UNIVERSIDADE ESTADUAL PAULISTA
Instituto de Biociências de Botucatu (IBB-UNESP)
São Paulo State University- Institute of Bioscience of Botucatu

TESE DE DOUTORADO

Seleção de fêmeas de *Pseudopimelodus mangurus, pimelodus maculatus* e *Prochilodus lineatus* aptas à reprodução através da ultrassonografia

Dilberto Ribeiro Arashiro

Médico Veterinário

Botucatu

2021

Seleção de fêmeas de *Pseudopimelodus mangurus, pimelodus maculatus* e *Prochilodus lineatus* aptas à reprodução através da ultrassonografia

DILBERTO RIBEIRO ARASHIRO

Orientador: Prof. Dr. José Augusto Senhorini

Coorientadores : Prof. Dr. George Shigueki Yasui

Prof. Dr. Luciano Andrade Silva

Tese apresentada ao curso de Pós Graduação em Ciências Biológicas – Instituto de Biociências da Universidade Estadual Paulista – Unesp – Campus de Botucatu, como parte dos requisitos para a obtenção do título de Doutor em Ciências Biológicas – Zoologia.

Botucatu

2021

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Arashiro, Dilberto Ribeiro.
Seleção de fêmeas de *Pseudopimelodus mangurus, Pimelodus maculatus* e *Prochilodus lineatus* aptas à reprodução através da ultrassonografia / Dilberto Ribeiro Arashiro. - Botucatu, 2021

Tese (doutorado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências de Botucatu
Orientador: José Augusto Senhorini
Coorientador: George Shigueki Yasui
Coorientador: Luciano Andrade Silva
Capes: 20400004

1. Aquicultura. 2, Peixes - Reprodução. 3. Sexagem. 4. Ovários - Maturação.

Palavras-chave: Não invasivo; Ovário; Reprodução; Sexagem.

Agradecimentos

Aos membros da Banca Examinadora: Nivaldo Ferreira do Nascimento, Diógenes Henrique Siqueira, Matheus Pereira dos Santos e Norberto Castro Vianna

Ao Centro Nacional de Pesquisa e Conservação da Biodiversidade Aquática Continental pela estrutura e material biológico que viabilizaram a execução deste trabalho

Ao programa de Pós Graduação em Zoologia e a Universidade Estadual Paulista “Júlio de Mesquita Filho” – UNESP/Botucatu.

Ao meu coorientadore George Shigueki Yasui, pela oportunidade, confiança e amizade e tudo que aprendi com ele.

Ao meu coorientador Luciano Andrade Silva e toda sua equipe por disponibilizarem o ultrassom que permitiu a execução deste trabalho.

Aos meus amigos e colegas de laboratório.

Ao meu amigo Zequinha, segundo melhor pescador de Bagre Sapo de Pirassununga.

Em especial ao meu orientador Prof. Dr. José Augusto Senhorini, que não foi apenas um professor, foi um grande amigo, companheiro de trabalho e viagens. Por sempre me apoiar nas decisões em que tomei e pelos conselhos, uma das melhores pessoas que conheci na minha vida e que sempre será referência de amizade, caráter e bom coração. Zezinho, dedico este trabalho para você.

A toda minha família e amigos.

Lista de Figuras

Lista de Tabelas

Sumário

Introdução

A região neotropical tem uma das maiores ictiofaunas do mundo, com mais de 4400 espécies de água doce descritas, que encontram uma grande diversidade de sistemas hidrográficos, onde podemos destacar as duas principais ordens, os Siluriformes e Characiformes, com mais de 2100 e 1800 espécies descritas respectivamente (Froese and Pauly 2015).

Dentre os as ordens dos Siluriformes e Characiformes existem espécies mais abundantes e com potêncial para aquicultura, como o *Pimelodus maculatus* (Lacépède, 1803) e *Prochilodus lineatus* (Valenciennes, 1837), porém também são encontradas espécies em risco de extinção, como é o caso do *Pseudopimelodus mangurus* (Valenciennes, 1835) que esteve categorizado como ameaçado de extinção no estado de São Paulo (Bressan, Kierulff et al. 2009, São Paulo 2014),

Estas espécies são reofílicas, e realizam migrações reprodutivas, conhecias como piracema, e que se concentram nos períodos quentes e chuvosos do ano.

O *P. mangurus*, também conhecido como 'peixe sapo', pertence à família Pseudopimelodidae, possui hábito alimentar carnívoro em seu meio natural, eles são importantes para manter o equilíbrio entre as espécies e servem como fonte de alimento para outras espécies de peixes, répteis, mamíferos e aves.

Embora *P. mangurus* não seja produzido em larga escala no Brasil, eles apresentam grande importância na alimentação das populações ribeirinhas.

Devido à qualidade da carne e à ausência de espinhos intramusculares, características de peixes Siluriformes como *Pseudoplatystoma spp*. e o *Sorubim trigonocephalus* (Smerman, Castro et al. 2002, de Oliveira Schuingues, de Lima et al. 2013), podendo fazer com que esta espécie tenha potencial para a aquicultura. No entanto, a produção massiva em cativeiro é uma etapa primordial, tanto para conservação, quanto para aquicultura de interesse comercial, e para tanto é necessário conhecer a sua biologia reprodutiva (Luz and Zaniboni Filho 2002, MURGAS[1], de Oliveira FELIZARDO[1] et al. 2012).

Já Curimbatá *P. lineatus* e o Mandi *P. meculatus* são espécies de fácil reprodução, larvicultura, manejo e criação em cativeiro.

O *P. lineatus* apresenta hábito alimentar iliófago e o *P. maculatus* apresenta hábito alimentar onívoro, embora ambas espécies aceitem facilmente dietas artificiais (LUZ and ZANIBONI-FILHO 2001, Galdioli, Hayashi et al. 2002), e por estas características são espécies bastante produzidas no Brasil (às Micro and Empresas–SEBRAE 2015).

São espécies que costumam migrar em grandes cardumes, de reprodução massiva e grande importância em seu ambiente natural pois suas larvas também servem de alimento para diversas espécies de peixes carnívoras, bem como para aves, répteis, anfíbios e outros organismos. Essas espécies apresentam bom potencial para aquicultura, e pela facilidade da reprodução em cativeiro, servem como modelo para estudos envolvendo reprodução de Characiformes e Siluriformes.

O Curimbatá e o Mandi são espécies muito capturadas, por serem muito apreciadas pela população ribeirinha, o que pode levar ao declínio de suas populações em seus ambientes, juntamente de outras ações antrópicas. Devido à importância ecológica mencionada acima, a redução dos estoques naturais pode afetar várias outras espécies de peixes e outros organismos que dependem de sua reprodução.

O *P. lineatus*, *P. mangurus, P. maculatus* e outras espécies reofílicas, nativas e de desova total, se reproduzem apenas uma vez ao ano, durante o período da piracema (Andrade and Yasui 2003).

Porém, não se reproduzem espontaneamente em ambientes lênticos, por falta de estímulos ambientais, sendo necessário uma indução hormonal artificial para induzir a maturação final dos gametas e subsequente ovulação das fêmeas em cativeiro.

Dentre os hormônios mais utilizados para indução à desova das fêmeas em cativeiro podemos dar ênfase à hipófise desidratada de carpa, que é o método mais utilizado nas pisciculturas (Streit Jr, Moraes et al. 2003), seguido por outros hormônios sintéticos, como aos análogos do GnRH, que atuam na liberação dos hormônios gonadotróficos durante a reprodução (Zohar and Mylonas 2001), e que pode estar associado a antagonistas de Dopamina que atuam no bloqueio dos receptores da dopamina, consequentemente aumentando a produção endógena de GnRH e Gonadotrofinas, como HCG e LH que atuam diretamente nas gônadas (Andrade and Yasui 2003).

Quando falamos em reprodução de peixes, já é conhecido que existe uma interação entre as condições ambientais como o fotoperíodo (Muniz, Catanho et al. 2008) e a temperatura da água (Firkus, Rahel et al. 2018) e com as condições fisiológicas, como o aumento dos níveis hormonais esteroides séricos (Shimizu 2003) que atuam no desenvolvimento das gônadas (Bapary and Takemura 2010).

A reprodução é um ponto chave para o sucesso da produção de espécies de peixes, tanto em risco de extinção quanto de interesse comercial. No entanto, existem alguns entraves, como a seleção de matrizes aptas à desova. Uma fêmea que ainda não atingiu o ponto de maturação, ou mesmo já passou do ponto de maturação gônadal ideal para sua reprodução não vai responder a indução hormonal à desova, sendo que estas condições não são controladas pelo produtor, fazendo-se necessário desenvolver métodos para predizer o momento certo a ser feita a indução hormonal.

Com a aproximação da estação reprodutiva dos peixes de reprodução sazonal, as gônadas ficam mais desenvolvidas e a maturação dos ovários e testículos podem ser avaliados através de técnicas que possibilitem tanto uma visualização macroscópica, quanto microscópica, possibilitando a visualização da morfologia dos gametas e predizer a sua viabilidade.

Algumas técnicas não invasivas e outras invasivas podem ser adotadas para avaliar o desenvolvimento gonadal, como biopsia ovariana (Ferraz and Cerqueira), canulação de oócitos intra ovárianos (AndradeTalmelli, Fenerich-Verani et al. 1999), histologia (Petochi, Di Marco et al. 2011, do Nascimento, Pereira-Santos et al. 2017) e ultrassonografia (Blythe, Helfrich et al. 1994, Evans, Fitzpatrick et al. 2004, Kohn, Lokman et al. 2013, Chiotti, Boase et al. 2016).

Um método eficiente que é utilizado para avaliar o desenvolvimento gonadal é a ultrassonografia, técnica que já é utilizada em peixes desde a década de 80 utilizado na sexagem e atividade ovariana (Hildebrandt and Saragusty 2015), é uma técnica não invasiva que viabiliza diferenciar estruturas moles em diferentes profundidades, apontando também tamanho, formato e localização de estruturas em tempo real. Por ser um método não letal, se torna uma alternativa muito importante na avaliação dos aspectos reprodutivos dos peixes, podendo substituir outros métodos invasivos que podem comprometer a saúde do peixe e o sucesso reprodutivo (Blythe, Helfrich et al. 1994, CREPALDI and ROTTA 2007).

O ultrassom é um equipamento portátil, que pode ser facilmente transportado até lugares de difícil acesso (Brizendine, Ward et al. 2018) podendo se obter os resultados em tempo real e em alta resolução. A sonda responsável pela captação das ondas refletidas e retransmitidas para a máquina pode ser utilizada submersa na água, ajudando no bem estar da matriz e reprodutor, causando um menor estresse (Chiotti, Boase et al. 2016). Para organismos aquáticos, essa característica é particularmente importante e se adequa perfeitamente às condições ambientais aquáticas.

A técnica da ultrassonografia, além de não ser invasiva, permite também a sexagem (Blythe, Helfrich et al. 1994, Evans, Fitzpatrick et al. 2004, Bryan, Wildhaber et al. 2007, Newman, Jones et al. 2008, Kohn, Lokman et al. 2013, Brizendine, Ward et al. 2018) e indicar se a fêmea está com o ovário maduro e apto à desova e o macho com o testículo pronto para espermiar. No caso da maioria das espécies nativas reofílicas só é possível realizar a sexagem durante o período reprodutivo.

O manejo geral para a seleção de matrizes e reprodutores muitas vezes é empírica e intuitiva, baseada em aspectos morfológicos e comportamentais. Dentre as características morfológicas das se destacam o abdômen abaulado e macio ao toque e papila avermelhada (MURGAS[1], de Oliveira FELIZARDO[1] et al. 2012), e os machos, além de não apresentarem o abdômen abaulado, liberam sêmen através de compressão abdominal no sentido crânio caudal e entre as características comportamentais os machos de algumas espécies, quando preparados, emitem sons (ronco) (Campeche, Balzana et al. 2011). Ainda não existe uma metodologia bem estabelecida de seleção de matrizes aptas à desova, de forma que, muitas vezes a matriz julgada pelas características externas como apta à desova não responde à indução hormonal artificial, onde os hormônios utilizados são de alto custo (Andrade and Yasui 2003), levando a uma grande perda de tempo e recursos com hormônios e manejo das matrizes que não responderam à indução hormonal, podendo até levar a perda de reprodutores.

Estabelecer o perfil do ovário da fêmea apta à desova através da imagem do ultrassom pode levar ao aumento da taxa de fêmeas induzidas que respondem à indução hormonal, uma vez já que nem todas fêmeas induzidas desovam quando selecionadas através de caracteres secundários como abdômen abaulado e macio ao toque e papila avermelhada (Zohar 1989).

