os túbulos seminíferos emitem prolongamentos que formam os cistos, envolvendo um grupo de células germinativas em mesma fase de desenvolvimento derivadas de uma única espermatogônia. Nos túbulos seminíferos são encontradas células germinativas em várias fases de desenvolvimento simultaneamente.

***Índice gonadossomático***

Índice gonadossomático, que é a relação entre o peso gonadal e o peso corporal, é um bom indicador da atividade reprodutiva de peixes, podendo ser utilizado na determinação dos estádios de maturação gonadal durante o ciclo reprodutivo, pois o peso gonadal aumenta simultaneamente ao desenvolvimento das células germinativas (Maddock & Burton, 1999; Lubzens et al., 2010).

***Fecundidade***

Fecundidade avalia o potencial reprodutivo de uma espécie, podendo ser estudada através do número de ovócitos vitelogênicos presentes nos ovários antes de iniciar a desova (Bagenal, 1978). Para eliminar a interferência do comprimento total, peso corporal e peso

gonadal, calcula-se a fecundidade relativa. A fecundidade é utilizada para avaliar o potencial reprodutivo das espécies, e pode variar entre indivíduos do mesmo tamanho e peso (Lowe-McConnell, 1987), e também em função da temperatura da água, disponibilidade de alimento e condição intrínseca de cada fêmea (Yoda & Yoneda, 2009; Arantes et al., 2010). Espécies que possuem longo período reprodutivo, com múltiplas desovas e fecundidade em lotes, a estimativa do potencial reprodutivo é comumente obtida contando-se o número de ovócitos vitelogênicos produzidos por lote (Yoda & Yoneda, 2009).

***Diâmetro folicular***

Vários estudos relacionam diâmetro dos folículos ovarianos com fecundidade, cuidado parental e migrações reprodutivas, sendo parâmetro importante porque a qualidade do gameta é diretamente ligada ao seu volume, avaliado pelo diâmetro (Suzuki et al., 2000; Kolm & Ahnesjö, 2005). O diâmetro folicular pode ser alterado quando os peixes estão sob condições adversas, influenciando negativamente o *fitness* reprodutivo, constituindo um parâmetro importante na avaliação de distúrbios reprodutivos (Yoda & Yoneda 2009; Arantes et al., 2010).

***Folículos pós ovulatórios***

Folículos pós ovulatórios são remanescentes dos folículos ovarianos após ovulação, sendo constituídos de lume, onde havia um folículo vitelogênico, delimitado pelas células foliculares e teca conjuntiva, esta estrutura é importante pois indica que ocorreu ovulação, e por conseqüência pode-se inferir que a espécie apresenta atividade reprodutiva completa, com desova (Drummond et al., 2000).

***Espécie em estudo***

A família Acestrorhynchidae da ordem Characiformes possui um único gênero, *Acestrorhynchus,* e 15 espécies de peixe cachorro popularmente conhecidas e identificadas. Estas espécies ocorrem nas bacias dos rios Amazonas, Orinoco, São Francisco, Paraná e Paraguai (Menezes, 2003). Esta família tem morfologia cônica de todos os dentes e possui fortes caninos na premaxila, região anterior da maxila e no dentário. O padrão de dentição dos Acestrorhynchidae torrna-os predadores especializados, utilizando os peixes como alimentação principal (Menezes, 2003; Silva & Goitein, 2009). O peixe cachorro *Acestrorhynchus lacustris* (Lütken, 1875) ocorre nas bacias dos rios São Francisco e Alto Paraná, e pode atingir 27 centímetros de comprimento padrão. O peixe cachorro é importante elo na manutenção da cadeia alimentar da ictiofauna onde ocorre, servindo de alimento para

os grandes peixes piscívoros de interesse comercial (Hahn et al., 2000; Silva & Goitein, 2009).

**Figura 1**: Exemplar de *Acestrorhynchus lacustris* (Lütken, 1875) com 18 cm de comprimento total, capturado no rio São Francisco, a jusante da barragem da usina hidrelétrica Três Marias.

## OBJETIVOS

### *Objetivo geral*

Verificar se ocorrem diferenças na reprodução de *A. lacustris* em dois trechos do rio São Francisco à jusante da barragem de Três Marias, através de analises comparativas dos principais parâmetros reprodutivos da espécie, capturada em cada trecho.

### *Objetivos específicos*

- Obter os parâmetros físico-químicos da água;
- Determinar os valores de comprimento total e peso corporal de machos e fêmeas nos dois trechos do rio;
- Determinar os estádios de maturação gonadal, e respectivas frequências, época de reprodução e tipo de desova;
- Calcular o índice gonadossomático e o fator de condição de Fulton;
- Determinar em lâminas histológicas o diâmetro e o índice de atresia dos folículos vitelogênicos;
- Calcular os valores da fecundidade absoluta e relativa ao peso corporal;
- Comparar comprimento total, peso corporal, índice gonadossomático, fator de condição de

Fulton diâmetro dos folículos vitelogênicos, fecundidade absoluta, fecundidade relativa e parâmetros físico-químicos da água entre os dois trechos estudados.

**ARTIGO A SER SUBMETIDO**

## REPRODUCTION OF THE DOGFISH *Acestrorhynchus lacustris* IN TWO SECTIONS OF THE SÃO FRANCISCO RIVER, DOWNSTREAM FROM THE TRES MARIAS DAM, SOUTH-EASTERN BRAZIL


### Abstract

Aquatic ecosystems are impacted worldwide, and among the impacts, the rivers damming directly affect fish populations by altering the physico-chemical and discharge of water released by hydroeletric power plants. To compare the reproduction of A. lacustris in two differently sections of the river São Francisco, 521 adult specimens adult specimens were captured between May 2011 to June 2012, with the aid of gillnets with 3-8 mm between opposite knots. The first section is located immediately downstream the Three Marias dam, and section 2, located at the confluence of the São Francisco and the Abaeté rivers. Of each specimen were recorded total length (TL), body weight (BW) and gonad weight (GW) in order to calculate the gonadosomatic index (GSI) and Fulton's condition factor (K). Fragments of gonads were fixed in Bouin's fluid for 8-12 hours and subjected to routine histological techniques. Fragments of mature ovaries were weighted and fixed in Gilson fluid in order to determine fecundity. The diameter of vitellogenic follicles (DFV) was measured on histological sections under light microscopy coupled with an micrometric ocular lens. The physico-chemical parameters of water were obtained with probe Horiba U-10. Statistical tests were performed for the variables analyzed. Fish captured in section 2 had higher values of TL and BW. The mean values of GSI and K of females and males showed no statistical differences between fish of each section, as well as the average values of DFV and batch fecundity. The presence of post ovulatory follicles was observed in the ovaries of females captured in the two samples sections which was associated with long spawning period and the occurrence of classes of varying sizes of vitellogenic follicles indicated that spawning of *A. lacustris* is the multiple type. The results showed that *A. lacustris* unlike other Neotropical migratory teleost, has its reproduction not a compromised reproduction due to the impact of

the Três Marias dam, where thermal and hydrological conditions of the river São Francisco are altered by water from the hypolimnion released by the hydroelectric power plant.

