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BIOLOGIA REPRODUTIVA DO BAGRE AMEAÇADO DE EXTINÇÃO *Genidens barbus* (LACÉPÈDE, 1803) (ARIIDAE, SILURIFORMES), EM AMBIENTE ESTUARINO NO SUL DO BRASIL.

PORTO ALEGRE
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Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Mestre em Biologia Animal. Área de concentração: Biodiversidade

Orientador: Prof. Dr. Ignacio Benites Moreno

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Aprovada em____de____________de_____.

BANCA EXAMINADORA

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Dra. Valéria Lemos

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Dr. João Paes Vieira

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Dr. Esteban Avigliano

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SUMÁRIO

RESUMO

A presente pesquisa descreve aspectos de biologia reprodutiva do bagre marinho ameaçado de extinção *Genidens barbus* (Lacépède, 1803), na região do estuário do rio Tramandaí, sul do Brasil, objetivando a compreensão acerca da biologia populacional da espécie e resiliência frente à pressão pesqueira, capaz de gerar de subsídios para o monitoramento e manejo pesqueiro da espécie na região. Os exemplares foram coletados a partir de capturas provenientes da pesca artesanal e e amostragem científica, de julho de 2020 a fevereiro de 2021. Seis fases reprodutivas foram atribuidas para cada sexo. Para as fêmeas, foi descoberta uma estrutura ainda não descrita em peixes, denominada de "zona de reserva", e a análise gonadal macroscópica (baseada em características externas e internas dos ovários, incluindo o comprimento da zona de reserva), foi integrada com dados histológicos para a descrição das fases reprodutivas. Para os machos, as características macroscópicas dos testículos provaram não ser confiáveis para definir a maiorias das fases, e o método histológico foi essencial para a descrição das fases reprodutivas. A fecundidade absoluta variou de 104 a 272 oócitos vitelogênicos hidratados, e correlacionou-se significativa e positivamente com o comprimento e peso total dos peixes. A análise da distribuição de frequências dos diâmetros dos oócitos sugere que a espécie apresenta desova do tipo total, fecundidade determinada e desenvolvimento sincrônico dos oócitos. A temporada reprodutiva da espécie (excluindo o cuidado parental) na região foi definida com base nos Índices Gonadossomático e Hepatossomático, e frequência mensal de fases reprodutivas, iniciando em outubro e terminando em fevereiro. O IHS mostrou-se útil como um indicador do período prévio à temporada reprodutiva para ambos os sexos, com base em diferenças entre os meses reprodutivos e os que os antecedem, além de variar inversamente ao IGS. Os resultados encontrados podem contribuir para a revisão do período defeso da espécie na região, bem como dos tamanhos mínimos e máximos de captura, e na elaboração de planos de manejo pesqueiro sustentáveis, contribuindo para a conservação da espécie, e, consequentemente, com a manutenção da biodiversidade e desenvolvimento socioeconômico regional.

Palavras-chave: Ciclo de vida; fecundidade; histologia; Lista Vermelha de Espécies Ameaçadas de Extinção; pesca artesanal; temporada reprodutiva.

ABSTRACT

This study describes reproductive biology aspects of the endangered white sea catfish *Genidens barbus* in the Tramandaí River Estuary region, southern Brazil, aiming the understanding of the species populational biology and resilience under fishery pressure, providing subsidies for the species local fishery monitoring and management. Fish were sampled from artisanal fisheries captures and scientific sampling, from July/2020 to February/2021. Six reproductive phases were assigned to each sex. For females, analysis of a new structure that was never described before for fish, named “reserve zone”, and macroscopic gonadal analysis (based on external and internal ovaries features, including reserve zone’s length) were integrated with histological data to phases description. For males, macroscopic features of the testis prove to be unreliable for most phases, and the histological method was essential for reproductive phases description. Absolute fecundity ranged from 104 to 272 hydrated vitellogenic oocytes and correlated significatively and positively with fish total length and weight. Analysis of frequency distribution of oocytes diameter suggests that the species is a total spawner, with group-synchrony spawn and determinate fecundity. The species spawning season (excluding parental care) in the region was defined as starting in October and ending in February, based on gonadal and liver indexes (GSI and HSI, respectively) and reproductive phases monthly frequencies. The HSI was useful as a pre-spawning indicator, showing differences between spawning months and the predecessors’ months for both sexes, and also varying inversely to GSI. The results found here can subsidize local sustainable fisheries management plans, supporting the species conservation and hence biodiversity maintenance and regional socioeconomic development.

Keywords: Brazilian Red List of Endangered Species; fecundity; histology; life cycle; small-scale fisheries; spawning.

CAPÍTULO I – Introdução Geral

Os bagres marinhos pertencem à familia Ariidae Bleeker, 1862, e constituem o único grupo da ordem dos Siluriformes distribuído circumglobalmente. A família é composta por mais de 150 espécies, agrupadas em duas subfamílias: (1) Galeichthynae, contendo apenas o gênero *Galeichtys* e quatro espécies, e (2) Ariinae, contendo os aproximadamente 30 gêneros restantes (Nelson et al. 2016). Os Ariidae ocorrem em regiões temperadas, tropicais e subtropicais, principalmente no ambiente marinho, mas algumas espécies podem completar seu ciclo de vida em ambientes aquáticos continentais (Fischer et al. 2011; Nelson et al. 2016; Condini et al. 2019). A ampla distribuição dos ariideos é explicada, principalmente, por eventos vicariantes associados à deriva continental, de regiões associadas à Gondwana, e por sua baixa capacidade de dispersão transoceânica, fatores que culminaram no alto grau de endemismo do grupo (Betancur-R et al. 2007). A monofilía do grupo foi confirmada com base em características morfológicas e moleculares (Betancur-R et al. 2007; Macerniuk and Menezes 2007 Macerniuk et al. 2012). Diversos ariideos são imporantes recursos pesqueiros para a pesca comercial, artesanal e de subsistência ao redor do mundo, devido a sua abundância e qualidade da carne (Menezes et al. 2003; Fischer et al. 2011; Nelson et al. 2016)

O gênero *Genidens* Castelnau, 1855 pertence à familia Ariide, sendo representado por quatro espécies: *G. genidens* (Cuvier, 1829), *G. planifrons* (Higuchi, Reis & Araújo, 1982), *G. machadoi* (Miranda-Ribeiro, 1918), e *G. barbus* (Lacépède, 1803) (Nelson et al. 2016; Fischer et al. 2011; Menezes et al. 2003), sendo a última a espécie alvo do presente trabalho. Conhecido popularmente como “bagre-branco”, *G. barbus* habita a plataforma continental, regiões estuarinas e rios, principalmente em águas de fundo raso e lodoso (Fischer et al. 2011). Sua distribuição ocorre do estado da Bahia, no Brasil, até a região norte da Patagonia, na Argentina (López e Bellisio 1965; Avigliano et al. 2020). A espécie é considerada um dos mais importantes recursos pesqueiros no Brasil, além de apresentar importância no Uruguai e na Argentina (Reis 1986b; Velasco et al. 2007; MINAGRI 2013), apresentando destacada importância pesqueira histórica na região sul-sudeste brasileira, onde já foi considerada abundante (Mishima e Tanji 1981; Araújo 1998; Seeliger et al. 1998; Soeth et al. 2014; Haimovici e Cardoso 2017). Entretanto, o bragre-branco é notadamente suscetivel à sobreexplotação pesqueira, devido aos complexos aspectos reprodutivos de sua história de vida, dificultando sua conservação (Mendonça et al. 2020).