Várias pesquisas envolvendo a reprodução do *P. lineatus, P. mangurus* e *P. maculatus* já foram realizadas (Fenerich-Verani, Godinho et al. 1984, RESENDE 1992, Vono, Silva et al. 2002, Moraes, STREIT JR et al. 2004, Lima-Junior and Goitein 2006, Hainfellner, De Souza et al. 2011, SOUZA 2011, Hainfellner, Souza et al. 2012, Sabinson, Rodrigues Filho et al. 2014, De Melo Dias, Cassel et al. 2017, Arashiro, Yasui et al. 2018, Arashiro, Shigueki Yasui et al. 2020) sendo que em vários casos foi necessário a eutanásia dos peixes para observação dos órgãos reprodutivos e gametas (Vono, Silva et al. 2002, Lima-Junior and Goitein 2006, Hainfellner, De Souza et al. 2011, Hainfellner, Souza et al. 2012, Sabinson, Rodrigues Filho et al. 2014, De Melo Dias, Cassel et al. 2017, Arashiro, Yasui et al. 2018).

Diante do exposto à cima, o presente trabalho propõe estabelecer um protocolo de seleção de fêmeas aptas à desova e sexagem através das imagens do ultrassom para as espécies *P. mangurus, P. maculatus* e *P. lineatus*.

Hipotese

Testar a efetividade do ultrassom para a sexagem e identificar a imagem do ovário que responderá à indução hormonal para de fêmeas de *P.* lineatus, *P. mangurus* e *P. maculatus*

Objetivos

O objetivo deste estudo foi estabelecer um protocolo de seleção de fêmeas aptas à desova e diferenciar os machos das fêmeas através da imagem de ultrassom para as espécies *P. mangurus*, *P. maculatus* e *P. lineatus*.

Capitulo 1: **Ultrasound a tool for sex identification and selection of females able to spawn in neotropical catfishes**

Running title: Ultrasound in catfish species reproduction

Keywords: Reproduction, Siluridae, Ovary, Sexing, Non-invasive.

Abstract

The aim of this study was to stablish a methodology to female able to spawn selection and sexing by ultrasonography for the neotropical catfishes *P. mangurus* and *P. maculatus.* Fish were anesthetized and then measured for its weight, length, height, width of the body giving rise to biometric data: 1.061 ± 0.452 kg, 34.886 ± 5.291 cm, 7.257 ± 1560 cm, 8.586 ± 2.34 cm respectively for *P. mangurus* who spawn and 482.500 ± 118.366 g, 28.329 ± 2.426 cm, 7.626 ± 0.972 cm and 5.261 ± 0.756 cm respectively for *P. maculatus* who spawn. Ultrasonography was made in ventral region measuring ovary height, ovary width and ovary area, giving rise to biometric data: 2.763 ± 0.793 cm, 2.693 ± 0.477 cm and 14.867 ± 6.873 cm^2 respectively for *P. mangurus* who spawn and 2.576 ± 0.583 cm, 1.917 ± 0.374 and 8.738 ± 2.824 cm^2 for *P. maculatus* who spawn. After ultrasonography, fish of both species were induced to spawn, being seventeen females of *P. mangurus* and forty-two *P. maculatus,* were only seven *P. mangurus* and thirty-four *P. maculatus* spawned, giving rise to the following reproductive: data: relative oocytes weight (117.373 ± 62.272 g), number of oocytes per gram (645 ± 80.060), total fecundity (67.770 ± 30.271), fertilization rate (90,58 ± 15,90%), and hatching rate (72,07 ± 31,09%) for *P. mangurus* and relative oocytes weight (47.517 ± 25.256 g), number of oocytes per gram (3.415 ± 3.415), total fecundity (79.082 ± 57.622%), fertilization rate (76,47 ± 22,31%), and hatching rate (39 ± 30,67%) for *P. maculatus*. For both species the ultrasound presented a highly accuracy for sexing at the reproductive season and can be applied in reproductive studies as a non-invasive technique replacing invasive methods that can compromise de reproductive success.

Introduction

The order of the siluriforms is the most abundant in number of fish species in neotropical region (Froese and Pauly 2015, Toussaint, Charpin et al. 2016). Some species are more abundant like *Pimelodus maculatus* (Lacépède, 1803) and others are in smaller number or in danger of extinction like *Pseudopimelodus mangurus* (Valenciennes, 1835) which was recently categorized in São Paulo State list of endangered animals (Bressan, Kierulff et al. 2009, São Paulo 2014). Both species present great importance in the feeding and economy of the riverside populations and have ecological relevance, serving as a food source for other species of fish, reptiles, mammals and birds. They can be found in Parana River basins and have migratory behavior, total spawning and reproducing once a year, between months of October to February in the period known as Piracema(Arashiro, Yasui et al. 2018).

P. maculatus is a species of interest for aquaculture and *P. mangurus* is a species who requires conservation efforts. Some techniques such as artificial propagation, re-stocking, genetics and biotechniques like chromosome manipulation (Piferrer, Beaumont et al. 2009, do Nascimento, Pereira-Santos et al. 2017) chimerism (Yasui, Fujimoto et al. 2011) and *ex situ* gene bank.

For aquaculture or conservation works, reproduction is a key step, being necessary basic studies with their reproductive biology (MURGAS[1], de Oliveira FELIZARDO[1] et al. 2012). Some methods can be adopted to follow the reproductive development of the fish, such as ovarian biopsy (Ferraz and Cerqueira), cannulation of intra ovarian oocytes (Andrade-Talmelli, Fenerich-Verani et al. 1999), histology

(Petochi, Di Marco et al. 2011, do Nascimento, Pereira-Santos et al. 2017), endoscopy (Bryan, Wildhaber et al. 2007) and ultrasonography (Blythe, Helfrich et al. 1994, Evans, Fitzpatrick et al. 2004, Kohn, Lokman et al. 2013, Chiotti, Boase et al. 2016).

From the techniques discussed above, ultrasonography can be highlighted, as it has been used in fish studies like carcass analysis (Perazza, PINAFFI et al. 2015) and reproduction, like sexing (Colombo, Wills et al. 2004, Bryan, Wildhaber et al. 2007, Newman, Jones et al. 2008, Kohn, Lokman et al. 2013) and follow reproductive development (Evans, Fitzpatrick et al. 2004, du Colombier, Jacobs et al. 2015, Chiotti, Boase et al. 2016, Næve, Mommens et al. 2018)

Ultrasonography is a very efficient technique to evaluate the stage of gonadal development of fish, allowing differentiating soft structures at different depths, pointing to size, shape and location of structures in real time (Blythe, Helfrich et al. 1994). As a non-invasive method, it becomes an important alternative in the evaluation of fish reproductive aspects, being able to substitute invasive methods that can compromise the reproductive success (Blythe, Helfrich et al. 1994, CREPALDI and ROTTA 2007).

Ultrasound may also point to different degrees of gonadal development in the reproductive cycle of a wild fish population (Blythe, Helfrich et al. 1994), and may point to females that are ovary mature and ready to spawn.

The general management of female selection for reproduction is empirical and intuitive, beginning in the selection of male and female that are able to spawn which is crucial to reproduction, this selection is based on morphological characteristics like reddish papilla and protuberant ventral region (Woynarovich and Horváth 1983, MURGAS[1], de Oliveira FELIZARDO[1] et al. 2012). However, often the selection by external morphological characteristics do not respond to hormonal induction, being necessary a more effective methodology to select females suitable for spawning.

Considering the aspects above, the objective of this work was to establish a protocol for selection of able to spawn females by ultrasound.

Material and methods

All procedures were conducted in the Laboratory of Fish Biotechnology (ICMBio/CEPTA, Pirassununga City, São Paulo State, Brazil). The experiments were conducted in accordance with the Ethics Committee for the Use of Laboratory Animals of the National Center for Research Conservation of Aquatic Biodiversity (CEUA / CEPTA;# 010/2015). The fish were collected in natural environments using the sampling license according to Brazilian law (Sisbio #55725-1).

Origin of broodstock and ultrasound

Adult males and females of *Pimelodus maculatus* were collected at Mogi Guassu River and stocked in 8x8 m^3 tanks with 62.000L. The fish were fed with a 6mm commercial fish pellet (45% crude protein, 3800 $kcal.kg^{-1}$) three times a week.

Adult males and females of *Pseudopimelodus mangurus* were collected from the Mogi Guassu River using hook-and-line fishing with a baitfish (hook >5cm) and stocked in a 5x1m^3 box with 3.000L. The fish were feed with dead adult *Astyanax lacustris* three times a week.

During the reproductive season between months of October to February 42 females of *P. maculatus* and 17 females *P. mangurus* were selected to spawning by external morphological characteristics like reddish papillae, increased and soft abdominal area, subsequently fish were anesthetized with clove oil at 150 mg L^{-1} (Biodinamica, Ibiporã, Brazil) and ultrasonography was done with the use of a digital ultrasound (Esaote S.p.a, MyLab 30 Vet, Genova, Italy) in B-mode (gray scale) with a linear multi-frequency transducer set at the frequency of 7.5 MHz and 60 mm wide beam field. All exams were made in the ventral region, under the pectoral fins for sexing and evaluate the ovaries. Data were documented in photos and videos for posterior analyses. From all ovaries, area (cm^2), length and height were measured.

After the ultrasonography, the females of both species were weighted and measure length, height and width of all individuals used in the experiment.

Induced spawning and artificial fertilization

The spawning of *P. mangurus* and *P. maculatus* was induced using crude carp pituitary extract (CCPE) at 6.6 mg kg^{-1} for *P. mangurus* and 5.5 mg kg^{-1} for *P. maculatus*, which was divided into two doses, 0.6 mg kg^{-1} and 6 mg kg^{-1} 6-hours afterwards for *P. mangurus* and 0.5 mg kg^{-1} and 5 mg kg^{-1} 6-hours afterwards for *P. maculatus*. The males were injected with a single dose of CCPE at 2 mg kg^{-1} for *P. mangurus* and 5 mg kg $^{-1}$ for *P. maculatus* at the same time that females received the second dose.

The male gametes were sampled prior to females at 165 hour-degrees after injection for both species. Males of both species were euthanized with an overdose of clove oil at 250 mg L^{-1} (Biodinamica, Ibiporã, Brazil), and the testis was removed and minced in Eagle's Minimum Essential Medium with the pH adjusted to 7.8 (E-MEM, Sigma #M0268, St. Louis, USA). A small aliquot was used to evaluate sperm motility, according to the procedures described by (Yasui, Senhorini et al. 2015). Females were anesthetized with clove oil at 150 mg L^{-1} (Biodinamica, Ibiporã, Brazil) and then stripping was performed in a dry glass container. The weight of oocytes was measured, and a small aliquot was weighed and fixed in 2% glutaraldehyde in Dulbecco's PBS

(Sigma #D5773, St. Louis, USA) in order to calculate the number of oocytes per gram. The diameter of the oocytes were measured with the stereomicroscope (Nikon SMZ 1500, Nikon, Tokyo, Japan) in combination with a CCD camera (Nikon DS-Fi, Nikon, Japan). The software NIS – AR Elements (Nikon, Tokyo, Japan) was used to capture and measure the diameter (µm) of the oocytes from the digital images.

For fertilization, the diluted sperm was placed on the oocyte mass and immediately activated by adding hatchery water. The resultant embryos were incubated at 26 °C, which is the best temperature to incubate *P. mangurus* and *P. maculatus* during early development (Arashiro, Yasui et al. 2018). A small aliquot was separated to calculate fertilization and hatching rates. Seven spawning of *P. mangurus* and thirty-four of *P. maculatus* were obtained.

Statistics

Data are shown as the mean ± standard deviation. Data were obtained with different females and each egg batches. Correlation equation was analyzed with Pearson and the other analysis with variance (ANOVA), followed by a Tukey multiple range test. In all analyses, the software STATISTICA (10.0, StatSoft, USA) was used with the probability set at 0.05.

Results

Ultrasound

The ultrasound image allowed differentiating males from females, observation of the testis of the males (Fig. 1A; Fig. 1B) and the ovaries of the females from both species.

There was a significant difference between the weight (P = 0.011), length (P = 0.027), height (P = 0.023), ovary height (P = 0.001) and ovary area (P = 0.000) of the females who spawn to females who did not spawn for *P. mangurus* as shown in Table 1.

In the case of *P. maculatus,* there was a significant difference between ovary height (P = 0.002), ovary width (P = 0.017) and ovary area (P = 0.006) as shown in table 2.