**Introduction**

River damming is considered to be one of the main impacts on the aquatic riverine ecosystems, due to the fact that it alters the physical and chemical parameters of the water and control the released discharge downstream, influencing the whole aquatic communities (Abell et al., 2008; Agostinho et al., 2008; Donaldson et al., 2008; Olden & Naiman 2010). Because fish are ectothermic, the cooler water temperature downstream from the reservoirs may affect their reproductive activity, altering gonadal development, ovarian follicle diameter and fecundity, besides impairing growth (Lukšienè & Svedang 1997; Gray et al., 2000; Kolm, & Ahnesjö 2005; Armstrong & Witthames 2012). In studies of fish reproduction, sampling sites are important since the reproductive activity may vary depending on immediate responses to the environmental conditions, thus allowing the comparison of populations of certain species from different locations (Gray et al., 2000).

Most species of the Neotropical ichthyofauna are sedentary and present a long reproductive period, multiple spawning and batch fecundity (Winemiller 1989; Godinho, Lamas & Godinho 2009), but few studies on Neotropical rivers assessed the impact of dams on the reproduction of sedentary fish (Donaldson et al., 2008).

The species *Acestrorhynchus lacustris* (Lütken, 1875) occurs in the basins of the Paraná and São Francisco Rivers, Brazil, and can reach 27 centimetres in standard length. The dogfish is an important link in the food chain, serving as food for large piscivorous fish of commercial interest (Hahn et al., 2000; Silva & Goitein 2009).

Same studies (Sato et al., 2005, Arantes et al., 2010; Domingos et al., 2012; Thomé et al., 2012) show that the reproduction of migratory teleosts is compromised in São Francisco River just downstream from the Três Marias Dam where the temperature and dissolved oxygen are lower in the summer, related to thermal stratification of the reservoir at this time of year. Downstream of the Três Marias dam the first large tributary is the Abaeté river, and the same studies (Sato et al., 2005, Arantes et al., 2010; Domingos et al., 2012; Thomé et al., 2012) suggest that in this region of the São Francisco River, influenced by the water characteristics of the tributary there are better conditions for the reproduction of migratory teleosts, these conditions are the higher temperature, dissolved oxygen and turbidity, of Abaeté river water.

Studies of the reproductive biology of this species in lotic environments influenced of a dam have not yet been undertaken. Therefore, this study aimed at assessing the reproduction of the dogfish *A. lacustris* in two sections of the São Francisco River downstream from the Três Marias dam, south-eastern Brazil.

## Material and Methods

### *Sampling*

Specimens of *A. lacustris* were captured between May 2011 and June 2012 on two sections of the São Francisco River: section 1, just downstream of the Três Marias dam (18°11'3.75"S, 45°14'51.54"W) and section 2, downstream of the dam, on the region of confluence with Abaeté river (18°2'3.94"S,45°11'0.83"W). The specimens were captured using gillnets with 3-8 centimetres between opposite knots. The nets were placed in the river late afternoon and removed early the following morning, totalling 12 hours of exposure. The specimens were immediately transported to the Três Marias Hatchery and Hidrobiology Station – CODEVASF. The fish, if alive, were killed by transversal section of the cervical medulla, following the ethical principles of animal handling established by the Brazilian College for Animal Experimentation (COBEA). For each specimen, the following parameters were recorded: total length (TL), body weight (BW) and gonadal weight (GW). While in the field, fragments of gonads were collected and fixed in Bouin's fluid for 8-12 hours for routine histological processing. Fragments of 40 mature ovaries were also weighed and fixed in Gilson's fluid for posterior determining fecundity after the dissociation of the ovarian follicles.

### *Stages of gonadal maturation, follicular diameter, biological indices and body-weight ratio*

The gonadal maturation stages were determined by observing the macro and microscopic features of gonads and the changes in the gonadosomatic index according to Carvalho et al., (2009). For microscopic analyses, fragments of gonads, previously fixed in Bouin's fluid, underwent routine histological techniques, embedding in paraffin, 5 µm thick microtome sections and haematoxylin-eosin (HE) stain.

The diameters of 50 vitellogenic follicles (VF) were obtained from histological slides using an ocular micrometer attached to a light microscope of mature ovaries for 10 specimens from each section of the river being studied. Only intact vitellogenic follicles, with little shrinkage and with the nucleus visible, were measured.

From each specimen, we determined: the gonadosomatic index (GSI= GW×100/BW and the length-weight relationship (BW= a. $TL^b$).

*Fecundity*

In order to determine the batch fecundity, samples from the middle region of mature ovaries of 20 females of each section of the river were fixed in modified Gilson's solution (100 ml of 60% ethanol, 880 ml distilled water, 15 ml of 80% nitric acid, 18 ml of glacial acetic acid and 20 g of mercury chloride) until the complete dissociation of the follicles. With the aid of a stereoscopic microscope, the vitellogenic follicles were visibly separated from the others by their opaque color and counted. The batch fecundity (BF) was determined by the expression: BF= FV×GW, in which FV = number of vitellogenic follicles per ovary gram and GW= gonadal weight. Relative fecundity is estimated by the expression: RF= BF/BW, where BF= batch fecundity, BW= body weight.

*Physical and chemical parameters of the water*

The physical and chemical parameters of the water of the two sections of the study were recorded on the same day of fish samples, during the rainy season (November to February) and the dry season (March to October). The following variables were analysed: pH, temperature, dissolved oxygen concentration and electric conductivity, taken with a Horiba U-10 probe. The water discharge values were supplied by Companhia Energética de Minas Gerais (CEMIG).

*Statistical Analyses*

We conducted Lilliefors normality test for all studied parameters. In order to analyse the variables between the sections, we used for independent samples the T-student test for the parametric data and the Mann-Whitney test for nonparametric data. For GSI comparison between the maturation stages, we used the Kruskal-Wallis test, followed by the post hoc Dunn test. Diameter classes of the vitellogenic follicles were verified through the one-way ANOVA test, followed by the post hoc Dunn test. A significance level of $P < 0.05$ was used for all analysed tests.

Biometric data, GSI, fecundity, K, vitellogenic follicles diameters, maturation stages and abiotic factors are expressed in tables and graphs with mean ± standard deviation.

**Results**

*Physical and chemical parameters of the water*

The parameters: temperature, dissolved oxygen and discharge presented higher mean values for section 2, with significant statistical differences in relation to the values of section 1 (Table 1).