A espécie apresenta elevada plasticidade migratória, e contingentes populacionais apresentando variados modos migratórios foram identificados, como anfidromia e residência em água doce, relacionados à ontogenia da espécie (Avigliano et al. 2017), além de anadromia reprodutiva proposta por Reis (1986a). Esse comportamento depende de variadas condições ambientais específicas e requerimentos fisiológicos transicionais, fatores os quais demandam elevado investimento energético (Wooton e Smith 2014). Após a fecundação externa, os machos incubam os ovócitos dentro de sua cavidade orobranquial, e não se alimentam durante o período incubatório. Diversos estoques pesqueiros foram descritos para a espécie, com base em microquímica de otólitos e espinhos dorsais, devendo a gestão pesqueira da espécie ser específica para cada estoque, os quais estão geograficamente associados a estuários e rios (Avigliano et al. 2020; Maciel et al. 2021). A espécie apresenta baixa fecundidade, crescimento lento e maturação sexual tardia, sendo considerada k-estrategista, e de recuperação populacional lenta frente à pressão pesqueira (Velasco et al. 2007), a qual impacta negativamente a espécie principalmente durante o período reprodutivo, chegando a representar até 90% dos bagres marinhos capturados pela frota pesqueira artesanal, em regiões estuarino-lagunares no sul do Brasil durante o período reprodutivo (Reis 1986a; Machado et al. 2012). O comprimento de primeira maturação sexual (C50) foi estimado em 415 mm (CT) para as fêmeas e 430 mm para os machos na Lagoa dos Patos – RS, com os menores indivíduos capazes de reprodução medindo 224 e 187 mm (fêmeas e machos, respectivamente). O C100 foi estimado em 730 e 630 mm para fêmeas e machos, respectivamente (Reis 1986a). Os mesmos parâmetros foram avaliados para a espécie na Foz do rio Itajaí-Açu – SC por Hostim-Silva (2001), variando significativamente: o C50 foi estimado em 166,79 mm para as fêmeas e 180,90 mm para os machos, e o C100 foi estimado em 245,98 mm e 243,45 mm para fêmeas e machos, respectivamente. A diferença significativa observada pode ser atribuida a diferença dos regimes abióticos de cada local, e corrobora com a hipótese de complexa estrutura populacional sugerida para a espécie, em anos posteriores, por Avigliano et al. (2020) e Maciel et al. (2021), bem como reforça a urgência de testar a hipótese de um estoque pesqueiro específico na região do estuário do rio Tramandaí, o que acarretaria em medidas de manejo específicas locais, fundamentais para a conservação ou recuperação dos estoques.

Apesar de sua importância comercial histórica, notadamente para a pesca de pequena escala do estado do Rio Grande do Sul (Reis 1986b), análises de desembarque pesqueiro demonstraram declíneos significativos desde 1960, diminuindo de nove mil toneladas registradas para o setor pesqueiro artesanal estuarino de emalhe em 1972 e 1973, para apenas 24 toneladas em 1999 no município de Rio Grande, principal área de pesca da espécie, caracterizando o colapso do estoque (Haimovici e Cardoso 2017). O cenário de colapso pesqueiro, aliado à importância comercial da espécie, culminaram na outorga de leis para regulamentação de suas capturas durante a decada de 1970, como períodos de defeso durante a época reprodutiva e tamanhos mínimos de captura (Haimovici e Cardoso 2017). Em 2014, a captura da espécie foi proibida no Rio Grande do Sul, e posteriormente totalmente banida no Brasil, devido a sua inclusão na Lista Vermelha de Espécies Ameaçadas de Extinção, na categoria “Em perigo”, critério “A4bcde” (MMA 2014). Atualmente, programas de monitoramento pesqueiro nos estados de São Paulo e Paraná permitem a pesca da espécie (Mendonça et al. 2020). No Litoral Norte do Rio Grande do Sul, o contubardo cenário legislativo gerou diversos conflitos sociais entre pescadores e outros atores sociais interessados no sistema pesqueiro de *G. barbus* (Vontobel e Beroldt 2020), culminando, junto a outros motivos, na criação do Projeto de Monitoramento Pesqueiro do Estuário do Rio Tramandaí (MOPERT), com ênfase na pesca dos bagres marinhos (JFRS 2018), no qual a presenta pesquisa insere-se.

Recursos pesqueiros são mundialmente importantes para a provisão de alimento, nutrição e subsistência de bilhões de pessoas (FAO 2020), porém a maioria dos estoques pesqueiros naturais permanecem não estudados e não manejados (Costello et al. 2012), e muitos são considerados totalmente explotados ou sobrexplotados (FAO 2020). Tendo em vista a sustentabilidade desses estoques, essencial para a sociedade (FAO 2020), é necessário que sejam estabelecidos pontos biológicos e pesqueiros de referência, para fins de monitoramento e manejo dos estoques. Tal fator, aliado à mudança global de uma gestão pesqueira focada na otimização da produtividade pesqueira, para o conservacionismo e recuperação dos estoques, levou a um crescente reconhecimento sobre a importância da biologia reprodutiva da espécies (Lowerre-Barbieri et al. 2011), essencial para predições de crescimento populacional dos recursos pesqueiros, bem como de sua resiliência frente à pressão pesqueira (King 2007; Lowerre-Barbieri 2009; Lowerre-Barbieri et al. 2011). Além disso, o conhecimento de aspectos reprodutivos das espécies é essencial para o estabelecimento de medidas de manejo pesqueiro baseadas em dados robustos (King 2007; Lowerre-Barbieri 2011).

O objetivo da presente pesquisa foi investigar aspectos de biologia reprodutiva de *G. barbus*, como desenvolvimento gonadal, fecundidade, estratégia reprodutiva e ciclo reprodutivo, na região do Estuário do Rio Tramandaí, que possam servir de base para o monitoramento populacional da espécie e compreensão ecológica frente à pressão pesqueira, capaz de subsidiar futuros planos e medidas de manejo sustentáveis para a espécie na área de estudo, bem como auxiliar futuras pesquisas de biologia reprodutiva da espécie ao longo de sua ampla distribuição geográfica. Os resultados encontrados também podem ser utilizados em futuras pesquisas que objetivem analisar se os indivíduos de *G. barbus* que habitam a região do estuário do rio Tramandaí compõe um estoque pesqueiro específico.

A partir da coleta de indivíduos provenientes da captura pesqueira profissional, e amostragem científica, diversos aspectos de biologia reprodutiva foram analisados. Foram descritas seis fases reprodutivas para cada sexo. Para as fêmeas, além da análise macroscópica externa, características macroscópicas internas, incluindo a descrição, caracterização e análise de uma estrutura nova denominada de "zona de reserva", foram utilizadas, integradas à análise histológica. Para os machos, o método histológico foi essencial, devido ao baixo grau de confiança nas características macroscópicas para distinção das fases. A fecundidade absoluta variou de 104 a 272 oócitos vitelogênicos hidratados, variando significativa e positivamente com o comprimento e peso total dos indivíduos. A análise de distribuição de frequência dos diâmetros dos oócitos sugere que a espécie apresenta desova total, desenvolvimento oócitário em grupo, e fecundidade determinada. A partir do IGS, IHS e variação da frequência mensal de fases reprodutivas, a temporada reprodutiva da espécie na região foi definida de outubro a fevereiro.

Referências bibliográficas

Avigliano, E., Volpedo, A.V., 2015. New records of anadromous catfish *Genidens barbus* (Lacépède, 1803) in the Paraná Delta (South America): Evidence of extension in the migration corridor? Mar. Biodivers. Rec. 8, 1–3. https://doi.org/10.1017/S175526721400147X

Avigliano, E., Leisen, M., Romero, R., Carvalho, B., Velasco, G., Vianna, M., Barra, F., Volpedo, A.V., 2017b. Fluvio-marine travelers from South America: Cyclic amphidromy and freshwater residency, typical behaviors in *Genidens barbus* inferred by otolith chemistry. Fish. Res. 193, 184–194. https://doi.org/10.1016/j.fishres.2017.04.011

Avigliano, E., Martínez, G., Stoessel, L., Méndez, A., Bordel, N., Pisonero, J. Volpedo, A, 2020. Otoliths as indicators for fish behavior and procurement strategies of hunther-gatherers in North Patagonia. Heliyon 6. https://doi.org/10.1016/j.heliyon.2020.e03438

Betancur-R., R., Acero P., A., Bermingham, E., Cooke, R., 2007. Systematics and biogeography of New World Sea catfishes (Siluriformes: Ariidae) as inferred from mitochondrial, nuclear, and morphological evidence. Mol. Phylogenet. Evol. 45, 339–357. https://doi.org/10.1016/j.ympev.2007.02.022.