For both species the ultrasound image was determinant to point a mature and ready to spawn ovary (Fig. 2A) for *P. mangurus*, (Fig. 2C) for *P. maculatus* and a nonmature ovary which is not ready to spawn (Fig. 2B) for *P. mangurus* and (Fig. 2D) for *P. maculatus.* Moreover, all females who spawned after hormonal induction presented an egg mass near from the papilla region as shown in (Fig. 3A) for *P. mangurus* and
(Fig. 3B) for *P. maculatus*.

Reproduction

The weight of the captured broodstock ranged from 325 to 1472 g (average 106.5 ± 48.71) for *P. mangurus* and 282 to 726 g (average 480 ± 115.37g) for *P. maculatus*. Seventeen *P. mangurus* and forty-two *P. maculatus* females were induced to spawn, which seven *P. mangurus* and thirty-four *P. maculatus* spawned successfully. The oocyte diameter obtained from the females ranged from 1224.940 ± 163.130 µm non-hydrated to 1541.187 ± 74.990 µm after hydration for *P. mangurus* and 650 ± 116.782 µm non-hydrated to 1161.462 ± 109.647 µm hydrated for *P. maculatus*. Giving rise to the following reproductive data: relative oocytes weight (117.373 ± 62.272 g), number of oocytes per gram (645 ± 80.060), total fecundity (67.770 ± 30.271), fertilization rate (90,58 ± 15,90 %), and hatching rate (72,07 ± 31,09%) for *P. mangurus* and relative oocytes weight (47.517 ± 25.256 g), number of oocytes per gram (3.415 ± 3.415), total fecundity (79.082 ± 57.622), fertilization rate (76,47 ± 22,31%), and hatching rate (39 ± 30,67 %) for *P. maculatus*.

There was no correlation between biometric and reproductive parameters for *P. mangurus* as show in table 3 and was found a weak correlation between the ovary area and relative extruded weight for *P. maculatus* as shown in table 4.

Discussion

The oocytes size wasn't evaluated by ultrasound due the fact neotropical fishes like *P. mangurus*, *P. maculatus*, *Zungaro jahu* (1600 µm) (Nogueira, Azevedo et al. 2012), *R. quelen* (16470 µm), *P. charus* (1660 µm) (Vieira Sampaio and Yoshimi 2006), *Astyanax altiparanae* (695.119 ± 3.20 µm) (Pereira-Santos, Yasui et al. 2016), have a smaller oocytes size when compared with cold water fishes like *Salmo salar* (Næve, Mommens et al. 2018), *Oncorhynchus mykiss* (Evans, Fitzpatrick et al. 2004) and *Scaphirhynchus albus* (Bryan, Wildhaber et al. 2007) being more difficult to visualize individuals oocyteswhich are only seen as a homogeneous egg mass into the ovaries.

In the reproductive season, the females have developed ovaries, which makes it easier to differentiate males from females by the ultrasound images. In others studies with *Maccullochella peelii peelii* (Newman, Jones et al. 2008), *Morone saxatilis* (Blythe, Helfrich et al. 1994) and *Cyprinus carpio* (Brizendine, Ward et al. 2018) was more difficult to differentiate males from females when the females had a non-developed ovaries, decreasing the accuracy in fish sexing through ultrasonography.

In this study, sexing by ultrasound was highly effective method as all females presented developed ovaries. High sexing accuracy using ultrasound has also been deported for *Morone saxatilis* (Blythe, Helfrich et al. 1994), *Maccullochella peelii peelii* (Newman, Jones et al. 2008) and *Scaphirhynchus platorynchus* (Colombo, Wills et al. 2004).

The ovaries of the female who spawn always presented a saculiform form filling a large space inside the abdominal cavity in ultrasound image. The spawned females of *P. mangurus* presented significate difference in ovary height and ovary area (p =

0.001 and p = 0.0006 respectively) when compared with the females who did not spawn. In addition, *P. maculatus* ovary height, width and area have a significant difference (p =

0.002, p = 0.017 and p = 0.006 respectively) from females who didn't spawn. The females who did not spawn can present an ovary with more irregular or circular borders.

Although they are both Siluriformes, they present a different body style, while the *P. mangurus* has the body wider than high and the ovaries higher than wide, the *P. maculatus* has the body higher than wide and the ovaries higher than wide. However, no biometric parameter was strong correlated with the relative extruded weight for both species.

In conclusion, the present study suggest the *P. mangurus* female with 1.061 ± 0.452 kg able to spawn should have 2.736 ± 0.793 cm of ovary height, 2.693 ± 0.477 cm of ovary width and 14.867 ± 6.873 cm^2 of ovary area and a *P. maculatus* female with 482.500 ± 118.366 g have 2.576 ± 0.583 cm of ovary height, 1.9917 ± 0.374 cm of ovary width and 8.738 ± 2.824 cm^2 of ovary area, where all female that spawned for both species presented through ultrasound image a circular-shaped ovaries and an egg mass in the papilla area. In addition, the ultrasound showed a highly effective method for sexing both species in reproductive season being an important noninvasive tool to apply in reproductive studies.

Acknowledments

The authors would like to thank the Instituto de Biociências (IBB) of Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil, in addition to the Laboratory of Fish Biotechnology, CEPTA/ICMBio, Pirassununga, Brazil.

References

Andrade-Talmelli, E., N. Fenerich-Verani and J. R. Verani (1999). "Fator de condição relativo (Kn): um critério para selecionar fêmeas de piabanha Brycon insignis (Steindachner, 1876)(Pisces: Bryconidae), para indução reprodutiva." Bol Inst de Pesca 25: 95-99.

Arashiro, D. R., G. S. Yasui, L. L. Calado, N. F. do Nascimento, M. P. dos Santos, S. C. A. do Santos, N. Levy-Pereira, P. S. Monzani, D. H. Siqueira-Silva and J. A. Senhorini (2018). "Synchronizing developmental stages in Neotropical catfishes for application in germ cell transplantation." Zygote: 1-14.

Blythe, B., L. A. Helfrich, W. Beal, B. Bosworth and G. S. Libey (1994). "Determination of sex and maturational status of striped bass (Morone saxatilis) using ultrasonic imaging." Aquaculture 125(1-2): 175-184.

Bressan, P. M., M. C. M. Kierulff and A. M. Sugieda (2009). "Fauna ameaçada de extinção no Estado de São Paulo." São Paulo: Fundação Parque Zoológico de São Paulo, Secretaria do Meio Ambiente.

Brizendine, M. E., D. L. Ward and S. A. Bonar (2018). "Effectiveness of Ultrasonic Imaging for Evaluating Presence and Maturity of Eggs in Fishes in Remote Field Locations." North American Journal of Fisheries Management 38(5): 1017-1026.

Bryan, B. J., M. Wildhaber, D. Papoulias, A. DeLonay, D. Tillitt and M. Annis (2007). "Estimation of gonad volume, fecundity, and reproductive stage of shovelnose sturgeon using sonography and endoscopy with application to the endangered pallid sturgeon." Journal of Applied Ichthyology 23(4): 411-419.

Chiotti, J. A., J. C. Boase, D. W. Hondorp and A. S. Briggs (2016). "Assigning sex and reproductive stage to adult lake sturgeon using ultrasonography and common morphological measurements." North American Journal of Fisheries Management 36(1): 21-29.

Colombo, R. E., P. S. Wills and J. E. Garvey (2004). "Use of ultrasound imaging to determine sex of shovelnose sturgeon." North American Journal of Fisheries Management 24(1): 322-326.

CREPALDI, D. and M. ROTTA (2007). "Uso do ultra-som em programas de reprodução de peixes nativos." Embrapa Pantanal-Comunicado Técnico (INFOTECA-E).

do Nascimento, N. F., M. Pereira-Santos, L. H. Piva, B. Manzini, T. Fujimoto, J. A. Senhorini, G. S. Yasui and L. S. O. Nakaghi (2017). "Growth, fatty acid composition, and reproductive parameters of diploid and triploid yellowtail tetra Astyanax altiparanae." Aquaculture. du Colombier, S. B., L. Jacobs, C. Gesset, P. Elie and P. Lambert (2015). "Ultrasonography as a non-invasive tool for sex determination and maturation monitoring in silver eels." Fisheries research 164: 50-58.

Evans, A. F., M. S. Fitzpatrick and L. K. Siddens (2004). "Use of ultrasound imaging and steroid concentrations to identify maturational status in adult steelhead." North American Journal of Fisheries Management 24(3): 967-978.

Ferraz, E. and V. Cerqueira "O USO DE TERAPIAS CRÔNICAS DE INDUÇÃO HORMONAL COM HCG E FSH, NA MATURAÇÃO GONADAL DO ROBALO-FLECHA, Centropomus undecimalis, EM CATIVEIRO."

Froese, R. and D. Pauly. (2015). "FishBase. World Wide Web electronic publication.

." Retrieved 06/2015, 2015, from www.fishbase.org.
Kohn, Y. Y., P. M. Lokman, A. Kilimnik and J. E. Symonds (2013). "Sex identification in captive hapuku (Polyprion oxygeneios) using ultrasound imagery and plasma levels of vitellogenin and sex steroids." Aquaculture 384: 87-93.
MURGAS[1], L. D. S., V. de Oliveira FELIZARDO[1], M. R. FERREIRA[1], G. C. VERAS[1], E. de Souza and D. A. d. J. P. ANDRADE[1] (2012). "Eficiência reprodutiva em espécies nativas de peixes de água doce."
Næve, I., M. Mommens, A. Arukwe and E. Kjørsvik (2018). "Ultrasound as a noninvasive tool for monitoring reproductive physiology in female Atlantic salmon (Salmo salar)." Physiological reports 6(9): e13640.
Newman, D. M., P. L. Jones and B. A. Ingram (2008). "Sexing accuracy and indicators of maturation status in captive Murray cod Maccullochella peelii peelii using noninvasive ultrasonic imagery." Aquaculture 279(1-4): 113-119.
Nogueira, L. B., P. G. Azevedo, M. R. Canelhas, A. G. Bedore, J. M. Lopes and H. P. Godinho (2012). "Induced spawning and early ontogeny in hatchery-reared catfish Zungaro jahu (Siluriformes: Pimelodidae)." Neotropical Ichthyology 10(1): 89-98.
Perazza, C. A., F. L. V. PINAFFI, L. A. Silva and A. W. S. HILSDORF (2015). "Evaluation of ultrasound imaging to predict loin eye area in tambaqui." BOLETIM DO INSTITUTO DE PESCA 41: 803-809.
Pereira-Santos, M., G. Yasui, P. Xavier, A. N. de Macedo, N. do Nascimento, T. Fujimoto, J. Senhorini and L. Nakaghi (2016). "Morphology of gametes, postfertilization events and the effect of temperature on the embryonic development of Astyanax altiparanae (Teleostei, Characidae)." Zygote (Cambridge, England): 1-13.
Petochi, B. T., P. Di Marco, V. Donadelli, A. Longobardi, I. Corsalini, D. Bertotto, M. Finoia and G. Marino (2011). "Sex and reproductive stage identification of sturgeon hybrids (Acipenser naccarii× Acipenser baerii) using different tools: ultrasounds, histology and sex steroids." Journal of Applied Ichthyology 27(2): 637-642.
Piferrer, F., A. Beaumont, J.-C. Falguiere, M. Flajshans, P. Haffray and L. Colombo (2009). "Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment." Aquaculture 293(3-4): 125-156.
São Paulo (2014). DECRETO Nº 60.133, DE 7 DE FEVEREIRO DE 2014.
Toussaint, A., N. Charpin, S. Brosse and S. Villéger (2016). "Global functional diversity of freshwater fish is concentrated in the Neotropics while functional vulnerability is widespread." Scientific reports 6: 22125.
Vieira Sampaio, E. and S. Yoshimi (2006). "Biologia reprodutiva e desova induzida de duas espécies de bagres (Osteichthyes: Siluriformes) da bacia do rio São Francisco." Acta Scientiarum. Biological Sciences 28(3).
Woynarovich, E. and L. Horváth (1983). A propagação artificial de peixes de águas tropicais: manual de extensão. Brasília, FAO/CODEVASP/CNPq.
Yasui, G., T. Fujimoto, S. Sakao, E. Yamaha and K. Arai (2011). "Production of loach () germ-line chimera using transplantation of primordial germ cells isolated from cryopreserved blastomeres." Journal of animal science 89(8): 2380-2388.
Yasui, G. S., J. A. Senhorini, E. Shimoda, M. Pereira-Santos, L. S. O. Nakaghi, T. Fujimoto, L. Arias-Rodriguez and L. A. Silva (2015). "Improvement of gamete quality and its short-term storage: an approach for biotechnology in laboratory fish." Animal 9(03): 464-470.