*Biometrics*

A total of 521 specimens were captured, of which there were 127 females and 116 males from section 1 and 134 females and 144 males from section 2. Females and males of section 2 showed statistically higher values of total length and body weight, compared to those captured in section 1 (Table 2 and 3). The length-weight relationship between fishes of the sections for females and males presented a higher correlation for fish caught in section 2. The value of the parameter b of the length-weight ratio was less than 3 for fish of the two sections, showing a negative allometric growth (Figure 2 A, B, C, D).

*Gonadal maturation stages*

The following maturation stages were identified for females: F1= resting: thin, translucent and less vascularised ovaries with follicles initial (O1) and advanced (O2) perinucleolar (Figure 3A); F2= initial maturation: voluminous, orange ovaries, and histologically containing O1, O2 and previtellogenic follicles (O3) (Figure 3B); F3= advanced maturation/mature: ovaries of maximum volume, with numerous vitellogenic follicles (O4) visible to the naked eye, dark brown in colour, besides follicles at all developmental stages (Figure 3C); F4A= partially spawned: flaccid, haemorrhagic ovaries with follicles at all developmental stages, besides post-ovulatory follicles (Figure 3D) and F4B= totally spawned ovaries: very flaccid and haemorrhagic, histologically containing O1, O2, O3, post-ovulatory follicles and residual yolk (Figure 3E).

The following maturation stages were identified for males: M2= early maturation, whitish testicles, with open lumen, containing a small amount of spermatozoa and seminiferous tubules wall with cysts of spermatogenic cells in all developmental stages (Figure 4A). M3= advanced maturation/mature, testes reach the maximum volume, are milky-white in colour and presenting the lumen of seminiferous tubules filled with spermatozoa (Figure 4B). M4A= partially spent, testicles are flaccid, haemorrhagic, with translucent and

milky-white areas, seminiferous tubules' lumen partially empty, containing few spermatozoa (Figure 4C). Early maturing males (M1) and totally spent ones (M4B) were not captured.

*Frequency of the maturation stages*

Females and males in reproductive activity, stages 2, 3, and 4A, were recorded throughout the year in both sampling sections (Figure 5 and 6). Through variance analysis, four classes of vitellogenic follicle diameters with statistical differences between the classes were observed, indicating asynchronous growth of the follicles (Figure 7). The long reproductive period and the presence of spawned females with post-ovulatory follicles and follicles in all developmental stages indicate that the species present batch spawning of the multiple type.

*Gonadosomatic index:*

The mean gonadosomatic index (GSI) of females and males did not present statistical differences between stages 3 and 4A (Figure 8). Comparing the two sampling sections, the mean GSI values by each maturation stages were not statistically different (Figure 8).

*Histometry*

The vitellogenic follicles of *A. lacustris* collected in section 1 had an average diameter of 768.1 ± 67.4 µm and, in section 2, 765.3 ± 82.3 µm, and no statistical difference between them (Table 2 - lowercase) was observed.

*Fecundity*

The batch fecundity (BF) (table 2) averaged 3712.2 ± 2615.5 vitellogenic follicles in fish caught in section 1 and 3710.6 ± 1821.2 in the fish of section 2, with no significant statistical differences between fish of each section. Relative fecundity (RF) to body weight averaged 41.5 ± 22.3 follicles per gram of fish from section 1 and 40.5 ± 20.5 for section 2, with no statistical differences between sections. The relative fecundity to body weight showed that the heavier specimens presented lower relative fecundity. The highest Pearson correlations for fecundity occurred between batch fecundity and gonadal weight followed by the gonadosomatic index in fish caught in section 2.

**Table 1:** Temperature, dissolved oxigen, pH, conductivity and discharge of 2 sections downstream of the Três Marias dam, on São Francisco river. Different letters represents statistically differences ($p<0.05$) between parameters on the samples sections.

| | Section 1 | | Section 2 | |
|---|---|---|---|---|
| | Mean ± SD | Range | Mean ± SD | Range |
| Temp. (°C) | 23.21 ± 0.57[a] | 22.68 - 24.00 | 24.31 ± 0.71[b] | 23.58 - 25.40 |
| Oxygen (mg/L) | 5.55 ± 1.67[a] | 4.13 - 8.12 | 7.97 ± 0.55[b] | 7.33 - 8.65 |
| pH | 5.83 ± 0.46[a] | 5.12 - 6.24 | 6.35 ± 0.51[a] | 5.62 - 7.02 |
| Cond. (µS/cm) | 68.6 ± 1.51[a] | 67.00 - 71.00 | 64.2 ± 7.19[a] | 56.00 - 74.00 |
| Discharge ($m^3/s$) | 693.22 ± 100.58[a] | 584.93 - 798.22 | 815.13 ±52.71[b] | 724.57 - 854.51 |

**Table 2:** Total length (TL), body weight (BW), gonadosomatic index (GSI), Fulton condition factor (K), vitelogenic follicle diameter (DFV), batch fecundity (BF), relative fecundity per unit of body weight (RF), r= Pearson correlation between batch fecundity and GSI, BF-GSI and between batch fecundity and gonadal weight, BF-GW of females of *A. lacustris* captured from May 2011 to June 2012 in two sections on São Francisco river downstream Três Marias dam. Different letters represents statistically differences ($p<0.05$) between parameters on the samples sections.

| Females | Section 1 | | Section 2 | |
|---|---|---|---|---|
| N | 127 | | 134 | |
| | Mean ± SD | Range | Mean ± SD | Range |
| TL | 20.0 ± 2.3[a] | 15.0 - 24.8 | 21.2 ± 2.6[b] | 14.4 - 25.9 |
| BW | 80.24 ± 27.9[a] | 25.8 - 158 | 94.9 ± 36.3[b] | 24.4 - 187.0 |
| GSI | 3.6 ± 2.3[a] | 0.03 - 10.5 | 2.7 ± 1.9 [a] | 0.14 - 9.4 |
| DVF | 768.1 ± 67.4 [a] | 628.6 - 915.8 | 765.3 ± 82.3 [a] | 532.2 - 918.7 |
| BF | 3712.2 ± 2615.5[a] | 1980 - 7150 | 3710.6 ± 1821.2[a] | 510 - 7363 |
| RF | 41.5 ± 22.3[a] | 10 - 93 | 40.5 ± 20.5[a] | 19 - 83 |
| r BF-GSI | 0.50 | | 0.60 | |
| r BF-GW | 0.71 | | 0.75 | |

**Table 3:** Total length (TL), body weight (BW), gonadosomatic index (GSI), Fulton condition factor (K), of males of *A. lacustris* captured from May 2011 to June 2012 in two sections on São Francisco river downstream Três Marias dam. Different letters represents statistically differences ($p<0.05$) between parameters on the samples sections.