Condini, M. V., Pereyra, P. E. R., Garcia, M. A., Saint'Pierre, T. D., Ceni, G., Lugo, R., Fontoura, N. F., Viera, J. P., Albuquerque, C. Q., 2019. Use of fresh water by an estuarine-resident marine catfish: evidence from gonadal and otolith chemistry analyses. Journal of the Marine Biological Association of the United Kingdom 1–8. https://doi.org/10.1017/S0025315419000493

Costello, C., Ovando, D., Hilborn, R., Gaines, S.D., Deschenes, O., Lester, S.E., 2009. Reports Status and Solutions for the World ' s Unassessed Fisheries. Sci. Express 1–4
FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

Fischer, L.G., Pereira, L.E.D., Vieira, J.P., 2011. Peixes Estuarinos Costeiros, 2nd ed. Rio Grande.

Haimovici, M., Cardoso, L.G., 2017. Long-term changes in the fisheries in the Patos Lagoon estuary and adjacent coastal waters in Southern Brazil. Mar. Biol. Res. 13, 135–150. https://doi.org/10.1080/17451000.2016.1228978.

Hostim-Silva, M, 2001. Reprodução de Genidens genidens (Valenciennes, 1839) e Netuma barba (Lacépède, 1803) (Siluriformes, Ariidae) da Foz do Rio Itajaí-Açu, Santa Catarina, Brasil. Tese de doutorado - Programa de Pós-Graduação em Ecologia e Recursos Naturais do Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos. São Carlos, SP.

IBAMA, 1999. Desembarque de pescados no Rio Grande do Sul. Annual Report, Centro de Pesquisa do Rio Grande, RS, Brasil.

Instituto Chico Mendes de Conservação da Biodiversidade, 2018. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. Brasília: ICMBio. 4162 p.

JFRS - Justiça Federal Seção Judiciária do Rio Grande do Sul, 2018. JFRS autoriza projeto de monitoramento para pesca do Bagre no Rio Tramandaí. Retrieved from: https://www2.jfrs.jus.br/noticias/jfrs-autoriza-projeto-de-monitoramento-para-pesca-do-bagre-no-rio-tramandai/

King, M.G., 2007. Fisheries biology, assessment, and management, 2nd ed. Blackwell Publishing Ltd, Oxford.

López, R. B., Bellisio, N. B., 1965. Contribución al conocimiento del *Tachysurus barbus* (Lacépède), bagre del mar argentino. (Pisces, Ariidae). Anuario del II Congreso Latinoamericano Zool. Vol. 2, p.145–153.

Lowerre-Barbieri, S., 2009. Reproduction in Relation to Conservation and Exploitation of Marine Fishes. Reprod. Biol. Phylogeny Fishes, Vol 8B 371–394. https://doi.org/10.1201/b10257-11.

Lowerre-Barbieri, S.K., Brown-Peterson, N.J., Murua, H., Tomkiewicz, J., Wyanski, D.M., Saborido-Rey, F., 2011. Emerging issues and methodological advances in fisheries reproductive biology. Mar. Coast. Fish. 3, 32–51. https://doi.org/10.1080/19425120.2011.555725.

Machado, R., Ott, P.H., Sucunza, F., Marceniuk, A.P., 2012. Ocorrência do bagre marinho *Genidens machadoi* (Siluriformes, Ariidae) na laguna Tramandaí, sul do Brasil. Neotrop. Biol. Conserv. 7, 214–219. https://doi.org/10.4013/nbc.2012.73.08.

Marceniuk, A.P., Menezes, N.A., 2007. Systematics of the family Ariidae (Ostariophysi, Siluriformes), with a redefinition of the genera. Zootaxa. Vol. 1416, p. 1–126.

Marceniuk, A.P., Menezes, N.A., Britto, M.R., 2012 Phylogenetic analysis of the family Ariidae (Ostariophysi: Siluriformes), with a hypothesis on the monophyly and relationships of the genera. Zoological Journal of the Linnean Society. Vol. 165, p. 534–669.

Mendonça, J.T., Balanin, S., Garrone-Neto, D., 2020. The marine catfish *Genidens barbus* (Ariidae) fisheries in the state of São Paulo, southeastern Brazil: Diagnosis and management suggestions. An. Acad. Bras. Cienc. 92, 1–16. https://doi.org/10.1590/0001-3765202020180450

Menezes, N.A., Buckup, P.A., Figueiredo, J.L. De, Moura, R.L. De, 2003. Catálogo das Espécies de Peixes Marinhos do Brasil, Book.

MINAGRI, 2013. Ministerio de Agricultura y Pesca de la Nación Argentina, Argentina. Disponible in: http://www.minagri.gob.ar/site/pesca/pesca_maritima/02-desembarques/anio.php?anio=2012.

Mishima, M., Tanji, S., 1983. Maturaçao e desova de bagres marinhos (Osteichthyes, Ariidae) do complexo estuarino da cananeia (25°S, 48°W). B. Inst. Pesca.

MMA - Ministério do Meio Ambiente, 2014. Portaria MMA n° 445/2014 (2014) Portarias. No 445 de 17 de dezembro de 2014. Ministério do Meio Ambiente - MMA. Diário Oficial da União-Seção 1, 245, 126; Retrieved from https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=18/12/2014&jornal=1&pagina=126&totalArquivos=144

Nelson, J.S., Grande, T., Wilson, M.V.H., 2016. Fishes of the World, 5th ed. John Wiley & Sons, Inc, New Jersey.

Reis, E.G. 1986b. A pesca artesanal de bagres marinhos (Siluriformes, Ariidae) no estuário da Lagoa dos Patos (RS), Brasil: Editora da FURG. Documentos Técnicos, Oceanografia, FURG

Reis, E.G., 1986a. Reproduction and feeding habits of the marine catfish *Netuma barba* (Siluriformes, Ariidae) in the estuary of Lagoa dos Patos, Brazil. Atlântica 8, 35–55.

Seeliger, U., Odebrecht, C., Castello, J. P., 1998. Ictiofauna. In: Os ecossistemas costeiros e marinho do extremo Sul do Brasil. Rio Grande: Ecoscientia. 341p.

Vasconcellos, M., Diegues, A. C., De Sales, R., 2008. Aspectos relevantes relacionados à pesca artesanal costeira nacional. In: COSTA, A. L. (Org.). Nas redes da pesca artesanal costeira. Ibama.

Velasco, G., Reis, E.G., Vieira, J.P., 2007. Calculating growth parameters of *Genidens barbus* (Siluriformes, Ariidae) using length composition and age data. J. Appl. Ichthyol. 23, 64–69. https://doi.org/10.1111/j.1439-0426.2006.00793.x

Vontobel, E, D., Beroldt, L., 2019. Análise de um sistema socioecológico pesqueiro, na perspectiva dos recursos de uso comum, em Imbé, Sul do Brasil. Boletim Geográfico do Rio Grande do Sul. 34, 52-77.

Wootton, R.J., Smith, C., 2015. Reproductive Biology of Teleost Fishes. John Wiley & Sons, Ltd., Oxford.

CAPÍTULO II - Reproductive biology of the endangered white sea catfish (*Genidens barbus*) in the region of the Tramandaí River Estuary, South Brazil, subsidizing its fishery management.

Title: Reproductive biology of the endangered white sea catfish (*Genidens barbus*) in the Tramandaí River Estuary region, Southern Brazil, subsidizing its fishery management.