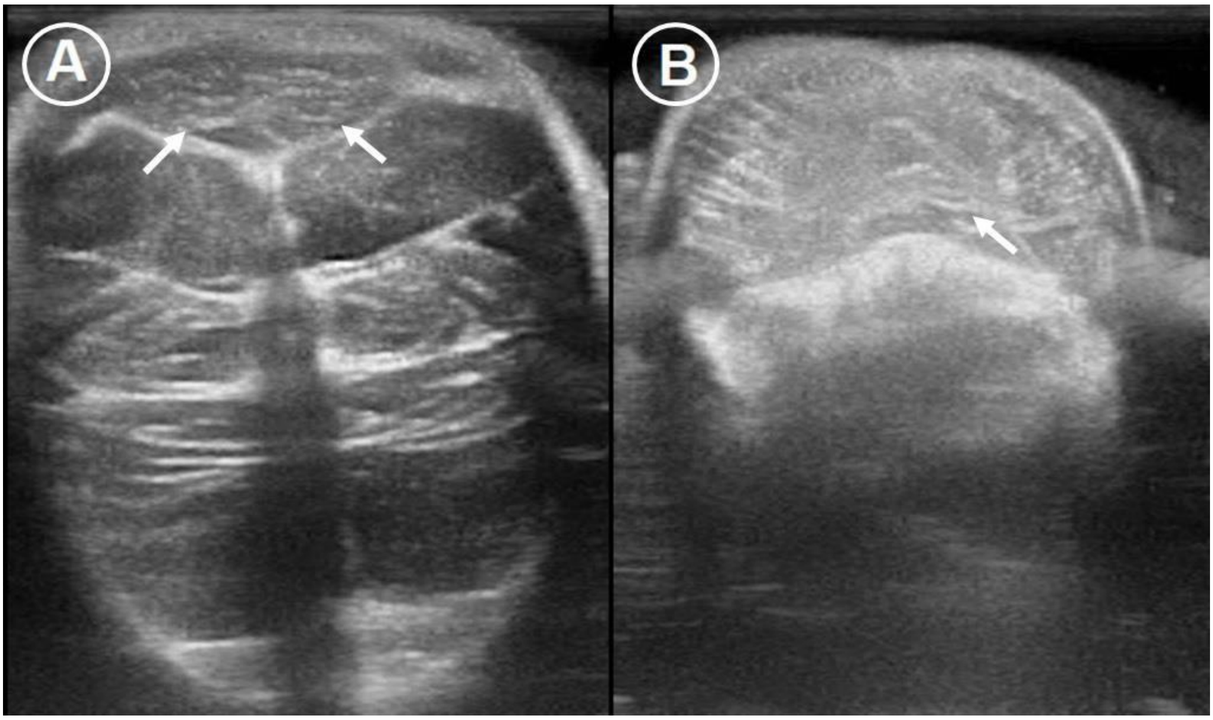

Fig. 1. Testis from males of *P. mangurus* and *P. maculatus.* (A) Testis of *P. mangurus* (arrows indicate the testis), (B) Testis of *P. maculatus* (arrow indicate the testis).

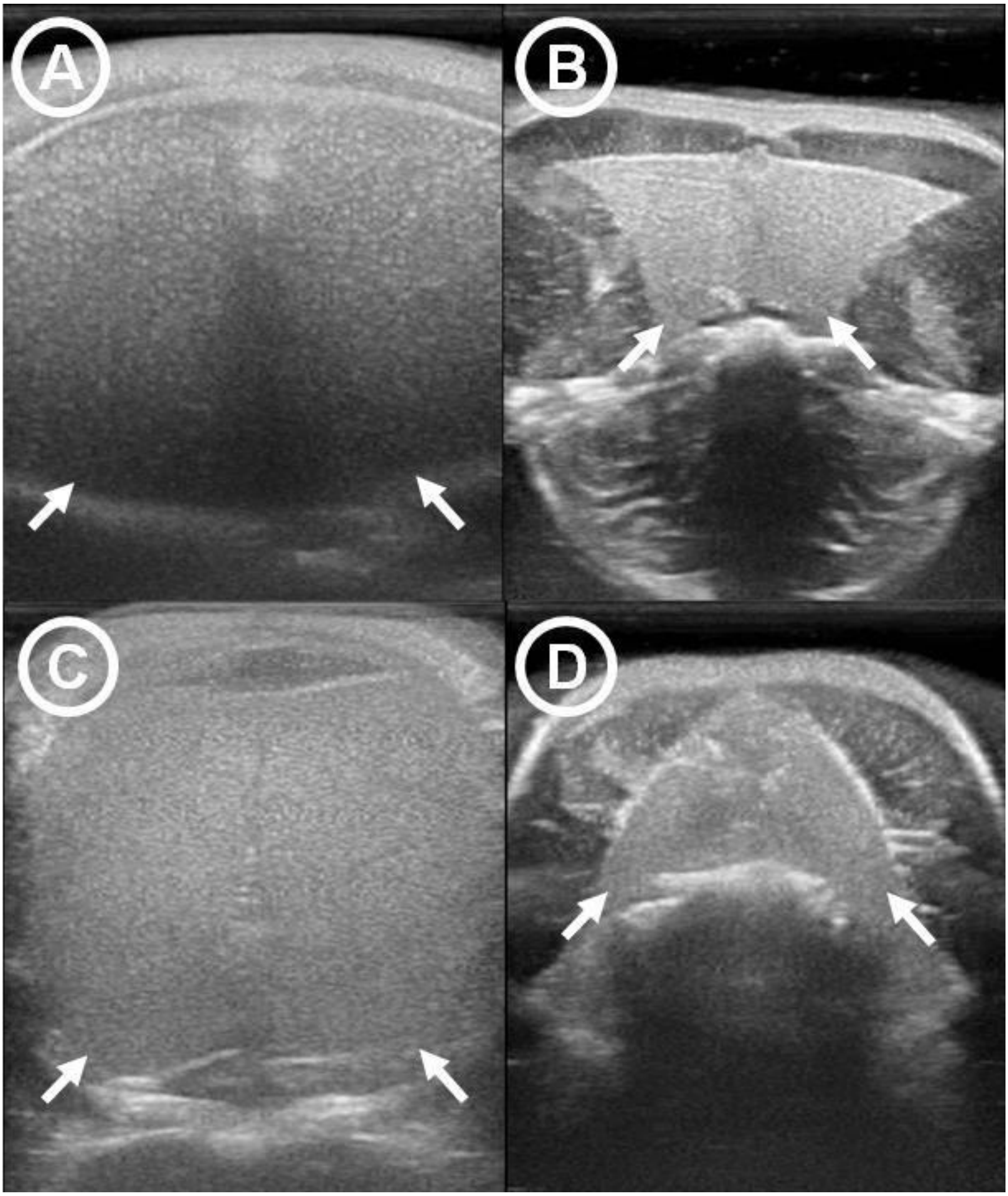

Fig. 2. Ovaries of females who spawned and do not spawned from *P. mangurus* and *P. maculatus.* (A) Ovaries of *P. mangurus* that spawned (arrows indicate the ovaries), (B) Ovaries of *P. mangurus* that not spawned (arrows indicate the ovaries), (C) Ovaries of *P. maculatus* that spawned (arrows indicate the ovaries), (D) Ovaries of *P. maculatus* that not spawned (arrows indicate the ovaries).

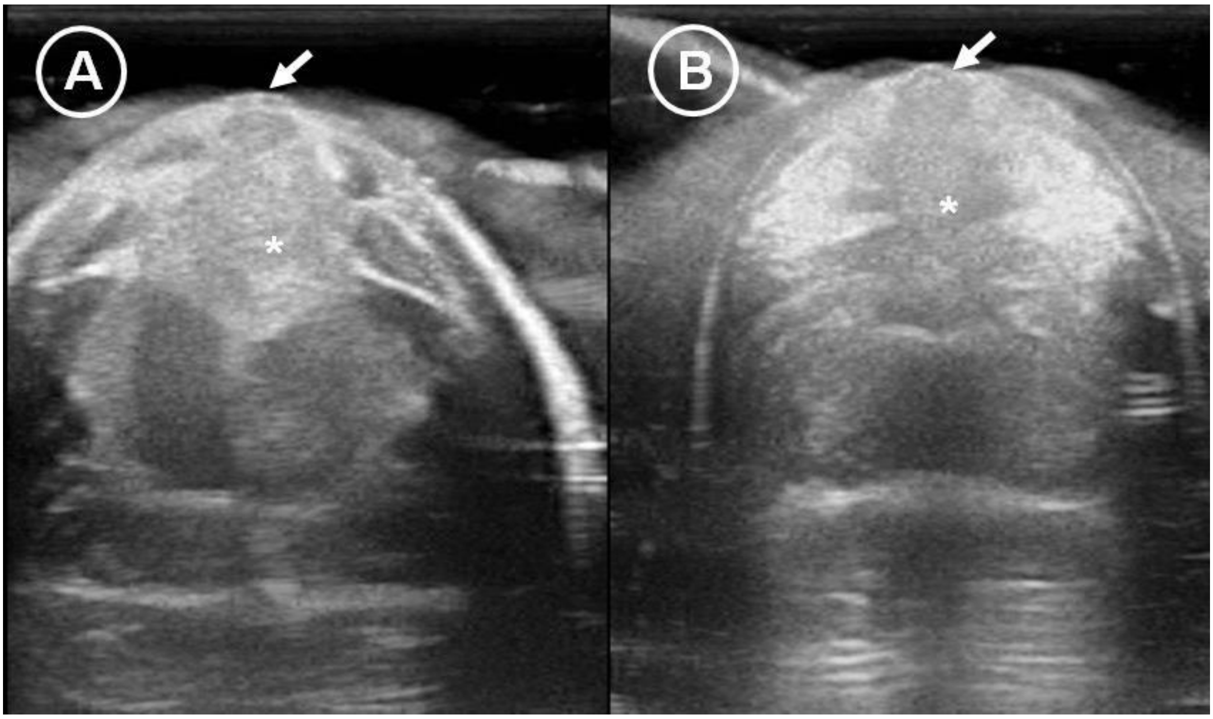

Fig. 3. Fig. 3. Papillae of females of *P. mangurus* and *P. maculatus* that spawned. (A) Papillae of *P. mangurus* (Arrow indicates the papilla and asterisk indicate the egg mass), (B) papilla of *P. maculatus* (arrow indicate the papilla and asterisk indicate the egg mass).

Table 1. Average, minimum and maximum values of biometric parameters of females of *P. mangurus* induced to spawn.

	Number of females		Weight (kg)	Length (cm)	Height (cm)	Width (cm)	Ovary height (cm)	Ovary width (cm)	Ovary area (cm^2)
Spawned females	7	Minimum	0.325	25.3	5	5.4	1.62	2.05	5.6
		Maximum	1.472	41.8	9.2	12.3	4.03	3.3	25.69
		Average	1.061	34.886	7.257	8.586	2.736	2.693	14.867
		SD	0.452	5.291	1.560	2.346	0.793	0.477	6.873
Did not spawned	10	Minimum	0.329	24.5	4.5	4.9	0.57	0.85	0.6
		Maximum	0.875	35	8	9.5	2.37	2.7	9.32
		Average	0.590	29.410	5.810	6.820	1.446	2.254	4.550
		SD	0.218	3.989	1.033	1.490	0.609	0.935	2.739
		P - Value	0.011	0.027	0.023	0.075	0.001	0.274	0.0006

Significance ($P < 0.05$) SD
= Standard deviation.

Table 2. Average, minimum and maximum values of biometric parameters of females of *P. maculatus* induced to spawn.

	Number of females		Weight (g)	Lenght (cm)	Height (cm)	Width (cm)	Ovary height (cm)	Ovary width (cm)	Ovary area cm^2
Spawned females	34	Minimum	282	23.4	5.4	4.1	1.1	1,17	2.33
		Maximum	726	33.1	9.2	6.7	3.64	2.8	13.58
		Average	482.500	28.329	7.626	5.261	2.576	1.917	8.738
		SD	118.366	2.426	0.972	0.756	0.583	0.374	2.824
Did not spawned	8	Minimum	382	24.3	6.4	4.4	0.47	0.5	0.59
		Maximum	790	33.2	8.8	6.6	3.05	2.67	10.74
		Average	485.25	28.275	7.575	5.475	1.800	1.481	5.376
		SD	137.264	2.679	0.889	0.74	0.788	0.693	5.527
		P - Value	0.954	0.955	0.8919	0.478	0.002	0.017	0.006

Significance ($P < 0.05$).