| Males | Section 1 | | Section 2 | |
|---|---|---|---|---|
| N | 116 | | 144 | |
| | Mean ± SD | Range | Mean ± SD | Range |
| TL | 16.4 ± 1.5[a] | 14.0 - 21.0 | 17.2 ± 1.9[b] | 13.8 - 25.5 |
| BW | 39.5 ± 14.0[a] | 15.0 - 85 | 45.6 ± 21.6[b] | 20.0 - 176.0 |
| GSI | 0.61 ± 0.43[a] | 0.02 - 2.3 | 0.56 ± 0.42[a] | 0.01 - 2.37 |

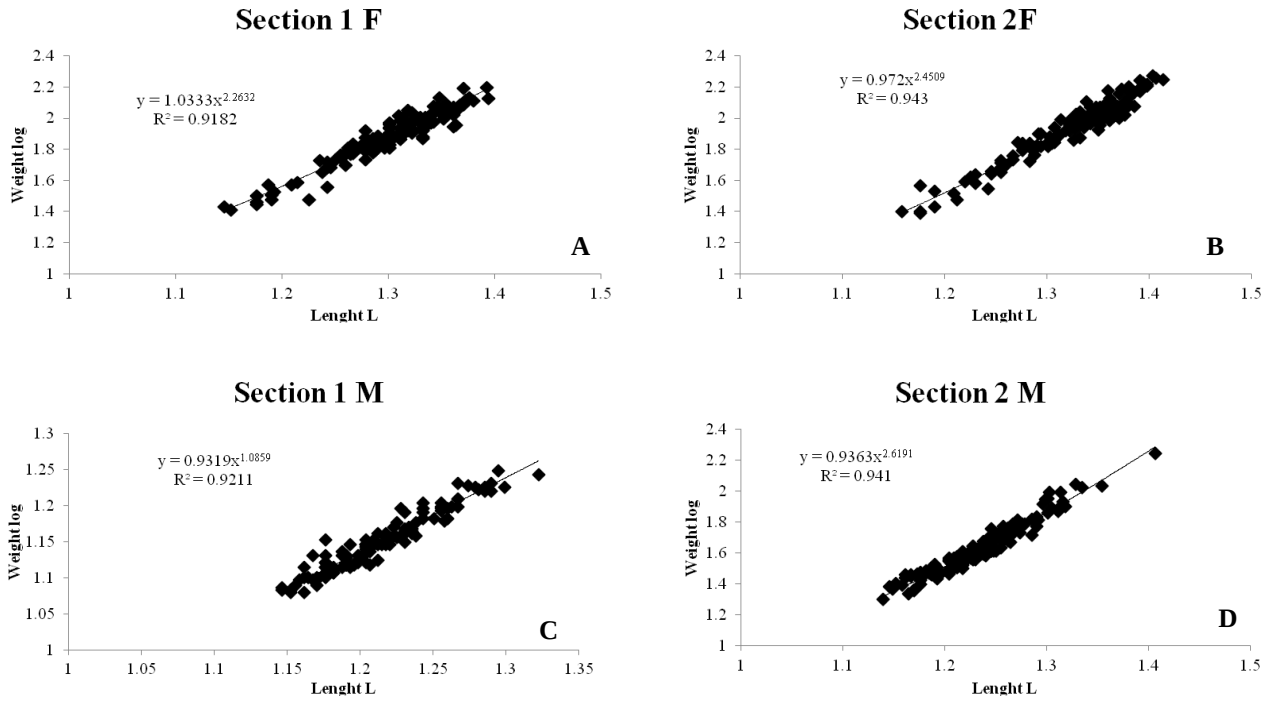


**Figure 2**: Lenght-weitgh relationship with logarithmic data of females, F, and males, M, captured from may 2011 to june 2012 in two sections on São Francisco river downstream Três Marias dam.

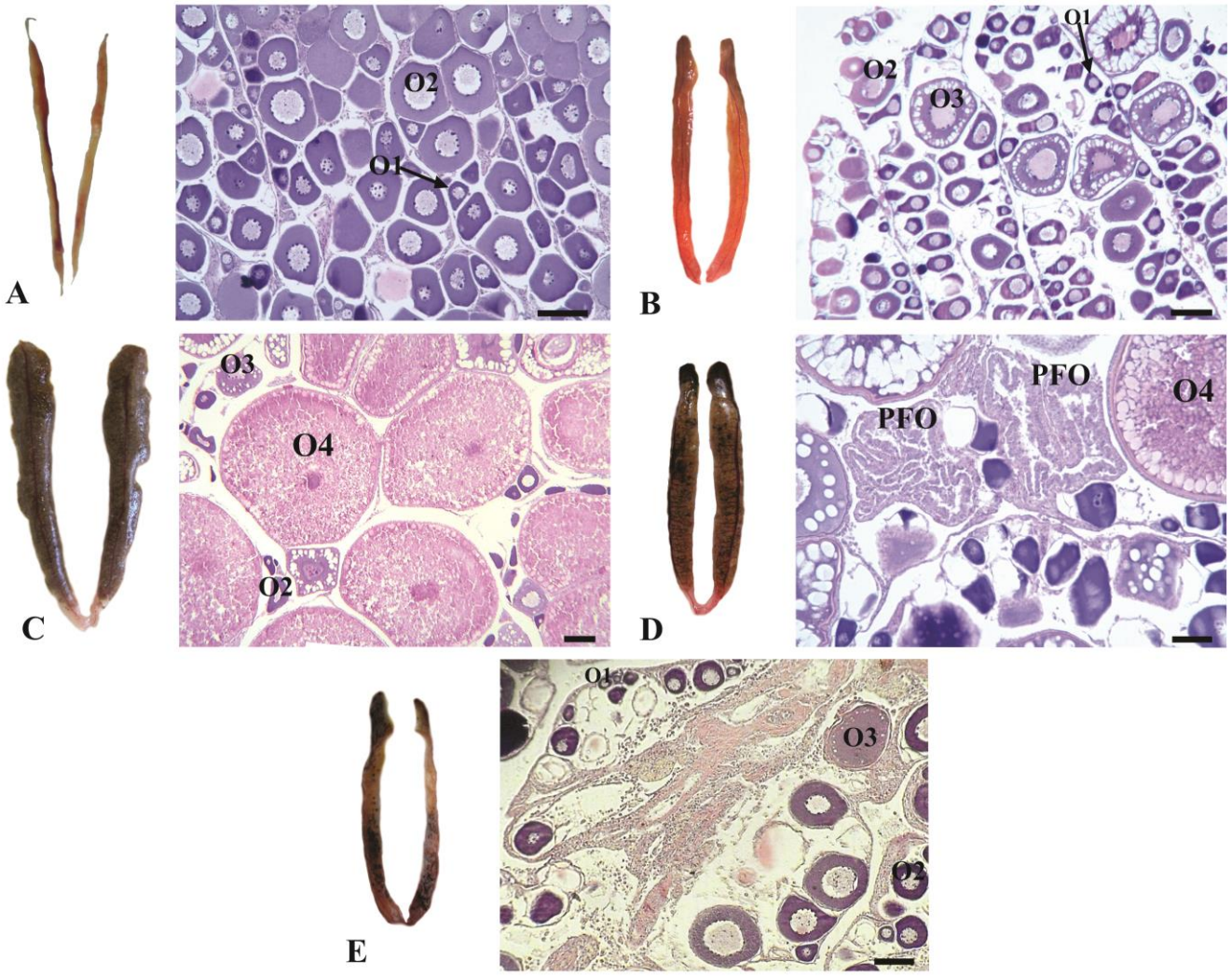


**Figure 3**: Ovaries in different maturation stages and the respective transversal sections stained by HE. A= resting; B= initial maturation; C= advanced maturation/mature; D= partially spawned, E= totally spawned ovaries. O1= early perinucleolar follicles; O2= late perinucleolar follicles; O3= previtellogenic follicles ; O4= vitellogenic follicles ; POF= post ovulatory follicle. Scales bars represent 100 µm.