ABSTRACT

This study describes reproductive biology aspects of the endangered white sea catfish *Genidens barbus* in the Tramandaí River Estuary region, southern Brazil, aiming the understanding of the species populational biology and resilience under fishery pressure, providing subsidies for the species local fishery monitoring and management. Fish were sampled from artisanal fisheries captures and scientific sampling, from July/2020 to February/2021. Six reproductive phases were assigned to each sex. For females, analysis of a new structure that was never described before for fish, named “reserve zone”, and macroscopic gonadal analysis (based on external and internal ovaries features, including reserve zone’s length) were integrated with histological data to phases description. For males, macroscopic features of the testis prove to be unreliable for most phases, and the histological method was essential for reproductive phases description. Absolute fecundity ranged from 104 to 272 hydrated vitellogenic oocytes and correlated significatively and positively with fish total length and weight. Analysis of frequency distribution of oocytes diameter suggests that the species is a total spawner, with group-synchrony spawn and determinate fecundity. The species spawning season (excluding parental care) in the region was defined as starting in October and ending in February, based on gonadal and liver indexes (GSI and HSI, respectively) and reproductive phases monthly frequencies. The HSI was useful as a pre-spawning indicator, showing differences between spawning months and the predecessors’ months for both sexes, and also varying inversely to GSI. The results found here can subsidize local sustainable fisheries management plans, supporting the species conservation and hence biodiversity maintenance and regional socioeconomic development.

Keywords: Brazilian Red List of Endangered Species; fecundity; histology; life cycle; small-scale fisheries; spawning.

INTRODUCTION

Fish resources provide food, nutrition, and livelihood worldwide, supporting billions of people especially in developing countries. Hence, fish stocks sustainability is essential (FAO 2020). Most world's natural fish stocks remain un-assessed and un-managed (Costello et al. 2012), several are fully or overexploited (FAO 2020), and fishing pressure is one of the main reasons causing yield's declines (Pauly et al. 2002), and the primary threat to ocean's biodiversity (Ripple et al. 2019; Yan et al. 2021). The small-scale fishing sector is a significant provider for world's food security, granting more than half of all fish consumed by society, and supporting local and national economies (World Bank 2012; FAO 2020). Therefore, is essential to establish biological and fishing reference points that support fish stocks assessment and management, successively contributing with world's food security and biodiversity maintenance. Fisheries management is shifting from yield optimization to conservation and recovery goals, with the recognition of a great number of overexploited fish stocks, which led to an increasing awareness of fish reproductive biology's importance, as reviewed by Lowerre-Barbieri et al. (2011). Understanding fish reproductive traits are fundamental to better predictions of populations growth and their resilience under fishing pressure (King 2007; Lowerre-Barbieri 2009; Lowerre-Barbieri et al. 2011). Harvest control strategies must also rely on robust reproductive biology aspects of the target species populations (King 2007; Lowerre-Barbieri 2011).

Many methods can be used to describe fish species' reproductive traits. Gonadal development analysis has been frequently applied, based on macroscopic or microscopic criteria (West 1990; Kjesbu et al. 2003; Lowerre-Barbieri et al. 2011). External macroscopical appearance of ovaries and testis and their Gonadosomatic Indexes (GSI) are more simple and rapid, and less costly and time-consuming alternatives (West 1990). On the other hand, microscopic histological analysis is considered the most accurate alternative, with the potential to produce the greatest amount of information, but it is more costly and requires more time (Hunter and Macewicz 1985; West 1990). Histology data is also helpful to validate simpler methods of gonadal development analysis (Lowerre-Barbieri et al. 2011), which can be important for monitoring and reassessing fish stocks status and health (FAO 2020). Along with that, the Hepatosomatic Index (HSI) can complement the previous methods (Skjaeraasen et al. 2010). Liver physiology relates to reproduction as many fish synthetize proteins and lipids in the liver for gonadal development, notably vitellogenin, yolk's precursor and thus essential for oocyte development. Hence, HSI can be seen as an index of energy storage and an indicator of fish spawning season. Reproductive strategies are also described based on fecundity estimates and spawning type. Fecundity is a critical parameter of population dynamics and for stock biomass estimates (Hunter et al. 1992) and monitoring its change overtime is necessary (Wright 2005).

The fishes of the Ariidae Bleeker, 1862 family (Ostariophysi: Siluriforms), also known as "sea catfishes", are the only world widely distributed Siluriforms, including more than 150 species in two subfamilies. Sea catfishes occur from warm temperate to tropical and subtropical regions, and although mainly attributed to the marine environment, some species can complete their life cycle in inland waters (Fischer et al., 2011; Nelson et al., 2016). Ariidae monophyly is supported by morphologic and molecular features (Betancur-R et al., 2007; Macerniuk and Menezes, 2007; Macerniuk et al., 2012), and many species are important fish resources for commercial and subsistence fisheries (Menezes et al. 2003; Fischer et al. 2011; Nelson et al. 2016). The genus *Genidens* Castelnau, 1855 is represented by four species. *Genidens barbus*, known as white sea catfish, inhabits the continental shelf, estuarine regions, and rivers, mainly of shallow and slimy waters, from the state of Bahia, in Brazil, to North Patagonia, in Argentina (López and Bellisio 1965; Avigliano et al. 2020). The species is considered one of the most important fish resources of Brazil, and important in Uruguay and Argentina (Reis 1986b; Velasco et al. 2007; MINAGRI 2013). In Brazil, *G. barbus* were once considered abundant in the Southern and South-eastern regions (Mishima and Tanji 1981; Araújo 1998; Seeliger et al. 1998; Soeth et al. 2014; Haimovici and Cardoso 2017). The species is especially susceptible to overexploitation, due to its complex life history reproductive aspects, which hamper its conservation (Mendonça et al. 2020). As the species shows high migratory plasticity, transiting between marine, estuarine and freshwater environments for several purposes (Reis 1986a; Avigliano et al. 2017), it depends on a variety of environmental conditions and transitional physiological responses that are energetically demanding (Wooton and Smith 2014). Several stocks were described for the species, associated with estuaries and rivers (Avigliano et al. 2015; 2016; 2017; 2019; Maciel et al. 2021), indicating that fishery management should be location specific. It is still unknown if the white sea catfish individuals inhabiting the Tramandaí River Estuary region compose a discrete stock. *Genidens barbus* is a K-strategy species that shows paternal mouthbrood incubation, low fecundity, slow-growing, late maturation, and, due to all these characteristics, slow stocks rebuild (Velasco et al. 2007).

The white sea catfish is historically of great commercial importance, notably for the small-scale fishery of the state of Rio Grande do Sul - Brazil (Reis 1986b), but its fishery production has shown a substantial decline since 1960 (Haimovici and Cardoso 2017). A scenario of major production declines and commercial importance led to several laws on its capture regulation during the 1970s, like fisheries closed periods during spawning season and minimum size limits (Haimovici and Cardoso 2017). A total ban on its capture was enacted for the whole country in 2014 due to the inclusion of the species in the Brazilian Red List of Endangered Species (MMA 2014). Such legislative scenario led to many social conflicts between fisherfolks and other social actors of the fishing system (Vontobel and Beroldt 2020). Currently, the species is considered "Endangered" (MMA 2022), but as a demand of the productive sector, nowadays monitored fishing projects permit *G. barbus* captures in the states of Paraná, São Paulo (Mendonça et al. 2020), and in the municipalities of Tramandaí and Imbé, in Rio Grande do Sul State (JFRS 2018).

The goal of the study was to investigate *G. barbus* reproductive development, fecundity, spawning strategy, and life cycle aspects, providing novel reproductive biology information in an important fishing area of this species, from artisanal fishery samples and scientific sampling. Results can subsidize fishery management measures for the species in the region and contribute with further investigation on the hypothesis of a discrete local stock by the use of reproductive biology parameters found herein, as well as provide a baseline for future reproductive biology research of *G. barbus* throughout its distribution.