SD = Standard deviation.

Table 3. Pearson correlation for biometric and reproductive parameters for *P. mangurus*.

Variables		Observations	Correlation	Significance
Lw	Wg	7	0.9895	0.0000
Lw	Ex	7	-0.4593	0.2997
Wg	Ex	7	-0.5148	0.2370
L	Ex	7	-0.4476	0.3138
W	Ex	7	-0.5671	0.1842
H	Ex	7	-0.4954	0.2581
Oh	Ex	7	-0.2794	0.5438
Ow	Ex	7	-0.0183	0.9689
Oa	Ex	7	-0.3099	0.4987

Obs: Lw = length/weight; Wg = weight; Ex = relative extruded weight; L = length; W = Width; H = height; Oh = ovary height; Ow = ovary width; Oa = Ovary area.
Significance: $P < 0.05$

Table 4. Pearson correlation for biometric and reproductive parameters of *P. maculatus*.

Variables		Observations	Correlation	Significance
Lw	Wg	34	0.9598	0.0000
Lw	Ex	34	0.3086	0.0757
Wg	Ex	34	0.2784	0.1108
L	Ex	34	0.174	0.3240
W	Ex	34	0.3231	0.0623
H	Ex	34	0.2730	0.1182
Oh	Ex	34	0.3062	0.0781
Ow	Ex	34	0.2516	0.1511
Oa	Ex	34	0.4619	0.0059

Obs: Lw = length/weight; Wg = weight; Ex = relative extruded weight; L = length; W = Width; H = height; Oh = ovary height; Ow = ovary width; Oa = Ovary area. Significance $P < 0.05$

33

Capitulo 2: **Ultrasound a tool for selection of females able to spawn in neotropical characiform**

Running title: Ultrasound in characiform species reproduction

Keywords: Reproduction, Characidae, Ovary, Non-invasive, *Pochilodus lineatus.*.

Abstract

The aim of this study was to stablish a methodology to female able to spawn selection and sexing by ultrasonography for the neotropical characiform *P. lineatus.* Fish were anesthetized and then measured for its weight, length, height, width of the body. Ultrasonography was made in lateral region measuring ovary height, ovary width and ovary area, giving rise to biometric data: weight 773 ± 292.91 g, lenght 30.86 ± 4.394 cm, height 10.593 ± 1.746 cm, width 5.153 ± 1.208 cm, ovary height 4.788 ± 0.703cm, ovary width 1.504 ± 0.303 cm and ovary area 11.657 ± 3.594 cm^2 for *P. lineatus* who spawn. After ultrasonography, fish were induced to spawn, being thirty seven females were only fifteen spawned, giving rise to the following reproductive: data: relative oocytes weight (225.867 ± 351.382 g), number of oocytes per gram (1122.084 ± 389.508), total fecundity (67.770 ± 30.271), fertilization rate (90,799 ± 12.775%), and hatching rate (67.288 ± 163.427%). The ultrasound presented a highly accuracy for sexing at the reproductive season and can be applied in reproductive studies as a noninvasive technique replacing invasive methods that can compromise de reproductive success.

Introduction

The Neotropical region has the largest ichthyofauna in the world, with approximately 4400 fish species, with the order of Characiformes, second largest in number, with approximately 1847 species, only behind the order of Siluriformes (Froese and Pauly 2015, Toussaint, Charpin et al. 2016).

Among the native Characifores fish species with migratory behavior, we can highlight the *Prochilodus lineatus* (Valenciennes, 1837), is a fish species which can pass the 7kg and present ecological relevance in their natural environment serving as food source for birds, reptiles and mammals. Present easy reproduction and breeding in captivity, with iliophage food habit (Moraes, Barbola et al. 1997), easily accepted artificial diets (Galdioli, Hayashi et al. 2002) and they meat appreciated by the riverside population.

P. lineatus larvaes are widely used in fish farms for first feeding of many carnivorous fish of commercial interest and endangered species like *Salminus brasiliensis* (Schütz and Nuñer 2007), *Brycon orbignyanus* (Pereira and de Oliveira Nuñer 2003), *Brycon spp.* (Costa, Pedreira et al. 2013)**,** *Pseudopimelodus mangurus* (Arashiro, Yasui et al. 2018)*,* and *Steindachneridion scriptum* (Schütz, Weingartner et al. 2008). For these characteristics is one of the most produced native species in Brazil (às Micro and Empresas–SEBRAE 2015) being an important native fish species.

P. lineatus performs total spawning and reproduce only once a year during the period of piracema (Andrade and Yasui 2003)**,** the broodstock selection is a key step for the reproductive success, since not all females judged as apt to spawn through the external morphological characteristics like reddish papilla, soft and bulging abdomen respond to the hormonal induction.

With the approach of the reproductive season of the seasonal reproduction fish, the gonads are more developed and some methods are adopted to evaluate the gonadal development, such as histology (Petochi, Di Marco et al. 2011, do Nascimento, Pereira-Santos et al. 2017), ovarian biopsy(Coward and Bromage 2002), intra ovarian oocyte cannulation (Andrade-Talmelli, Fenerich-Verani et al. 1999) and ultrasonography (Blythe, Helfrich et al. 1994, Colombo, Wills et al. 2004, Evans, Fitzpatrick et al. 2004, Newman, Jones et al. 2008, Kohn, Lokman et al. 2013, Chiotti, Boase et al. 2016, Brizendine, Ward et al. 2018).

An non-lethal method like the ultrasonography becomes very important to evaluate the reproductive aspects of the fish, and may substitute other invasive methods that may compromise fish health and reproductive success (Blythe, Helfrich et al. 1994, CREPALDI and ROTTA 2007). The ultrasonography besides being noninvasive technique also allows sexing (Newman, Jones et al. 2008, Kohn, Lokman et al. 2013, Brizendine, Ward et al. 2018) and indicates if the female has a mature ovary and is able to spawn.

In the case of most migratory native species, it is only possible to perform sexing during the reproductive period, where the females have a reddish papilla, a soft and bulging abdomen (Solis-Murgas, Felizardo et al., 2011) but it is not possible to know by these characteristics if the ovary is mature and ready for spawning.

Establishing the ovary profile of the female able to spawn through ultrasound imaging may lead to an increase in the rate of induced females who respond to hormonal induction, since not all induced females spawn when selected through external characteristics such as bulging and soft abdomen to the touch and reddish papilla (Zohar 1989).

Considering the aspects above the aim of this study was to establish the ovary profile of the female able to spawn for *P. lineatus* by ultrasound.

Material and methods

Origin of broodstock and ultrasound

All procedures were conduced in Laboratory of Fish Biotechnology (ICMBio/CEPTA, Pirassununga City, São Paulo State, Brazil). The experiments were conducted in accordance with the Ethics Committee for the Use of Laboratory Animals of the National Center for Research Conservation of Aquatic Biodiversity (CEUA / CEPTA;# 010/2015).

Adult males and females of *P. lineatus* were captured in earth tanks in CEPTA/ICMBio and selected by external morphological characteristics such as reddish papilla, bulging and soft abdomen to the touch and reddish papilla, during the reproductive season between months of October to February.

Thirty-seven females and seventy-four males *P. lineatus* were selected to reproduction, subsequently fish were anesthetized with clove oil at 150 mg L^{-1} (Biodinamica, Ibiporã, Brazil) and ultrasonography were done with the use of a digital ultrasound (Esaote S.p.a, MyLab 30 Vet, Genova, Italy) in B-mode (gray scale) with a linear multi-frequency transducer set at the frequency of 7.5 MHz and 60 mm wide beam field. All exams were made in the lateral region, below the middle of the dorsal fin to evaluate the ovaries. Data were documented in photos and videos to posterior analyses. Of all ovaries were measured area (cm^2), length and height.

After the ultrasonography, the females were weighted and measured the length, height and width of all individuals used in the experiment.

Induced spawning and artificial fertilization

Fish were induced with crude carp pituitary extract (CCPE) 5.5 mg kg^{-1} which was divided into two doses, 0.5 mg kg^{-1} and 5 mg kg^{-1} 8-hours afterwards. The males were injected with a single dose of CCPE at 2 mg kg^{-1} at the same time as the females received the second dose.

The fish were stocked for semi-natural spawning in the proportion of two males to one female in boxes of 3.000 l with continuous water renewal. From six to eight hours after the second dose the females began to spawn in the box and a small aliquot was separated to calculate fertilization and hatching. Subsequently they were withdrawn from the box, anesthetized with clove oil at 150 mg L^{-1} (Biodinamica, Ibiporã, Brazil) and then stripped the rest of the oocytes without being hydrated in a dry glass container. The weight of oocytes was measured, and a small aliquot was weighed and fixed in 2% glutaraldehyde in Dulbecco's PBS (Sigma #D5773, St. Louis, USA) in order to calculate the number of oocytes per gram.

The resultant embryos were incubated at 26 °C, which is the best temperature to incubate *P. lineatus* during early development (Coelho, Isaac et al. 2019).

Statistics

Data are shown as the mean ± standard deviation. Data were obtained with different females and each egg batches. Correlation equation was analyzed with Pearson and the other analysis with variance (ANOVA), followed by a Tukey multiple range test. In all analyses, the software STATISTICA (10.0, StatSoft, USA) was used with the probability set at 0.05.

Results

Ultrasound

The lateral view allowed a good visualization of the ovaries and the sexing accuracy for females selected to spawn by external morphologic characteristics was 100% proven by ovaries visualization with ultrasound images, where all females that not spawn shown a undeveloped ovaries with with irregular shape (FIG. 1A) and all females that spawn shown a developed ovary with a circular shape (FIG. 2A). As shown in Table 1 there was no significant difference between the weight, length, height, ovary height and ovary area from females of *P. lineatus* who spawned to females who didn't spawned.

The ventral view with the use of the ultrasound didn't allowed a good visualization of the ovaries (FIG. 2A) but allowed a good visualization form the papilla area, where all females who had a mature ovaries and spawned presented an egg mass under the papilla, as shown in (FIG. 2B).

Reproduction

The weight of the *P. lineatus* females who spawn ranged from 293 to 1280 g (average 773.6 ± 292.911). Fifty-teen from thirty-seven females spawned successfully and the oocyte diameter obtained from the females ranged from 1159.301 ± 240.670 µm non-hydrated to 3637.801 ± 389.508 µm after hydration, giving rise to the following reproductive data: relative oocytes weight (224.712 ± 213.977 g), number of oocytes per gram (1122.084 ± 163.427), total fecundity (225.867 ± 351.382), fertilization rate (90.799 ± 12.775 %), and hatching rate (67.288 ± 163.427%) .

There was no correlation between biometric and reproductive parameters for *P. lineatus* as show in table 2.

Discussion

The ultrasound allowed visualizing different degrees of maturation of the ovaries in the reproductive season, which helps to differentiate males from females in other migratory fish species like *P. maculatus* and *P. mangurus.* In others studies was difficult to differentiate males from females when females had a non-developed ovaries like with *Cyprinus carpio* (Brizendine, Ward et al. 2018*)*. However, in this study the ultrasound was highly effective for *P. lineatus* sexing by the fact all females had a developed ovaries, which high sexing accuracy using ultrasound has been reported for *Morone saxatilis* (Blythe, Helfrich et al. 1994)*, Scaphrirhynchus platorchus* (Colombo, Wills et al. 2004).

The ultrasound did not allow to measure the oocytes with precision, since the oocytes of neotropical migratory species like *Prochilodus lineatus,* have very smaller oocytes when compared to other characiform species like *Salmo salar* (Næve, Mommens et al. 2018) and *Oncorhynchus mykiss* (Evans, Fitzpatrick et al. 2004).

The shape of the body from the Characiform *P. lineatus* is thinner when compared to the siluriform species like *P. mangurus* and *P. maculatus* however the shape of the ovaries from spawned females was circular and occupied a large space within the abdominal cavity of the fish, which pointed out to be a mature ovary as well for both siluriforms and for the characiform.

Although no correlation were observed between the biometric parameters with relative extruded weight for *P. lineatus.*

In conclusion, the present study suggest the *P. lineatus* female with 0.773 ± 0.292 kg able to spawn should have 4.788± 0.703 cm of ovary height, 1.546 ± 0.303 cm of ovary width and 11.657 ± 3.594 cm^2 of ovary area, where all female that spawned presented through ultrasound image a circular-shaped ovaries and an egg mass in the papilla area. In addition, the ultrasound showed a highly effective method for sexing and evaluate the development of the ovaries for this Characiform specie in reproductive season being an important non-invasive tool to apply in reproductive studies.