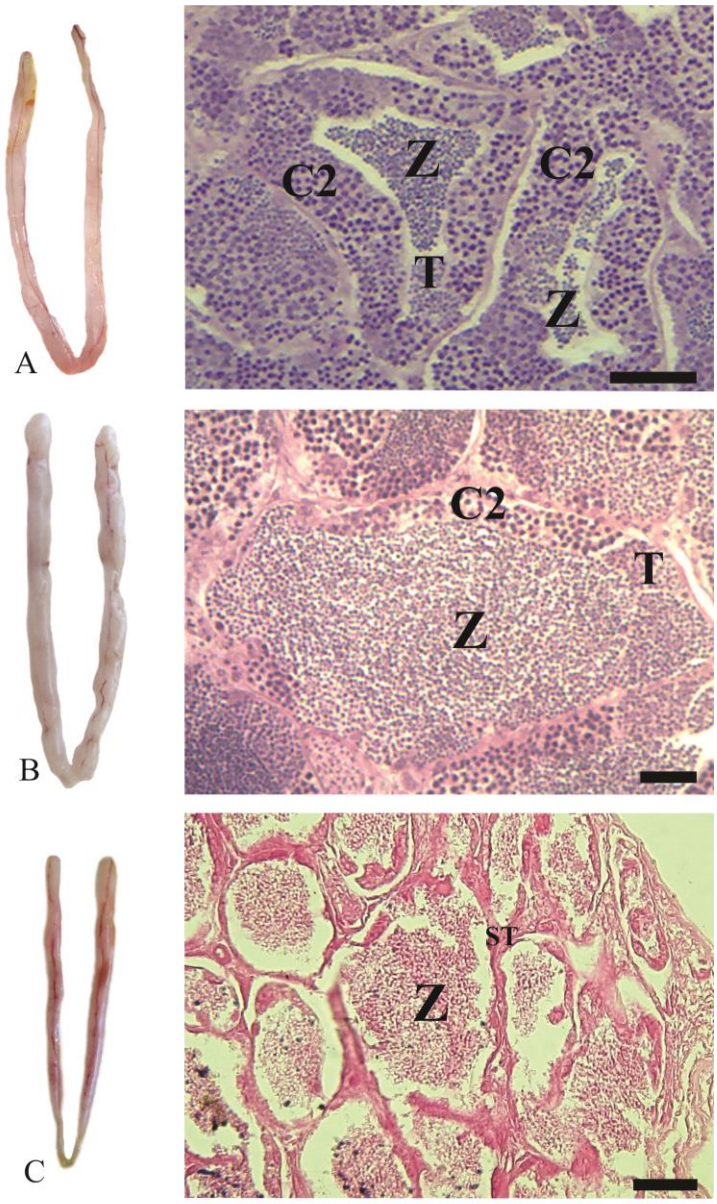


**Figure 4:** Testes in different maturation stages and respective transversal sections stained by HE. A= early maturation; B= advanced maturation/mature; C= partially spent; Z= spermatozoa; C2= secondary spermatocytes T= spermatids; ST= seminiferous tubules. Scales bars represents A= 100 µm, B= 200 µm and C= 500 µm.