MATERIAL AND METHODS

Study site and fish sampling

The study integrates the Fishing Monitoring Project of Tramandaí River Estuary (MOPERT) and was conducted during 2020 and 2021 in the municipalities of Tramandaí (30° 0′ 38″ S, 50° 9′ 8″ W) and Imbé (29° 57’ 37” S, 50° 7’ 43” W), state of Rio Grande do Sul, Brazil (Fig. 1). The Tramandaí River Estuary (TRE) is located at the state North Shore, within the Tramandaí River Hydrographic Basin (TRHB), which includes sandy beaches, an estuarine channel connecting the Atlantic Ocean and a lagoon system, lakes, and rivers (Fig. 1).

Fish were monthly collected from July 2020 to February 2021, in the TRE through professional fishers captures or scientific sampling (authorizations numbers: SISBIO 76612-1, MMA-BR 60978-8, CEUA/UFRGS 23078.502645/2019-01). As diverse fisheries are monitored by MOPERT, specimens were sampled as they were available by fishers and using diverse fishing methods such as gillnets, cast nets and line fishing. Scientific sampling activities were carried out during the white-sea-catfish fishing prohibition period (from December 15th to March 31st) mostly at the Tramandaí lagoon (gillnets), and complementarily at the estuarine channel and Tramandaí river (cast net and inshore line fishing). After sampled and identified (Macerniuk 2005a, 2005b), fish were kept in thermal boxes with ice for transportation.

Laboratorial procedures

Cooled individuals’ total length (TL, in millimeters) and total weight (TW, in kilograms) were measured, and a ventral incision was made to gonad and liver extraction. Ovaries and testis were sexually determined and classified macroscopically following the scale proposed by Brown-Peterson et al. (2011), to provide a reproductive phase assign for fresh gonads. This first classification was based on color, form, vascular irrigation intensity, and gonadal volume of abdominal cavity occupation. Photographs of the gonads were taken to reassessment after histological analysis, aiming to improve accuracy and further method analysis. Following, gonads and livers were weighted (GW and LW respectively, in grams) and fixated in formaldehyde solution (10%) for subsequent analysis

Reproductive phases description and assignment

A subsample of gonads was selected for histological analysis aiming a microscopic characterization of gonadal maturational phases based on Brown-Peterson et al. (2011). To investigate possible histological differences along the gonad’s length, proximal, medial, and distal sections were extracted from the gonads of 20 individuals. Gonadal segments were dehydrated in ethanol crescent series and embedded in historesin (glycol methacrylate resin Leica). Transverse and sagittal sections, three to five µm thick were made in a microtome (Leica, model RM 2145). Sections were stained with Toluidine Blue.

As hydrated vitellogenic oocytes of *G. barbus* are too large for histological procedure, internal macroscopical gonadal analysis were also performed for females to investigate reproductive markers such as the presence and abundance of vitellogenic oocytes, atretic oocytes, and post-ovulatory follicles (POF), as well as ovarian wall thickness and general internal development.

During ovaries analysis, a new structure, never described before for fishes, and named here as "reserve zone" had its morphology and development also investigated macroscopically and histologically. 12 females were analyzed, comprehending all reproductive phases except "Immatures". Ovarian Longitudinal Total Length (OLTL) and Reserve Zone Length (RZL) were measured. Analysis was performed based on the percentage of RZL relative to OLTL, through different gonadal development phases, and one-way ANOVA was applied to test significance on percentage variation. Logistic regression was applied to RZL data to test differences in RZL between reproductive phases

Fecundity and spawning type

The absolute fecundity (AF) was estimated counting all the hydrated vitellogenic oocytes (HVO) present in the ovaries of "Spawning capable" females. Relative fecundity (RF) was determined by the number of HVO per female's TW. Linear correlation between AF and TL, and AF and TW were tested through Pearson's coefficient. A subsample of 200 oocytes was removed from nine "Spawning capable" females. Each oocyte diameter was obtained with the use of a digital caliper, for a diameter frequency graphic plotation, aiming to establish the species' spawning strategies.

Spawning season

Gonadossomatic Index (GSI) were estimated for males and females by the formula GSI = GW x 100/TW (Santos, 1978), converting TW from kilograms to grams. For the species' reproductive period establishment, monthly variation of mean GSI and monthly variation of gonadal maturation phases relative frequencies were analyzed for males and females. Hepatosomatic Index (HSI) for males and females was obtained using the formula HSI = LW x 100/TW (Santos, 1978), converting TW from kilograms to grams. Mean HSI monthly variation was also determined to assess reproductive period. Tukey tests were applied to check significant variances of both indexes between months.

RESULTS

Sampling

Overall, 208 specimens of *G. barbus* (Fig. 2) were used in this study: 87 females (TL from 120 to 1000.3 mm, and TW from 0.014 to 15.12 kg) and 121 males (TL from 133 to 841 mm, and TW from 0.02 to 6.076 kg). Frequencies of sampled individuals by Total Length are indicated in Figure 3. Sample size of each location is described in Table 1.

Reproductive phase's description and assignment

G. barbus is a gonochoristic species. The gonads are paired elongated structures, one on either side of the fish midline, located ventrally to kidneys and swim bladder, in the abdominal cavity, separated by connective tissues. Anterior to ovarian orifice, the reserve zone, a new structure described for the species, can be observed in mature individuals (Fig. 4 b-f). Cystovarian ovaries are suspended from the dorsal wall of the peritoneum by the mesovarium. Tubular anastomosed testes are organized similarly to ovaries, also suspended from the dorsal wall of the peritoneum, by the mesorchium.

From macroscopic and microscopic analysis of the gonads (N=124), six reproductive phases were characterized for the females (Immature - 1, Early developing - 2, Late developing - 3, Spawning capable - 4, Regressing - 5 and Regenerating - 6) (Table 2), and six for the males (Immature - 1, Early developing - 2, Mid developing - 3, Late developing – 4, Spawning capable – 5, and Regressing - 6) (Table 3). As no significant differences were observed between proximal, medial, and distal sections, the issue will not be addressed.

After histological analysis, males' macroscopical features proved to be unreliable for developmental phase determination, as phases 1, 2 and 3 could not be distinguished, as well as phases 4 and 5, and 5 and 6 between themselves. For this reason, males phase determination was based only on the histologically analyzed subsample, with exception for cases of doubtless macroscopic phase attribution (e.g., very small and immature individuals. For females, ovaries macroscopic (internal and external) features were useful for reproductive phase determination, being analyzed along with histological results.

In *G. barbus*, macroscopic and microscopic features of a distinct portion of the ovary's named as "reserve zone" were explored, related to reproductive phase assignment and development. As reproductive development progress, the oocyte reserve zone can provide oocytes for the ovary, acting like a "second ovary", containing oocytes that precede the next ovarian reproductive phase. Such hypothesis is based on differences observed between reserve zone's length of different reproductive phases, as well as on histologic analysis. Immature individuals' reserve zone does not show any sign of development (being oocyte free), but as soon as fish reach "Early developing" phase, it is already possible to observe a longer reserve zone with the presence of oogonia and PG oocytes (indicating germinal activity). When the ovaries become occupied by vitellogenic oocytes (during "Late developing" and "Spawning capable" phases), the reserve zone length proportion goes down. As fish enter the "Regressing" phase, the ovaries shrink, and the reserve zone become smaller and much less distinct probably due the decrease of its germinal activity. After spawn has occurred, ovaries begin to shrink and reserve zone length percentage grows, as females regress. As the next cycle starts, the reserve zone will move as a "running machine" inside the ovaries, providing new oocyte batches that will develop until next spawning season.

Ovarian Longitudinal Total Length (OLTL) ranged from 76.5 to 503.76 mm, Reserve zone's length (RZL) ranged from 25.76 to 171.06 mm, and proportion ranged from 33.67% to 63.15%. One-way ANOVA did not point a significant variation ($p = 0.054$) between RZL proposition and reproductive phases. According to the boxplots (Fig. 7), it is possible to see that grouped phases have a similar range of RZL proportion. For the logistic regression, two groups were artificially separated to test the difference in RZL proportion. "Late development" and "Spawning capable" phases were grouped and are here cited as "Group 1". "Early development" and "Regressing" were also grouped and named as "Group 2". The groups were separated based on the distribution of RZL proportions according to each phase. The "Regenerating" phase was excluded because it is an intermediate state between phases that could be seen as a late "Regressing" phase or a preceding “Early developing” and develop in an unknow singular manner, and by taking these arguments into account, to simplify the logistic regression, aiming preliminary results.