Acknowledments

The authors would like to thank the Instituto de Biociências (IBB) of Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil, in addition to the Laboratory of Fish Biotechnology, CEPTA/ICMBio, Pirassununga, Brazil.

References

Andrade-Talmelli, E., N. Fenerich-Verani and J. R. Verani (1999). "Fator de condição relativo (Kn): um critério para selecionar fêmeas de piabanha Brycon insignis (Steindachner, 1876)(Pisces: Bryconidae), para indução reprodutiva." Bol Inst de Pesca **25**: 95-99.

Andrade, D. R. and G. S. Yasui (2003). "O manejo da reprodução natural e artificial e sua importância na produção de peixes no Brasil." Revista Brasileira de Reprodução Animal **27**(2): 166-172.

Arashiro, D. R., G. S. Yasui, L. L. Calado, N. F. d. Nascimento, M. P. d. Santos, S. C. Alves do Santos, N. Levy-Pereira, P. S. Monzani, D. H. Siqueira-Silva and J. A. Senhorini (2018). "Synchronizing developmental stages in Neotropical catfishes for application in germ cell transplantation." Zygote: 135-148. às Micro, S. B. d. A. and P. Empresas–SEBRAE (2015). Aquicultura no Brasil: série estudos mercadológicos, Brasília.

Blythe, B., L. A. Helfrich, W. Beal, B. Bosworth and G. S. Libey (1994). "Determination of sex and maturational status of striped bass (Morone saxatilis) using ultrasonic imaging." Aquaculture **125**(1-2): 175-184.

Brizendine, M. E., D. L. Ward and S. A. Bonar (2018). "Effectiveness of Ultrasonic Imaging for Evaluating Presence and Maturity of Eggs in Fishes in Remote Field Locations." North American Journal of Fisheries Management **38**(5): 1017-1026.

Chiotti, J. A., J. C. Boase, D. W. Hondorp and A. S. Briggs (2016). "Assigning sex and reproductive stage to adult lake sturgeon using ultrasonography and common morphological measurements." North American Journal of Fisheries Management **36**(1): 21-29.

Coelho, G. C., S. Y. Isaac, T. M. Mira-López, P. S. Monzani, D. R. Arashiro, T. Fujimoto, J. A. Senhorini and G. S. Yasui (2019). "Preparation of a fish embryo for micromanipulation: staging of development, removal of the chorion and traceability of PGCs in Prochilodus lineatus." International Journal of Developmental Biology **63**(12): 57-65.

Colombo, R. E., P. S. Wills and J. E. Garvey (2004). "Use of ultrasound imaging to determine sex of shovelnose sturgeon." North American Journal of Fisheries Management **24**(1): 322-326.

Costa, D. C., M. M. Pedreira, M. V. Coraspe-Amaral, A. E. Dupim, U. Barral and E. Duarte (2013). "Larvicultura de piabanha-do-pardo em aquários de cores diferentes." Pesquisa Agropecuária Brasileira **48**(8): 1005-1011.

Coward, K. and N. R. Bromage (2002). "Quantification of ovarian condition in fish: a safer, more precise alternative to established methodology." Aquatic Living Resources **15**(4): 259-261.

CREPALDI, D. and M. ROTTA (2007). "Uso do ultra-som em programas de reprodução de peixes nativos." Embrapa Pantanal-Comunicado Técnico (INFOTECA-E).

do Nascimento, N. F., M. Pereira-Santos, L. H. Piva, B. Manzini, T. Fujimoto, J. A. Senhorini, G. S. Yasui and L. S. O. Nakaghi (2017). "Growth, fatty acid composition, and reproductive parameters of diploid and triploid yellowtail tetra Astyanax altiparanae." Aquaculture.

Evans, A. F., M. S. Fitzpatrick and L. K. Siddens (2004). "Use of ultrasound imaging and steroid concentrations to identify maturational status in adult steelhead." North American Journal of Fisheries Management **24**(3): 967-978.

Froese, R. and D. Pauly. (2015). "FishBase. World Wide Web electronic publication. ." Retrieved 06/2015, 2015, from www.fishbase.org.

Galdioli, E. M., C. Hayashi, C. M. Soares, V. R. B. Furuya and A. Faria (2002). "Substituição da proteína do farelo de soja pela proteína do farelo de canola em rações para alevinos de curimbatá (Prochilodus lineatus V.)." Revista Brasileira de Zootecnia **31**(2): 552-559.

Kohn, Y. Y., P. M. Lokman, A. Kilimnik and J. E. Symonds (2013). "Sex identification in captive hapuku (Polyprion oxygeneios) using ultrasound imagery and plasma levels of vitellogenin and sex steroids." Aquaculture **384**: 87-93.

Moraes, M., I. F. Barbola and E. Guedes (1997). "Alimentação e relações morfológicas com o aparelho digestivo do" curimbatá", Prochilodus lineatus (Valenciennes)(Osteichthyes, Prochilodontidae), de uma lagoa do sul do Brasil." Revista brasileira de zoologia **14**(1): 169-180.

Næve, I., M. Mommens, A. Arukwe and E. Kjørsvik (2018). "Ultrasound as a noninvasive tool for monitoring reproductive physiology in female Atlantic salmon (Salmo salar)." Physiological reports **6**(9): e13640.

Newman, D. M., P. L. Jones and B. A. Ingram (2008). "Sexing accuracy and indicators of maturation status in captive Murray cod Maccullochella peelii peelii using noninvasive ultrasonic imagery." Aquaculture **279**(1-4): 113-119.

Pereira, A. S. and A. P. de Oliveira Nuñer (2003). "Utilização de diferentes densidades, dietas e formatos de tanque na larvicultura da piracanjuba, Brycon orbignyanus Valenciennes, 1849 (Characiformes, Characidae." Acta Scientiarum. Biological Sciences **25**(1): 55-61.

Petochi, B. T., P. Di Marco, V. Donadelli, A. Longobardi, I. Corsalini, D. Bertotto, M. Finoia and G. Marino (2011). "Sex and reproductive stage identification of sturgeon hybrids (Acipenser naccarii× Acipenser baerii) using different tools: ultrasounds, histology and sex steroids." Journal of Applied Ichthyology **27**(2): 637-642.

Schütz, J. H. and A. P. d. O. Nuñer (2007). "Growth and survival of dorado Salminus brasiliensis (Pisces, Characidae) post-larvae cultivated with different types of food and photoperiods." Brazilian Archives of Biology and Technology **50**(3): 435-444. Schütz, J. H., M. Weingartner, E. Zaniboni-Filho and A. d. O. NUÑER (2008). "Crescimento e sobrevivência de larvas de suruvi Steindachneridion scriptum nos primeiros dias de vida: influência de diferentes alimentos e fotoperíodos." Boletim do Instituto de Pesca **34**(3): 443-451.

Toussaint, A., N. Charpin, S. Brosse and S. Villéger (2016). "Global functional diversity of freshwater fish is concentrated in the Neotropics while functional vulnerability is widespread." Scientific reports **6**: 22125.

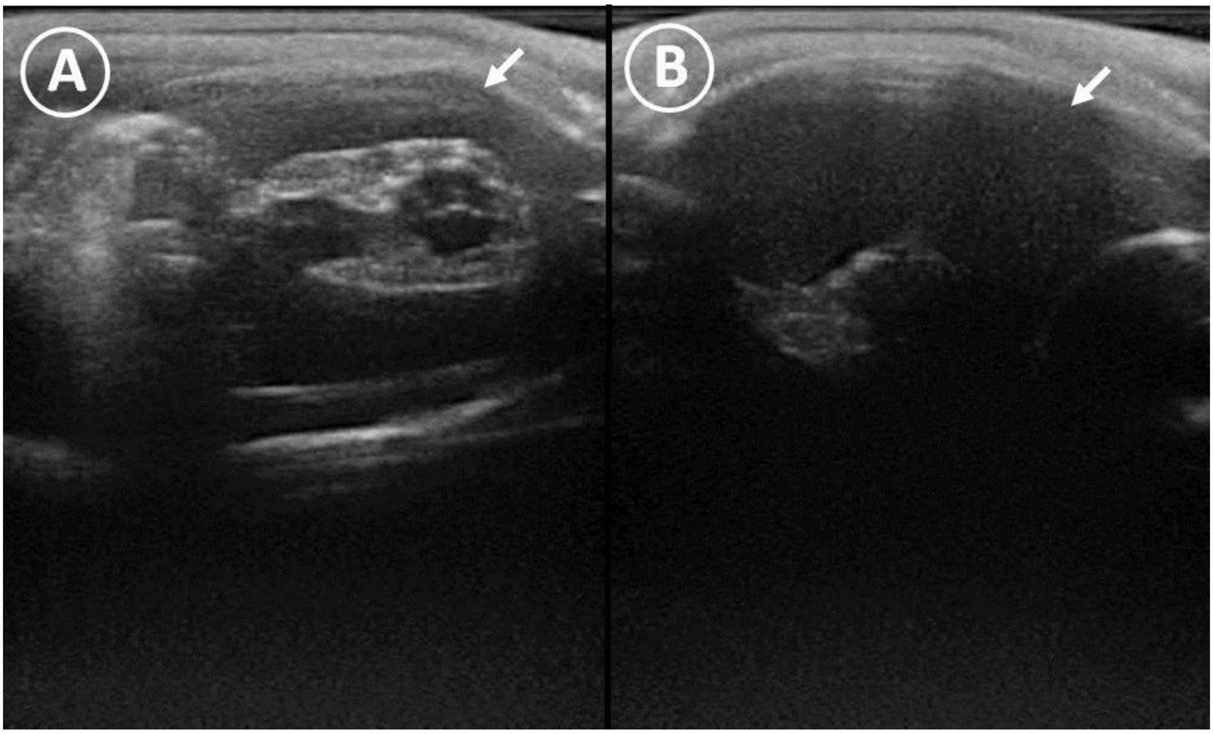

Fig. 1. Ovaries of females who spawned and do not spawned from *P. lineatus* (A) Ovariy of *P. lineatus* that not spawned (arrow indicate the ovary), (A) Imature ovary of *P. lineatus* that spawned (arrows indicate the ovary), (B) Mature ovary of *P. lineatus* that spawned (arrows indicate the ovary)

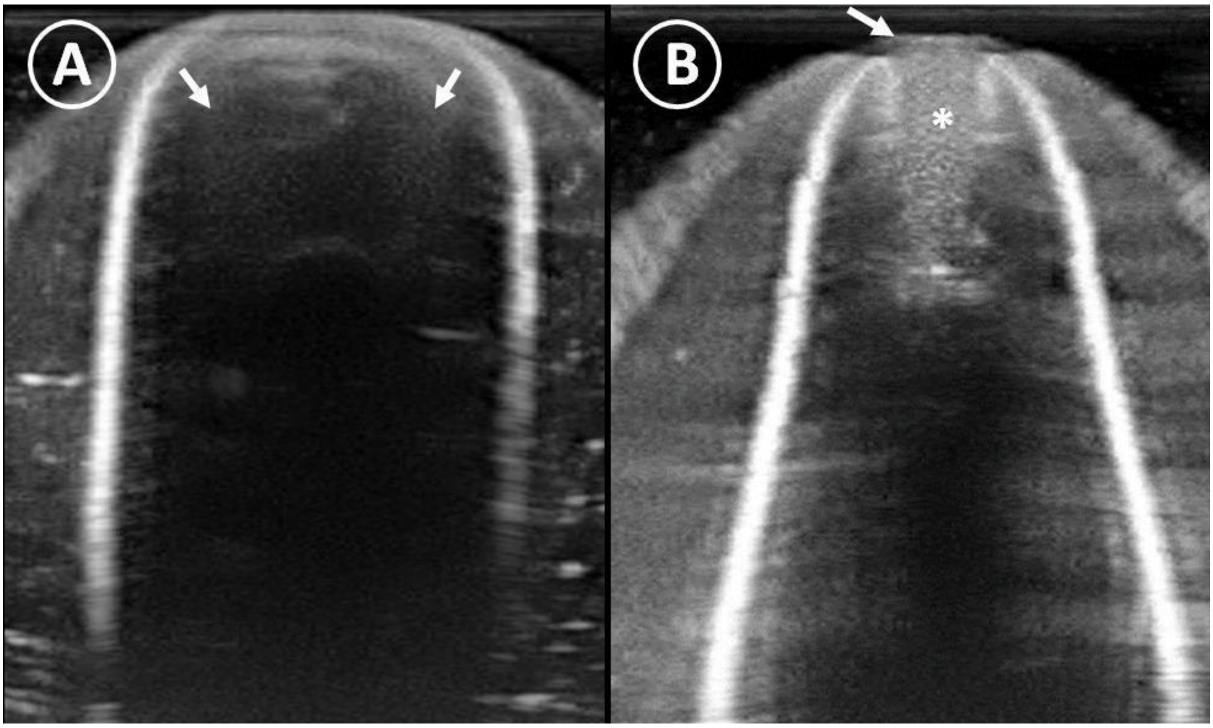

Fig. 2. (A)Ventral view of females of *P. lineatus* that spawned (Arrows indicates the ovaries), (B) Ventral view of female of papilla of *P. lineatus* (arrow indicate the papilla and asterisk indicate the egg mass).