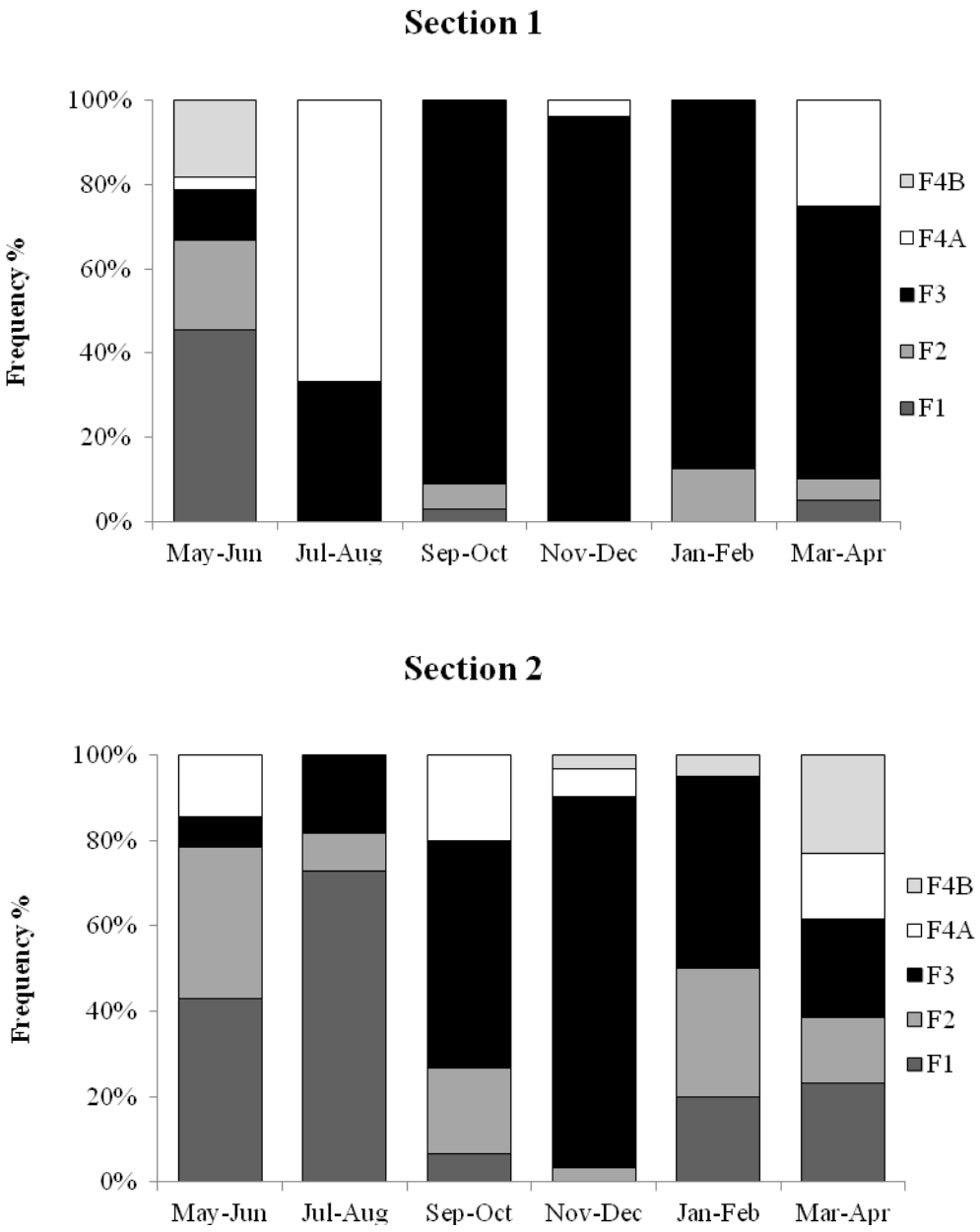


**Figure 5**: Frequency of the maturation stages of the *A. lacustris* reproductive cicle captured from may 2011 to june 2012 in two sections on São Francisco river downstream Três Marias dam. Gonadal maturation stages of females: F1= resting, F2= initial maturation, F3= advanced maturation/mature, F4A= partyally sapawned and F4B= totally sapawned.

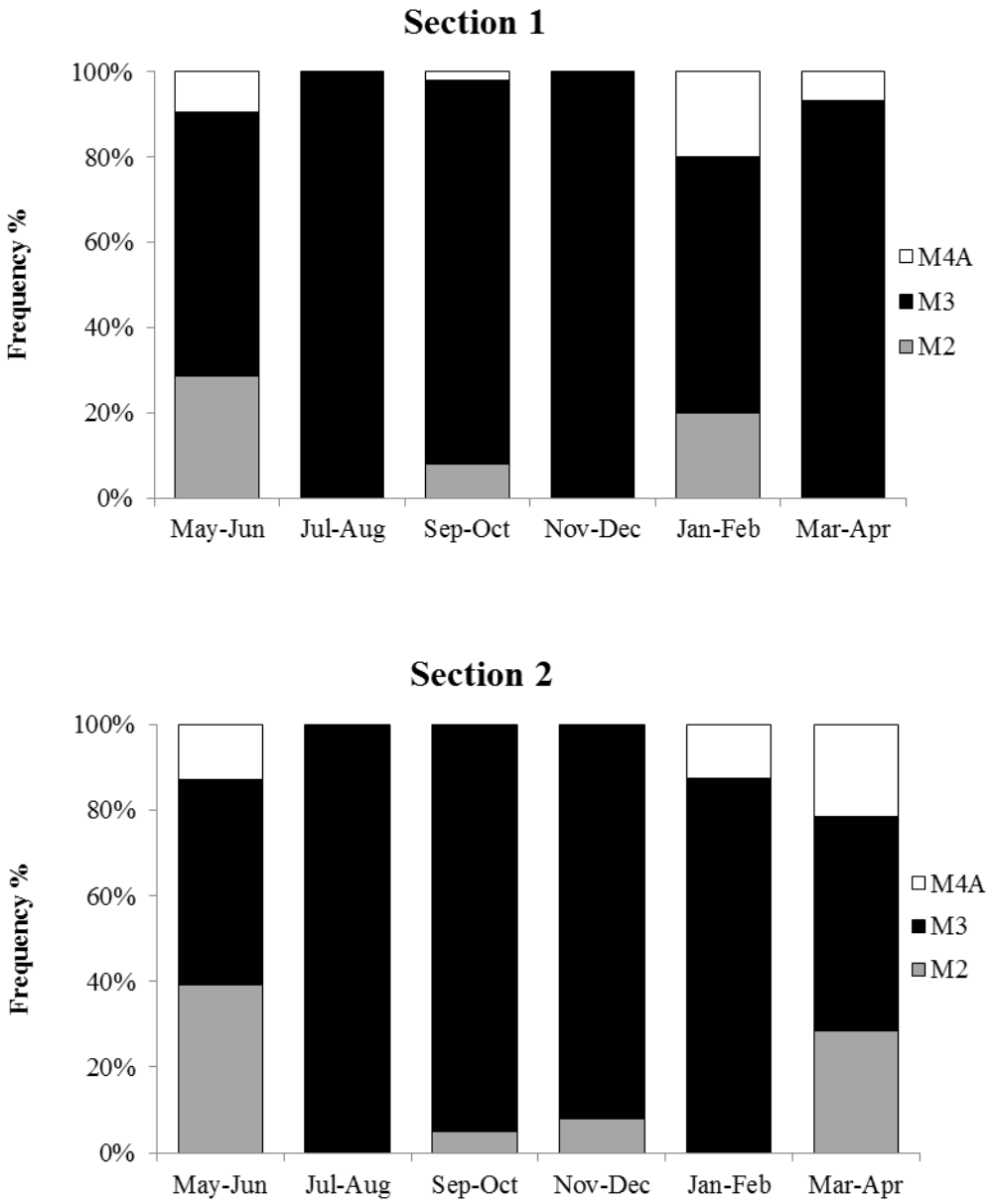


**Figure 6**: Frequency of the maturation stages of the *A. lacustris* reproductive cicle captured from may 2011 to june 2012 in two sections on São Francisco river downstream Três Marias dam. Gonadal maturation stages of males: M2= initial maturation, M3= advanced maturation/mature and M4A= partyally spent.