After logistically regressing Groups 1 and 2 against the RZL proportion (Fig. 8), we observe that the two groups can be significantly ($p < 0.05$) separated by the RZL proportion. At approximately 35% of RZL proportion, 100% of individuals belong to Group 1, and at approximately 55%, 100% individuals belong to Group 2.

Fecundity and spawning type

Minimum, maximum, and mean values of absolute fecundity were 104, 272 and 169 hydrated vitellogenic oocytes, respectively (Table 4). Relative fecundity ranged from 21,31 to 31,36 oocytes/kg, and the mean was equal to 25,10 oocytes/kg (0,0213 to 0,0313 oocytes/mg, mean = 0,0251 oocytes/mg). Pearson’s correlation test showed a positive correlation between absolute fecundity and individual’s total length ($r = 0.956$; $p < 0.001$) and a positive correlation between absolute fecundity and individual’s total weight ($r = 0.984$; $p < 0.001$). Frequency distribution analysis of oocytes diameter (ranging from 1.25 millimeters minimum to 21 millimeters maximum) suggests total spawn and iteroparity, as two main groups were identified, a more frequent one representing reserve oocytes (diameter ranging from approximately 2-3 millimeters), and a less frequent representing hydrated vitellogenic oocytes (diameter ranging from approximately 14-21 millimeters) (Fig. 9). Oocyte development pattern suggests group-synchrony spawn, which corresponds to determinate fecundity.

Spawning season

Spawning season aspects were based on three analyzed features: reproductive phases monthly frequencies, mean GSI and HSI monthly variation. For females, “Early developing” individuals were observed in all months, with the first register of “Late developing” in September, and the first “Spawning capable” in October, increasing greatly in November and December. In December, “Regressing” and “Regenerating” individuals were registered. In January, only “Early developing” individuals were observed (Fig. 10a). For males, only a “Mid developing” individual was observed in July. From August to October, “Early developing” and “Mid developing” were observed, with the first register of a “Late developing” in October. The first observation of a “Spawning capable” individual occurred in November, the presence lasting until January. In February, a “Regressing” individual was observed (Fig. 10b).

Mean GSI monthly variations of females showed small values from July to September, increasing greatly in October, and peaking in December (Fig. 11a). For males, values were small from July to December. High values were observed in January, dropping again in February (Fig. 11b). HSI variations were significant for females between July and December, October and December, and November and December (Fig. 12a), and for males between August and February, October and January, October and February, and November and December (Fig. 12b).

DISCUSSION

Reproductive phases description and assignment

Ovaries

As more detailed aspects of fish species reproductive biology are investigated and given attention, there is growing recognition on species-specific maturational scales importance, not only for life history understanding, but also for fish resource management (Lowerre-Barbieri et al., 2011). Previous research on *G. barbus* reproductive biology (Reis 1986a; Chaves et al. 2017; and Vidal et al. 2020) did not cite their source for reproductive phase determination, or description of cited phases, utilizing a not standardized terminology, hampering its use by scientists, managers and fisherfolks throughout the species distribution.

Ovaries are more frequently utilized in reproductive biology studies, due to their larger sizes and more observable external transformation during development compared to testis, and therefore easier to macroscopic assess (Brown-Peterson et al., 2011; Lowerre-Barbieri et. 2011). Reproductive biology markers such as oocytes, ovarian wall thickness and POFs can be used for *G. barbus* macroscopic reproductive phase assignment due to their large size, although usually analyzed by histology techniques for other species. Of the same genus, only *G. genidens* have its reproductive phases described, including histological analysis (Gomes and Araújo, 2004), while Machado et al (2012) assessed *G. machadoi* reproductive biology preliminarily, determining the sex of 19 individuals, and pointing the fact that 11 females were "mature" (i.e., Spawning capable), but without describing macroscopic reproductive phases. As presented herein, a six-phase maturational scale was described by Gomes and Araújo (2004) for *G. genidens* and *Sciadeichthys luniscutis*, and by Muro-Torres and Amezcua (2011) for *Bagre panamensis*, while Sang et al. (2019) described five reproductive phases for sagor catfish *Hexanematichthys sagor*. In general, macroscopic features of ovaries of sea catfishes undergo similar changes from small and filiform when immature, to large and lobular during developing, ending wrinkled and less lobular as the cycle restarts (Gomes and Araújo, 2004; Muro-Torres and Amezcua 2011).

Histology technique, although time consuming, is considered the most accurate method for reproductive phase assignment (Kjesbu et al. 2003). Immature individuals are those that do not participate in the reproductive cycle yet, and its gonadal development is gonadotropin independent (Brown-Peterson et al. 2011). In *G. barbus*, immature ovaries show only nests of oogonia, chromatin nucleolar and perinucleolar oocytes, a pattern observed in other ariid species like *G. genidens* and *S. luniscutis* (Gomes and Araújo, 2004), *Cathorops spixii* (Fávaro et al. 2005), many other Ostariophysi species of characiforms and siluriform families (Quagio-Grassiotto and Ishiba (2013). Since *G. barbus* "Early developing" phase is characterized by previous PG oocytes, CA, and primary vitellogenic oocytes, differing from what was described for other ariid fishes (Gomes and Araújo, 2004), it is possible that vitellogenin prematurely starts to be produced in the species. Reserve zone could be receiving vitellogenin inputs very early on *G. barbus* cycle triggering primary vitellogenesis, as a mean of improving nourishment and hence survival of the offspring, as the species shows low fecundity, granting yolk production before transition between marine and freshwater, a critical and physiologically demanding period for the females. The "Late developing" and "Spawning capable" phases also show traits observed earlier than in many species of ariid and other siluriform families (Gomes and Araújo, 2004; Brown-Peterson et al., 2011). Transition from

"Regenerating" to "Early developing" phases is a gradual process that can be observed through specific features described herein. Sang et al. (2019), studying *H. sagor* (Ariidae) and Chandran and Prasad (2018), studying *Horabagrus brachysoma* (a siluroid) did not report a "Regenerating" phase, while Gomes and Araújo (2004) found oogonias and PG oocytes in *G. genidens* and *S. luniscutis*, and fibrous nodules that could hypothetically be muscle bundles.

Reserve zone

As explored herein, *G. barbus* ovaries undergo drastic size and form variation through the species life cycle. The development of the reserve zone might be an advantageous trait for the species reproduction, as the ovaries can be an inadequate place for oocyte development and replenishment, as morphological changes occur, and linked with the species low fecundity. Also, as the species show signs of quick early development, and a possible rapid transition between "Regenerating" and "Early developing" reproductive phases, an active reserve zone that ensures oocyte provision efficiently can be adaptative. The reserve zone was not described before for other fish species. Investigating its role might be enlightening for understanding fish reproductive biology and evolution, notably for ariid species, as it is plausible to believe that other *Genidens* and ariid species also contain it.

Testis

Spermatogenesis is not studied as widely as oogenesis (Lowerre-Barbieri et al. 2011), due to the larger size of oocytes in comparison to males' germinal cells (notably for *G. barbus*), consequently making testis macroscopical development less evident than ovaries. Weak reliability on macroscopic testis features of *G. barbus* were observed, as well as in Reis (1986a). After histological checking, it is possible to point that "Immature" and "Early developing" testis cannot always be macroscopically distinguished. "Mid developing" testis can be externally similar to "Early developing" but might show external features that can be observed on "Late developing" phase. When late developed, testis can be similar to "Spawning capable" phase" (when showing robust and thicker appearance). Nevertheless, "Spawning capable" individuals might be externally equal to "Regressing" individuals, when flattened.