Table 1. Average, maximum e minimum values of biometric parameters of females of *P. lineatus* induced to spawn.

	Number of females		Weight (g)	Lenght (cm)	Height (cm)	Width (cm)	Ovary width (cm)	Ovary height (cm)	Ovary area cm^2
Spawned females	15	Maximum	1280	36.8	13.4	8.5	5.96	2.09	18.94
		Minimum	293	22.4	7.5	3.6	3.68	1.30	5.7
		Average	773.6	30.68667	10.593	5.153	4.788667	1.546	11.657333
		SD	292.911	4.39478	1.7466	1.208	0.703338	0.3032161	3.5945007
Did not spawned	22	Maximum	2324	41.5	17	8.1	7.59	2.21	19.76
		Minimum	236	21.2	7.1	3.1	2.54	0.5	2.21
		Average	664.364	28.83182	10.36818	4.709091	4.7613636	1.3445455	10.786818
		SD	4597.765	6.165525	2.709663	1.367622	1.4777373	0.5698014	7.356494
		P - Value	0.9145	0.323	0.7785	0.3167	0.9474	0.2195	0.6743

Significance ($P < 0.05$) SD
= Standard deviation.

Table 2. Pearson correlation for biometric and reproductive parameters of *P. lineatus.*

Variables		Observations	Correlation	Significance
Lw	Wg	15	0.9918	0.0000
Lw	Ex	15	0.3783	0.1643
Wg	Ex	15	0.4164	0.1225
L	Ex	15	0.3115	0.2583
W	Ex	15	0.0857	0.7612
H	Ex	15	0.3855	0.1557
Oh	Ex	15	0.2247	0.3793
Ow	Ex	15	0.2575	0.3541
Oa	Ex	15	0.1614	0.5653

Obs: Lw = length/weight; Wg = weight; Ex = relative extruded weight; L = length; W = Width; H = height; Oh = ovary height; Ow = ovary width; Oa = Ovary area. Significance $P < 0.05$.

Considerações finais

As espécies nativas migradoras como o *P. maculatus*, *P mangurus* e *P. lineatus* possuem oócitos muito pequenos quando comparamos com outras espécies de água gelada ou de ambientes lênticos, o que dificultou uma visualização individual dos oócitos através do ultrassom imagens do ultrassom Esaote S.p.a, MyLab 30 Vet.

Entretanto o ultrassom apresentou 100% de acurácia na sexagem dos peixes das espécies *P. maculatus*, *P mangurus* e *P. lineatus* durante a estação reprodutiva, pois permitiu uma visualização dos ovários em diversos níveis de maturação e desenvolvimento, também possibilitando a visualização dos testículos dos machos.

Para os Siluriformes como o *P. maculatus* e o *P mangurus*, o formato cônico de seus corpos permitiram que os exames fossem feitos pelo plano ventral, o que possibilitou que em apenas uma imagem fosse possível a visualização dos dois ovários, enquanto para os Characiformes como o *P. lineatus* que tem o formato do corpo fusiforme e alongado o melhor plano para visualização dos ovários foi o plano lateral, além do que os peixes precisavam de uma imagem de ultrassom de cada lado para ter uma visualização individual de cada ovário, pois a imagem do segundo ovário não ficava muito bem definida no plano lateral.

Outra vantagem dos Siluriformes para a ultrassonografia é que os mesmos não possuem escamas, que acabam por atrapalhar na análise das imagens.

O ovário das fêmeas de *P. maculatus*, *P mangurus* e *P. lineatus* aptos à desova possuíram formato circular e ocuparam um grande espaço dentro da cavidade abdominal, enquanto os ovários das fêmeas que não desovaram apresentaram ovários mais estreitos e com bordas irregulares nas imagens do ultrassom. Para os

Siluriformes foram encontradas diferenças significativas nos parâmetros biométricos entre as fêmeas que desovaram para as fêmeas que não desovaram, como altura e área do ovário (*P. maculatus* e *P mangurus*) e largura do ovário (*P. maculatus*). Para o Characiforme *P. lineatus* não foram encontradas diferenças significativas entre seus parâmetros biométricos das fêmeas que desovaram para as fêmeas que não desovaram.

Embora *P. maculatus* e *P mangurus* sejam ambos Siluriformes eles possuem um estilo corporal diferente, o *P. mangurus* apresenta um corpo mais largo do que alto e seus ovários são mais altos do que largos, enquanto o *P.maculatus* possui o corpo e seus ovários mais altos do que largos. Já o Characiforme *P. lineatus* possuem o corpo e ovários mais altos do que largos.

Nenhum parâmetro biométrico das três espécies estudadas apresentou uma correlação forte, apenas a área do ovário do *P. maculatus* mostrou uma correlação positiva, porém foi uma correlação fraca com o peso do extrusado.

Então podemos concluir com os resultados obtidos neste trabalho que as fêmeas aptas à desova de *P. mangurus* com 1.061 ± 0.452 kg, deverão possuir ovários com os seguintes parâmetros: 2.736 ± 0.793 cm de altura, 2.693 ± 0.477 cm de largura e 14.867 cm^2 de área, já as fêmeas de *P. maculatus* com 482.500 ± 118.366 g deverá possuir ovário com 2.576 ± 0.583 cm de altura, 1.9917 ± 0.374 cm de largura e 8.738 ± 2.824 cm^2 de área, e as fêmeas de *P. lineatus* com 0.773 ± 0.292 kg deveram ter um ovário com , 4.788± 0.703 cm de altura, 1.546 ± 0.303 cm de largura e 11.657 ± 3.594 cm^2 área. Além do que, todas as fêmeas que responderam à indução hormonal apresentavam um ovário desenvolvido, com formato circular, bordas regulares e uma massa de ovos era presente na região da papila. A sexagem durante o período reprodutivo do *P. lineatus*, *P.mangurus* e *P. maculatus* pode ser feita através da ultrassonografia com alta acurácia.

Os resultados obtidos no presente trabalho podem servir de modelo para outras espécies, tanto espécies de potencial econômico, quanto para espécies em risco de extinção, podendo substituir técnicas invasivas que possam comprometer o sucesso reprodutivo ou até mesmo levar o indivíduo ao óbito.

Referências

Andrade-Talmelli, E., N. Fenerich-Verani and J. R. Verani (1999). "Fator de condição relativo (Kn): um critério para selecionar fêmeas de piabanha Brycon insignis (Steindachner, 1876)(Pisces: Bryconidae), para indução reprodutiva." Bol Inst de Pesca **25**: 95-99.

Andrade, D. R. and G. S. Yasui (2003). "O manejo da reprodução natural e artificial e sua importância na produção de peixes no Brasil." Revista Brasileira de Reprodução Animal **27**(2): 166-172.

Arashiro, D. R., G. Shigueki Yasui, L. L. Calado, N. F. d. Nascimento, S. C. A. d. Santos, G. F. Shiguemoto, P. S. Monzani and J. A. Senhorini (2020). "Capturing, induced spawning, and first feeding of wild-caught Pseudopimelodus mangurus, an endangered catfish species." Latin american journal of aquatic research **48**(3): 440-445.

Arashiro, D. R., G. S. Yasui, L. L. Calado, N. F. do Nascimento, M. P. dos Santos, S. C. A. do Santos, N. Levy-Pereira, P. S. Monzani, D. H. Siqueira-Silva and J. A. Senhorini (2018). "Synchronizing developmental stages in Neotropical catfishes for application in germ cell transplantation." Zygote: 114.

Arashiro, D. R., G. S. Yasui, L. L. Calado, N. F. d. Nascimento, M. P. d. Santos, S. C. Alves do Santos, N. Levy-Pereira, P. S. Monzani, D. H. Siqueira-Silva and J. A. Senhorini (2018). "Synchronizing developmental stages in Neotropical catfishes for application in germ cell transplantation." Zygote: 135-148.

às Micro, S. B. d. A. and P. Empresas–SEBRAE (2015). Aquicultura no Brasil: série estudos mercadológicos, Brasília.

Bapary, M. A. J. and A. Takemura (2010). "Effect of temperature and photoperiod on the reproductive condition and performance of a tropical damselfish Chrysiptera cyanea during different phases of the reproductive season." Fisheries Science **76**(5): 769-776.

Blythe, B., L. A. Helfrich, W. Beal, B. Bosworth and G. S. Libey (1994). "Determination of sex and maturational status of striped bass (Morone saxatilis) using ultrasonic imaging." Aquaculture **125**(12): 175-184.

Bressan, P. M., M. C. M. Kierulff and A. M. Sugieda (2009). "Fauna ameaçada de extinção no Estado de São Paulo." São Paulo: Fundação Parque Zoológico de São Paulo, Secretaria do Meio Ambiente.

Brizendine, M. E., D. L. Ward and S. A. Bonar (2018). "Effectiveness of Ultrasonic Imaging for Evaluating Presence and Maturity of Eggs in Fishes in Remote Field Locations." North American Journal of Fisheries Management.

Brizendine, M. E., D. L. Ward and S. A. Bonar (2018). "Effectiveness of Ultrasonic Imaging for Evaluating Presence and Maturity of Eggs in Fishes in Remote Field Locations." North American Journal of Fisheries Management **38**(5): 1017-1026.

Bryan, B. J., M. Wildhaber, D. Papoulias, A. DeLonay, D. Tillitt and M. Annis (2007). "Estimation of gonad volume, fecundity, and reproductive stage of shovelnose sturgeon using sonography and endoscopy with application to the endangered pallid sturgeon." Journal of Applied Ichthyology **23**(4): 411-419.

Campeche, D., L. Balzana, R. Figueiredo, M. dos BARBALHO, F. d. S. REIS and J. Melo (2011). "Peixes nativos do Rio São Francisco adaptados para cultivo." Embrapa Semiárido-Documentos (INFOTECA-E).

Chiotti, J. A., J. C. Boase, D. W. Hondorp and A. S. Briggs (2016). "Assigning sex and reproductive stage to adult lake sturgeon using ultrasonography and common morphological measurements." North American Journal of Fisheries Management **36**(1): 21-29.

Coelho, G. C., S. Y. Isaac, T. M. Mira-López, P. S. Monzani, D. R. Arashiro, T. Fujimoto, J. A. Senhorini and G. S. Yasui (2019). "Preparation of a fish embryo for micromanipulation: staging of development, removal of the chorion and traceability of PGCs in Prochilodus lineatus." International Journal of Developmental Biology **63**(1-2): 57-65.

Colombo, R. E., P. S. Wills and J. E. Garvey (2004). "Use of ultrasound imaging to determine sex of shovelnose sturgeon." North American Journal of Fisheries Management **24**(1): 322-326.

Costa, D. C., M. M. Pedreira, M. V. Coraspe-Amaral, A. E. Dupim, U. Barral and E. Duarte (2013). "Larvicultura de piabanha-do-pardo em aquários de cores diferentes." Pesquisa Agropecuária Brasileira **48**(8): 1005-1011.

Coward, K. and N. R. Bromage (2002). "Quantification of ovarian condition in fish: a safer, more precise alternative to established methodology." Aquatic Living Resources **15**(4): 259-261.

CREPALDI, D. and M. ROTTA (2007). "Uso do ultra-som em programas de reprodução de peixes nativos." Embrapa Pantanal-Comunicado Técnico (INFOTECA-E).