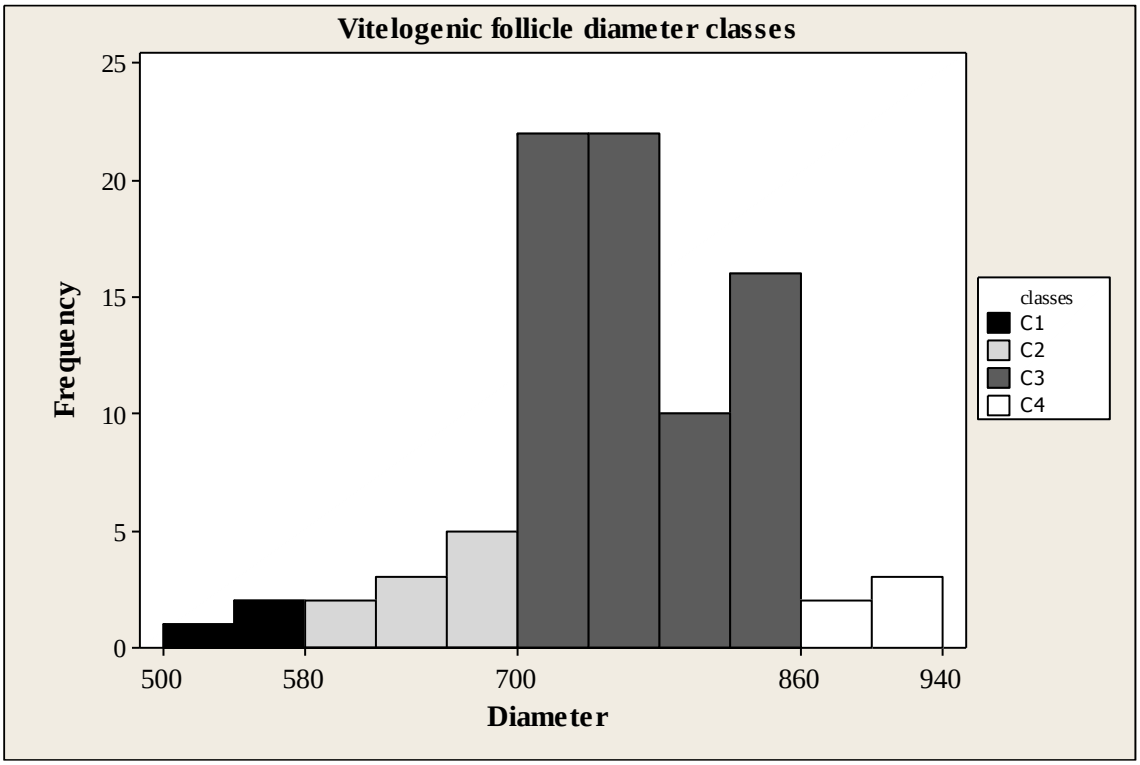


**Figure 7**: Diameter in micrometers (µm) of 100 vitelogenic follicles from histologic sections of mature ovaries of *A. lacustris.* Different bar colors indicates signifficant statistycaly differences between the diameter classes ($p < 0.05$).

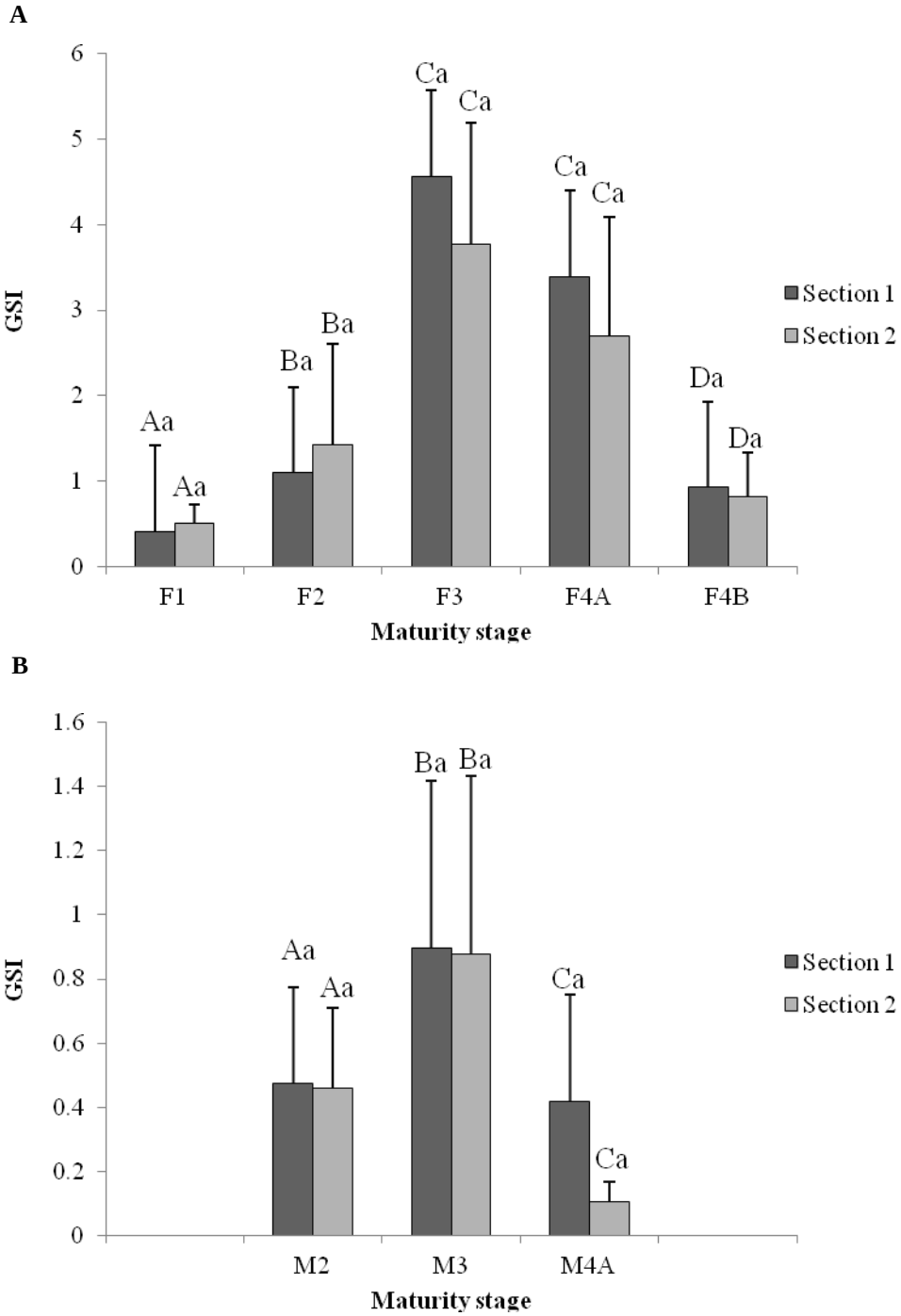


**Figure 8**: Gonadosomatic index (GSI) (mean ± sd) by gonadal maturation stages of females (F), A, and males (M), B, of *A. lacustris* captured from may 2011 to june 2012 in two sections on São Francisco river downstream Três Marias dam, section 1 – dark bars, section 2 – bright bars. Different capital letters indicates signifficant statistycaly differences between gonadal maturation stages, different lowercase letters indicate signifficant statistycaly differences between fishes captured in each section of study area ($p<0.05$).

**Discussion**

The results of this study showed that in the São Francisco River, section1 immediately downstream from Três Marias dam, *A. lacustris* presents reproductive success where the physical and chemical conditions of the water are not favourable to reproduction of others large migratory species (Sato et al., 2005; Arantes et al., 2010; Domingos et al., 2012, Thomé et al., 2012).