A "Regenerating" phase was not observed in the present study, as recorded by Gomes and Araújo (2004) for *G. genidens* and *S. luniscutis*, in the testis of individuals sampled, probably mainly due to the species' life cycle. Based on Gomes and Araújo (2004), and Sang et al. (2019), it is possible to point that *G. barbus* large body size hampers a better macroscopical testis differentiation, as these authors observed a variation on testis body occupation proportion as they develop, a feature not utilized in the present study. Many limitations on males' reproductive phase assignment were described and emphasizing the need of histological analysis for monitoring fisheries. Although limited, macroscopic inferences might be useful for discrimination of immatures and adults, a tool that can be explored for stock assessment and management (King 2007).

Fecundity and spawning type

Absolute fecundity in the study area were similar to that found by Reis (1986a), being the maximum identical. Minimum varied significantly (whereas she found 32 hydrated vitellogenic oocytes, a minimum of 104 hydrated vitellogenic oocytes was found here), and this occurred probably due to divergence on reproductive phase assignment. As Reis (1986a) sample showed a TL variation of 310 to 980 mm, and in this study a variation of 745 to 995 mm was observed, it is possible that the authors utilized “Late developing” individuals when considering “Ripe” individuals, therefore reducing absolute fecundity minimum. As found in Reis (1986a), Chaves et al. (2017) and Vidal et al. (2020), there is a positive correlation between absolute fecundity and body size (TL and TW) in *G. barbus*, a common pattern for fishes (Froese 2004; Palumbi 2004). As a result, unmanaged fishing activities (that usually aim larger individuals), can act diminishing fishes’ fitness (Conover 2007). As pointed out by Chaves et al. (2017), for *G. barbus*, maximum length capture could be used as a fishery management tool, viable for line fishing and maybe for gillnet if individual is still alive. Such approach also must account for senility, as not necessarily the bigger sizes would be the right for conservation (since fecundity will eventually start to decay in older fish) (Fitzhugh et al. 2012). Results could suggest that, based on fecundity analysis, fishery-induced evolution cannot be observed yet (as an induction of fisheries, that aim capturing bigger individuals, led to reduced size at maturity and consequently reduced absolute fecundity). Nonetheless, such assumption can only be confirmed with new fecundity analysis of Patos Lagoon *G. barbus* population, as the species might be geographically structured, consisting of several stocks (Avigliano et al. 2017a; Maciel et al. 2021), and therefore showing each their own fecundity dynamics, or with data suggesting that Patos Lagoon and TRE populations are the same. The lack of variation on absolute fecundity (based on equal maximum values) could also be occurring due to the large generational time of *G. barbus*, which could me masking an aspect of fishery induced evolution. It is important that species’ fecundity variations are incorporated in spawning stock assessment, as they can influence significatively (He et al. 2015).

As expected, based on previous ariid reproductive strategy observations (Mishima and Tanji 1983; Reis 1986a; Gomes et al. 1999), oocyte diameter frequency and development pattern analysis, as well as POFs analysis, *G. barbus* is: (1) A total spawner, releasing eggs in a single episode or over a short period; (2) Iteroparous, as it can breed multiple times throughout life; (3) Its development is group-synchrony, occurring in two-groups (considering only ovaries, excluding the reserve zone) and (4) shows determinate fecundity, meaning that recruitment of oocytes from PG to secondary growth occurs before the spawning period (Murua and Saborido-Rey 2003). These specific reproductive strategies must be considered by life history hypothesis and stock assessment, especially due to its great impact on recruitment and other population dynamic traits (Lowerre-Barbieri 2011).

Spawning season

Reis (1986a) proposed that *G. barbus* life cycle occurred through migration movements between marine and estuarine environments. Adult fish would enter in the Patos Lagoon estuary at austral late winter-early spring, coming from the sea, and during the austral summer, spawn should take place. The abundance of young fish near to marine waters in February (Araújo 1983) led to the assumption that mouth-breeder males would slowly return to the sea during incubation, releasing the yolk sac free young there, at a point that females had already returned to the sea (Reis 1986a). Avigliano et al. (2017; 2020) agreed with the life cycle suggested by Reis et al (1986a), although these authors also suggest that this is only one of many possible patterns of *G. barbus* reproductive migratory behavior, as the species shows a high migratory plasticity and complex life history, including residency and cyclical migration between freshwater, estuarine and marine environments, together with ontogenetic pattern changes.

Recently new data has been published concerning *G. barbus* great freshwater dispersal capacity, fish being registered up to more than 200 km and 300 km distant from estuarine waters in the Guaíba Lake (Fontoura et al. 2019) and Paraná River (Avigliano et al. 2015), respectively, and approximately 125 upstream in the Uruguay River (Vidal et al. 2020). In agreement with these results and associated with the species high migratory plasticity proposed by Avigliano et al. (2017), results found here indicate that *G. barbus* spawning season ranges from October to January in the study area. For females, mean GSI increase, and reproductive phases variation suggest the beginning in October, ending in December when mean GSI peaks, followed by a great drop in January, and a great number of “Spawning Capable” individuals, along with a few individuals in post-spawn phases, indicating the end. These results are differing from Reis (1986a), that proposed the spawning season for females to begin in the end of November and early December but were similar for the end in December. For the males, the season begins in November, peaking from December to January, based on a great increase in GSI and frequency of “Spawning capable” individuals, ending in February, as GSI drops significatively, and a “Regressing” individual was observed. The absence of “Regenerating” males, as well as low numbers of females in this phase, can be explained by the species’ migratory behavior. Fish will leave the estuarine region during regression and regeneration, to restart the cycle in an adequate environment, which could mean more availability of resources and less competition. Otolith analysis of the histologically sampled population could also reveal insights into how spatial distribution over different habitat and reproductive behavior are linked. Reis (1986a) suggested that maturation period of both sexes would occur at the same time and highlighted the active spermiogenesis at the end of the year, resting during the remaining months, but the males’ GSI peak were observed to be in January, and active spermiogenesis until January. Gomes et al (1999) also observed the greatest proportion of “Spawning capable” males of *G. genidens* a month later than females at the same reproductive phase. Divergences between studies are expected to occur when applying different sampling methods or abiotic regimes, but results of this study along with Reis (1986a), Gomes et (1999) and Vidal et al (2020) underline the occurrence of *Genidens* sp. spawning season during late spring and summer months.

The definition of spawning season and space limits can be useful for *G. barbus* fishery management plans, as it can support capture closed seasons and areas, notably due to the species behavior of spawning aggregations, which make them vulnerable to overfishing, evidently needing a more precautionary approach (Arendse et al. 2007; De Mitcheson 2016), even dealing with the Hilborn and Walters (1992) “hyperstability”, that is, the maintenance of biomass during spawning aggregation, masking stock decline due to overfishing. Efficiency of such management tool rely on other aspects of the fishing systems, like enforcement, reduced fishing effort outside capture closing limits, along with biological knowledge on different temporal and spatial scales (De Mitcheson 2016). Currently there is a total prohibition on *Genidens barbus* capture in Brazil, but the closed season for the species (from January 1st to March 31st) (MMA, 2004) is still necessary for fishing monitoring programs. In the TRE, the closed season was later modified, ranging from January 15th to March 31st (MMA, 2004). As “Spawning capable” individuals were first registered in October, it is therefore suggested a reassessment on the beginning of the closed season in the TRE if managed fisheries perdure, as the protection of these individuals are essential for the stock health and hence sustainable fisheries. It is compulsory that any alteration on closed season limits is aligned with the fishing sector socioeconomic dynamics, as species conservational goals must meet balance with fisherfolks communities' subsistence. Further analysis on the extension of the incubation period is needed to assess the adequate date on the closed season's ending for the area.