De Melo Dias, G. C., M. Cassel, L. W. Oliveira De Jesus, S. R. Batlouni and M. I. Borella (2017). "Spermatogonia, Germline Cells, and Testicular Organization in the Characiform Prochilodus lineatus Studied Using Histological, Stereological, and Morphometric Approaches." The Anatomical Record **300**(3): 589-599. de Oliveira Schuingues, C., M. G. de Lima, A. R. Lima, D. dos Santos Martins and G. de Medeiros Costa (2013). "Anatomia da cavidade bucofaringeana de Sorubim trigonocephalus (Siluriformes, Osteichthyes)." Pesquisa Veterinária Brasileira **33**(10): 1256-1262. do Nascimento, N. F., M. Pereira-Santos, L. H. Piva, B. Manzini, T. Fujimoto, J. A. Senhorini, G. S. Yasui and L. S. O. Nakaghi (2017). "Growth, fatty acid composition, and reproductive parameters of diploid and triploid yellowtail tetra Astyanax altiparanae." Aquaculture. du Colombier, S. B., L. Jacobs, C. Gesset, P. Elie and P. Lambert (2015). "Ultrasonography as a noninvasive tool for sex determination and maturation monitoring in silver eels." Fisheries research **164**: 50-58.

Evans, A. F., M. S. Fitzpatrick and L. K. Siddens (2004). "Use of ultrasound imaging and steroid concentrations to identify maturational status in adult steelhead." North American Journal of Fisheries Management **24**(3): 967-978.

Fenerich-Verani, N., H. M. Godinho and M. Y. Narahara (1984). "The size composition of the eggs of curimbatá, Prochilodus scrofa Steindachner 1881, induced to spawn with human chorionic gonadotropin (HCG)." Aquaculture **42**(1): 37-41.

Ferraz, E. and V. Cerqueira "O USO DE TERAPIAS CRÔNICAS DE INDUÇÃO HORMONAL COM HCG E FSH, NA MATURAÇÃO GONADAL DO ROBALO-FLECHA, Centropomus undecimalis, EM CATIVEIRO." Firkus, T., F. J. Rahel, H. L. Bergman and B. D. Cherrington (2018). "Warmed Winter Water Temperatures Alter Reproduction in Two Fish Species." Environmental management **61**(2): 291-303. Froese, R. and D. Pauly. (2015). "FishBase. World Wide Web electronic publication. ." Retrieved 06/2015, 2015, from www.fishbase.org.

Galdioli, E. M., C. Hayashi, C. M. Soares, V. R. B. Furuya and A. Faria (2002). "Substituição da proteína do farelo de soja pela proteína do farelo de canola em rações para alevinos de curimbatá (Prochilodus lineatus V.)." Revista Brasileira de Zootecnia **31**(2): 552-559.

Hainfellner, P., T. De Souza, T. Nascimento, G. Freitas and S. Batlouni (2011). "Heterogeneous distribution of oocytes in the ovaries of Prochilodus lineatus." Indian Journal of Science and Technology **4**(S8): 111-112.

Hainfellner, P., T. G. d. Souza, R. G. Moreira, L. S. O. Nakaghi and S. R. Batlouni (2012). "Gonadal steroids levels and vitellogenesis in the formation of oocytes in Prochilodus lineatus (Valenciennes)(Teleostei: Characiformes)." Neotropical Ichthyology **10**(3): 601-612.

Hildebrandt, T. B. and J. Saragusty (2015). "Use of ultrasonography in wildlife species." Fowler's Zoo and Wild Animal Medicine; Elsevier BV: Amsterdam, The Netherlands **8**: 714-723.

Kohn, Y. Y., P. M. Lokman, A. Kilimnik and J. E. Symonds (2013). "Sex identification in captive hapuku (Polyprion oxygeneios) using ultrasound imagery and plasma levels of vitellogenin and sex steroids." Aquaculture **384**: 87-93.

Lima-Junior, S. E. and R. Goitein (2006). "Fator de condição e ciclo gonadal de fêmeas de Pimelodus maculatus (Osteichthyes, Pimelodidae) no rio Piracicaba (SP, Brasil)." CEP **13506**: 900.

LUZ, R. K. and E. ZANIBONI-FILHO (2001). "Utilização de diferentes dietas na primeira alimentação do mandi-amarelo (Pimelodus maculatus, Lacépéde)." Acta Scientiarum **23**: 483-489.

Luz, R. K. and E. Zaniboni Filho (2002). "Larvicultura do mandi-amarelo Pimelodus maculatus Lacépède, 1803 (Siluriformes: Pimelodidae) em diferentes densidades de estocagem nos primeiros dias de vida." Revista Brasileira de Zootecnia **31**(2): 560-565.

Moraes, G., D. P. STREIT JR, R. P. RIBEIRO, E. S. SAKAGUTI, E. Souza and J. A. POVH (2004). "Ação de diferentes indutores reprodutivos hormonais no aparecimento de anormalidades morfológicas em espermatozóides de piavuçu (Leporinus macrocephalus), curimbatá (Prochilodus lineatus) e carpa comum (Cyprinus carpio)." Boletim do Instituto de Pesca **30**(2): 109-116.
Moraes, M., I. F. Barbola and E. Guedes (1997). "Alimentação e relações morfológicas com o aparelho digestivo do" curimbatá", Prochilodus lineatus (Valenciennes)(Osteichthyes, Prochilodontidae), de uma lagoa do sul do Brasil." Revista brasileira de zoologia **14**(1): 169-180.
Muniz, J., M. Catanho and A. d. Santos (2008). "Influência do fotoperíodo natural na reprodução induzida do tambaqui, Colossoma macropomum (CUVIER, 1818)." Boletim do Instituto de Pesca **34**(2): 205-211.
MURGAS[1], L. D. S., V. de Oliveira FELIZARDO[1], M. R. FERREIRA[1], G. C. VERAS[1], E. de Souza and D. A. d. J. P. ANDRADE[1] (2012). "Eficiência reprodutiva em espécies nativas de peixes de água doce."
Næve, I., M. Mommens, A. Arukwe and E. Kjørsvik (2018). "Ultrasound as a noninvasive tool for monitoring reproductive physiology in female Atlantic salmon (Salmo salar)." Physiological reports **6**(9): e13640.
Newman, D. M., P. L. Jones and B. A. Ingram (2008). "Sexing accuracy and indicators of maturation status in captive Murray cod Maccullochella peelii peelii using non-invasive ultrasonic imagery." Aquaculture **279**(1-4): 113-119.
Nogueira, L. B., P. G. Azevedo, M. R. Canelhas, A. G. Bedore, J. M. Lopes and H. P. Godinho (2012). "Induced spawning and early ontogeny in hatchery-reared catfish Zungaro jahu (Siluriformes: Pimelodidae)." Neotropical Ichthyology **10**(1): 89-98.
Perazza, C. A., F. L. V. PINAFFI, L. A. Silva and A. W. S. HILSDORF (2015). "Evaluation of ultrasound imaging to predict loin eye area in tambaqui." BOLETIM DO INSTITUTO DE PESCA **41**: 803-809.
Pereira-Santos, M., G. Yasui, P. Xavier, A. N. de Macedo, N. do Nascimento, T. Fujimoto, J. Senhorini and L. Nakaghi (2016). "Morphology of gametes, post-fertilization events and the effect of temperature on the embryonic development of *Astyanax altiparanae* (Teleostei, Characidae)." Zygote (Cambridge, England): 1-13.
Pereira, A. S. and A. P. de Oliveira Nuñer (2003). "Utilização de diferentes densidades, dietas e formatos de tanque na larvicultura da piracanjuba, Brycon orbignyanus Valenciennes, 1849 (Characiformes, Characidae." Acta Scientiarum. Biological Sciences **25**(1): 55-61.
Petochi, B. T., P. Di Marco, V. Donadelli, A. Longobardi, I. Corsalini, D. Bertotto, M. Finoia and G. Marino (2011). "Sex and reproductive stage identification of sturgeon hybrids (Acipenser naccarii× Acipenser baerii) using different tools: ultrasounds, histology and sex steroids." Journal of Applied Ichthyology **27**(2): 637-642.
Piferrer, F., A. Beaumont, J.-C. Falguiere, M. Flajshans, P. Haffray and L. Colombo (2009). "Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment." Aquaculture **293**(3-4): 125-156.
RESENDE, E. (1992). "Bioecologia do curimbatá, Prochilodus lineatus no Pantanal do MirandaAquidauana, Mato Grosso do Sul, Brasil." Acta Limnol. Brasil. Vol. IV **261**: 276.
Sabinson, L., J. Rodrigues Filho, A. Peret and J. Verani (2014). "Growth and reproduction aspects of Pimelodus maculatus Lacépède, 1803 (Siluriformes, Pimelodidae) of the Cachoeira Dourada reservoir, state of Goiás and Minas Gerais, Brazil." Brazilian Journal of Biology **74**(2): 450-459.
São Paulo (2014). DECRETO Nº 60.133, DE 7 DE FEVEREIRO DE 2014.
Schütz, J. H. and A. P. d. O. Nuñer (2007). "Growth and survival of dorado Salminus brasiliensis (Pisces, Characidae) post-larvae cultivated with different types of food and photoperiods." Brazilian Archives of Biology and Technology **50**(3): 435-444.
Schütz, J. H., M. Weingartner, E. Zaniboni-Filho and A. d. O. NUÑER (2008). "Crescimento e sobrevivência de larvas de suruvi Steindachneridion scriptum nos primeiros dias de vida: influência de diferentes alimentos e fotoperíodos." Boletim do Instituto de Pesca **34**(3): 443-451.

Shimizu, A. (2003). "Effect of photoperiod and temperature on gonadal activity and plasma steroid levels in a reared strain of the mummichog (Fundulus heteroclitus) during different phases of its annual reproductive cycle." General and comparative endocrinology **131**(3): 310-324.
Smerman, W., J. G. D. Castro, J. d. Toledo, C. d. Rosa and D. d. Godoi (2002). "Larvicultura de pintado (Pseudoplatystoma sp) em Alta Floresta–Mato Grosso." Revista de biologia e ciências da terra **2**(1): 1331.
Solis-Murgas, L., V. Felizardo, M. Ferreira, E. Andrade and G. Veras (2011). "Importância da avaliação dos parâmetros reprodutivos em peixes nativos." Rev Bras Reprod Anim **35**(2): 186-191.
SOUZA, T. G. (2011). Biologia reprodutiva e qualidade do sêmen de reprodutores de curimbatá, Prochilodus lineatus, em condições distintas de manejo Dissertação (mestrado em Aquicultura), Universidade Estadual Paulista – UNESP.
Streit Jr, D. P., G. Moraes, R. RIBEIRO, W. Caçador, E. S. Sakaguti, J. A. Povh and E. Souza (2003). "Estudo comparativo da indução hormonal da espermiação em piavuçu (Leporinus macrocephalus) com extrato de hipófise de frango, coelho e carpa." Acta Scientiarum, Animal Sciences **25**(2): 261-266.
Toussaint, A., N. Charpin, S. Brosse and S. Villéger (2016). "Global functional diversity of freshwater fish is concentrated in the Neotropics while functional vulnerability is widespread." Scientific reports **6**: 22125.
Vieira Sampaio, E. and S. Yoshimi (2006). "Biologia reprodutiva e desova induzida de duas espécies de bagres (Osteichthyes: Siluriformes) da bacia do rio São Francisco." Acta Scientiarum. Biological Sciences **28**(3).
Vono, V., L. G. M. Silva, B. P. Maia and H. P. Godinho (2002). "Biologia reprodutiva de três espécies simpátricas de peixes neotropicais: Pime/odus macu/atus Lacépede (Siluriformes, Pimelodidae), Leporinus amb/yrhynchus Garavello & Britski e Schizodon nasutus Kner (Characiformes, Anostomidae) do recém-formado Reservatório de Miranda, Alto Paraná."
Woynarovich, E. and L. Horváth (1983). A propagação artificial de peixes de águas tropicais: manual de extensão. Brasília, FAO/CODEVASP/CNPq.
Yasui, G., T. Fujimoto, S. Sakao, E. Yamaha and K. Arai (2011). "Production of loach () germ-line chimera using transplantation of primordial germ cells isolated from cryopreserved blastomeres." Journal of animal science **89**(8): 2380-2388.
Yasui, G. S., J. A. Senhorini, E. Shimoda, M. Pereira-Santos, L. S. O. Nakaghi, T. Fujimoto, L. AriasRodriguez and L. A. Silva (2015). "Improvement of gamete quality and its short-term storage: an approach for biotechnology in laboratory fish." Animal **9**(03): 464-470.
Zohar, Y. (1989). "Endocrinology and fish farming: aspects in reproduction, growth, and smoltification." Fish Physiology and Biochemistry **7**(1-6): 395-405.
Zohar, Y. and C. C. Mylonas (2001). Endocrine manipulations of spawning in cultured fish: from hormones to genes. Reproductive Biotechnology in Finfish Aquaculture, Elsevier**:** 99-136.