Previous studies have shown that some physical and chemical parameters of the São Francisco River water show statistically significant differences between the two sections analysed: immediately downstream from the dam and after the confluence of the São Francisco River and the Abaeté River (Esteves et al., 1985, Sato et al., 2005; Arantes et al., 2010; Domingos et al., 2012; Thomé et al., 2012). In this study, the low values of temperature and dissolved oxygen in the section of the São Francisco River immediately downstream of the dam may be due to thermal stratification of the reservoir water during the summer and also the release of hypolimnion water through the turbines as well as the anthropogenic impact of flow control (Esteves et al., 1985; Santos et al., 2012). The impacts due to the release of colder water, which is low in dissolved oxygen, were also detected downstream of several dams in different parts of the world (Hubbs 1972; Clarkson & Childs 2000; Bunn & Arthington 2002; Preece & Jones 2002; Graf 2006; Donaldson et al., 2008; Mantel & Hughes 2010).

In this study, for both sections analysed, we observed that females were larger than males. This sexual dimorphism seems to be a common feature for most Characiformes (Azevedo 2010). Length and weight of fish in section 1 were statistically lower than those of section 2 and these differences may also be due to the negative environmental impacts of the dam just downstream of the Três Marias dam.

The length-weight relationship and Fulton condition factor may indicate differences between populations of the same species (Froese 2006, Gray et al., 2000), and they depend on the environmental conditions to which the fish are subjected (Clarkson & Childs 2000; Campos-mendoza & Mcandrew 2004; Armstrong & Witthames 2012). In both sections of the São Francisco River analysed in this study, *A. lacustris* showed negative allometric growth; in section 2, however, we observed a length-weight ratio greater than that of section 1, probably due to the better conditions for somatic growth in section 2, where better quality water in the São Francisco River was found.

Accurate classification of fish gonadal maturation stages is important for the correct analysis of the reproductive biology and the use of histological diagnoses is critical and necessary in these analyses (Núñez & Duponchelle 2009). In this study, through macro and microscopic analysis, five main maturation stages were found: resting, early maturation, advanced maturation, partially spawned to females and partially spent to males and fully spawned to females and fully spent and males. These gonadal maturation stages show marked variations in the macroscopic and microscopic gonadal structure, following the GSI changes during the reproductive cycle. The classification into five stages of maturation showed that the *A. lacustris* reproductive cycle is similar in both sampling sections, presenting the mature gonadal maturation stage throughout the year with reproductive activity peak in the rainy season, which has also been observed in migratory and totally spawn species, in the same sampling sections of this study (Sato et al., 2005, Arantes et al., 2010; Thomé et al., 2012; Domingos et al., 2012). *Acestrorhynchus lacustris* showed reproductive activity during almost the whole year peaking during the rainy season, between November and February, as also observed by Bazzoli & Godinho 1991 who studied the reproductive biology of the species in a lentic environment, i.e. the Três Marias dam. The absence of the resting and fully spermed males was also noted in the work of Bazzoli & Godinho (1991), which may be indicative of fast spent period.

The GSI may reflect environmental quality, since it is directly linked to the energetic reserves available for reproduction (Frenkel & Goren 1997; Yoda & Yoneda 2009; Thomé et al., 2012). In this study, there were no statistical differences in mean values of maximum GSI between fish from the two sections analysed, contrary to was found for migratory species (Sato et al., 2005).

The histometric analysis also showed that there are no differences in the vitellogenic follicle diameter of fish caught in the two sections analysed, unlike was observed by Sato et al., (2005) and Arantes et al., (2010) in a Neotropical species of group synchronous follicle development and total spawning. The vitellogenic follicle diameter is reported as an important parameter related to reproductive fitness because larger follicles produce offspring with more energy reserves, thus being of better quality (Kinnison et al., 1998; Kolm & Ahnesjö 2005; Lubzens et al., 2010) and may vary during the reproductive cycle depending on fecundity and environmental factors such as water temperature (Kinnison et al., 1998; Donaldson et al., 2008; Armstrong & Witthames 2012). Therefore, our results showed that the physical and chemical conditions of the water in section 1 did not negatively affect the vitellogenic

follicles' growth, as occurs for other teleosts species studied under the same conditions (Sato et al., 2005; Arantes et al., 2010).

Through histometric analysis, it was also observed that there are four classes of vitellogenic follicle diameter (Figure 6) simultaneously occurring in mature females of *A. lacustris*, showing asynchronous folliculogenesis development with distinct populations of vitellogenic follicles, forming batches, as observed in other teleost with batch fecundity (Melo et al., 2011; Armstrong & Witthames 2012). Asynchronous folliculogenesis is typical of fish with long reproductive periods, multiple spawning and batch fecundity (Kurita & Kjesbu 2009; Yoda & Yoneda 2009; Núñez & Duponchelle 2009; Lubzens et al., 2010; Armstrong & Witthames 2012). Fish with these characteristics generally have greater biological plasticity, greater tolerance to variations in the environmental conditions they are subjected to, compared to fish with distinctive seasonal reproductive activity, such as the Neotropical migrators (Luks & Svedäng 1997; Campos-Mendoza & Mcandrew 2004; Sato et al., 2005; Godinho et al., 2009; Arantes et al., 2010; Thomé et al., 2012).

Fecundity is reported as a parameter which is strongly linked to the environmental conditions in which females live before spawning, and can be physiologically regulated (Armstrong & Witthames 2012). Food availability and water temperature are reported as major factors capable of influencing the reproductive potential through the number and diameter of vitellogenic follicles (Luks & Svedäng 1997; Kinnison et al., 1998; Yoda & Yoneda 2009; Armstrong & Witthames 2012). In this study, batch fecundity and fecundity relative to weight showed no statistically significant differences between fish collected from the two sampling sections, showing that, related to the diameter of vitellogenic follicles, fecundity was not affected by the conditions of the São Francisco River in section 1. This shows that *A. lacustris* maintain their reproductive potential even in environments with lower temperature and dissolved oxygen, a parameters that, for other fishes, alters fecundity.

Regarding spawning frequency, *A. lacustris*, as well as other multiple spawning fish, may present a greater number of spawning when the water temperature is higher, such as in section 2, as also reported for species living in environments with better water quality, especially with respect to temperature (Campos-Mendoza & Mcandrew 2004; Yoda & Yoneda 2009; Armstrong & Witthames 2012).

Although damming negatively impacts the reproduction of several fish species, especially migratory ones, the results of this study showed that for the sedentary species *A. lacustris* in the São Francisco River, downstream from the Três Marias dam, such damming

did not alter their reproductive activity even in the section immediately downstream from the dam, where the physical and chemical conditions of the water are notoriously unfavourable to fish reproduction, such fact could be related to the species reproductive strategy.

## CONCLUSÕES

- No rio São Francisco, a jusante da barragem de Três Marias não foi registrado colapso reprodutivo para *Acestrorhynchus lacustris*;

- A estratégia reprodutiva de *A. lacustris* está diretamente associada ao modo como a espécie responde às condições ambientais;

-Na região da confluência do rio São Francisco com o rio Abaeté *A. lacustris* encontra melhores condições para o crescimento somático.

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