The HSI analysis supported the index use as a valid tool for *G. barbus* life cycle analysis, as significant variations were observed between pre-spawning season and spawning season months, and negative correlation with GSI, for both sexes. For females, results varied accordingly as expected, as important precursors of reproductive development are synthesized in the liver, like vitellogenin and zona pellucida proteins, along with energy stored prior to active spawning (Wooton and Smith 2014). Males' variations also varied significatively between pre-spawning and spawning season months, at first due to migration energy use. It is still necessary to biochemically check these physiology specific hypothesis of liver activity, as well as to further investigate possible correlations between males fasting - as according to Reis (1986a) and Rimmer and Merrick (1982), male marine catfish are usually found to be with more stomach content than females because of mouthbrood - and liver energy storage. Other studies on ariid HSI showed that it can vary inversely in relation to GSI (as observed in the present study), as for *Occidentarius platypogon* (Amezcua and Muro-Torres 2012), or positively in relation to GSI, as for *B. panamensis* (Zavala-Leal et al. 2019). Variations highlight the physiology diversity and plasticity of the family, as both species were investigated in the Gulf of California.

A single and short spawn period is commonly reported for marine catfish species (Sang et al. 2019; Fávaro et al. 2005; Queiroga et al. 2015; Gomes and Araújo 2004). According to Rimmer and Merrick (1982), marine catfish are expected to spawn during warm season or associated with increasing temperatures, and Vidal et al. (2020) highlighted the importance of river discharge for reproductive migration and period of *G. barbus* in Uruguay. Gomes et al. (1999) reported that *G. genidens* and *C. spixii* spawning seasons are correlated with higher transparency and temperatures of water, and low salinity. Further abiotic analysis is needed for our study area in relation to *G. barbus* spawning season, as it is plausible that each *G. barbus* population has its own spawning specificities, related to specific estuarine abiotic regimes. Based on reproductive phases monthly variation and GSI, it can be suggested that, in the TRE region, fish congregate to spawn and can disperse after that to other areas, for purposes like those suggested by Avigliano et al. (2017), such as reproductive or feeding functions. As described before, possibilities of migratory behavior range from return to sea and offshore or near coastal behaviors, to freshwater residency (Fig. 13). It is necessary to amplify sampling to other south and northward regions and investigate its reproductive biology. *G. barbus* is considered "Endangered" (MMA 2022), and as its fisheries collapsed in the Patos Lagoon Estuary, historically the species main fishery area in Brazil (Reis 1986b; Haimovici and Cardoso 2017), to understand its life cycle is a basic aspect when aiming sustainable fisheries for the future (Jackson et al. 2011). As *G. barbus* is a migratory species, describing its life cycle might be specially challenging, and alternative methods might be useful. Acoustic telemetry allows to follow individuals over space and time, being useful to understand reproductive biology and ecological aspects, as well as how theses aspects can impact fisheries (Lowerre-Barbieri et al., 2016). Tag and recapture methods can also provide enlightening aspects on fish species large-scale patterns such as area utilization and population's movements (Espeland et al. 2008). Hughes et al (2022) investigated the efficiency of tagging programs by cooperative citizen-science, which can be a possibility within *G. barbus* fishing monitoring programs throughout its distribution, through cooperation between scientists, managers and fisherfolks.

References

Acero, A., 2002. Family Ariidae. living Mar. Resour. West. Cent. Atl. 831–852.

Acero P. A., Betancur-R., R., 2007. Monophyly, affinities, and subfamilial clades of sea catfishes (Siluriformes: Ariidae). Ichthyol. Explor. Freshwaters 18, 133–143.

Alonso-Fernández, A., Alós, J., Grau, A., Domínguez-Petit, R., Saborido-Rey, F., 2011. The use of histological techniques to study the reproductive biology of the hermaphroditic Mediterranean fishes *Coris julis, Serranus scriba,* and *Diplodus annularis*. Mar. Coast. Fish. 3, 145–159. https://doi.org/10.1080/19425120.2011.556927

Amezcua, F., Muro-Torres, V., 2012. Biología reproductiva del bagre cominate *Occidentarius platypogon* (Pisces: Ariidae) en el sureste del golfo de California. Lat. Am. J. Aquat. Res. 40, 428–434. https://doi.org/10.3856/vol40-issue2-fulltext-17

Araújo, F.G., 1988. Distribuição, abundância relativa e movimentos sazonais de bagres marinhos (Siluriformes, Ariidae) no estuário da Lagoa dos Patos (RS), Brasil. Rev. Bras. Zool. 5, 509–543. https://doi.org/10.1590/s0101-81751988000400002

Arendse, C., J., Govender, A., Branch, G., M. 2007. Are closed fishing seasons an effective means of increasing reproductive output? A per-recruit simulation using the limpet Cymbula granatina as a case history. Fisheries Research 85, 93-100.

Avigliano, E., Carvalho, B., Velasco, G., Tripodi, P., Vianna, M., Volpedo, A.V., 2017a. Nursery areas and connectivity of the adults anadromous catfish (*Genidens barbus*) revealed by otolith-core microchemistry in the south-western Atlantic Ocean. Mar. Freshw. Res. 68, 931–940. https://doi.org/10.1071/MF16058

Avigliano, E., Leisen, M., Romero, R., Carvalho, B., Velasco, G., Vianna, M., Barra, F., Volpedo, A.V., 2017b. Fluvio-marine travelers from South America: Cyclic amphidromy and freshwater residency, typical behaviors in *Genidens barbus* inferred by otolith chemistry. Fish. Res. 193, 184–194. https://doi.org/10.1016/j.fishres.2017.04.011

Avigliano, E., Miller, N., Maichak de Carvalho, B., Gironde, S.C., Tombari, A., Volpedo, A. V., 2020. Fin spine metals by LA-ICP-MS as a method for fish stock discrimination of *Genidens barbus* in anthropized estuaries. Fish. Res. 230. https://doi.org/10.1016/j.fishres.2020.105625

Avigliano, E., Martínez, G., Stoessel, L., Méndez, A., Bordel, N., Pisonero, J. Volpedo, A, 2020. Otoliths as indicators for fish behavior and procurement strategies of hunther-gatherers in North Patagonia. Heliyon 6. https://doi.org/10.1016/j.heliyon.2020.e03438

Avigliano, E., Pisonero, J., Méndez, A., Tombari, A., Volpedo, A. V., 2021. Habitat use of the amphidromous catfish *Genidens barbus*: first insights at its southern distribution limit. New Zeal. J. Mar. Freshw. Res. 0, 1–7. https://doi.org/10.1080/00288330.2021.1879178

Avigliano, E., Velasco, G., Volpedo, A. V., 2015. Assessing the use of two southwestern Atlantic estuaries by different life cycle stages of the anadromous catfish *Genidens barbus* (Lacépède, 1803) as revealed by Sr: Ca and Ba: Ca ratios in otoliths. J. Appl. Ichthyol. 31, 740–743. https://doi.org/10.1111/jai.12766

Avigliano, E., Volpedo, A.V., 2015. New records of anadromous catfish *Genidens barbus* (Lacépède, 1803) in the Paraná Delta (South America): Evidence of extension in the migration corridor? Mar. Biodivers. Rec. 8, 1–3. https://doi.org/10.1017/S175526721400147X

Betancur-R., R., Acero P., A., Bermingham, E., Cooke, R., 2007. Systematics and biogeography of New World sea catfishes (Siluriformes: Ariidae) as inferred from mitochondrial, nuclear, and morphological evidence. Mol. Phylogenet. Evol. 45, 339–357. https://doi.org/10.1016/j.ympev.2007.02.022

Brown-Peterson, N.J., Wyanski, D.M., Saborido-Rey, F., Macewicz, B.J., Lowerre-Barbieri, S.K., 2011. A standardized terminology for describing reproductive development in fishes. Mar. Coast. Fish. 3, 52–70. https://doi.org/10.1080/19425120.2011.555724

Campuzano-Caballero, J.C., Uribe, M.C., 2017. Functional morphology of the gonoduct of the viviparous teleost Poeciliopsis gracilis (Heckel, 1848) (Poeciliidae). J. Morphol. 278, 1647–1655. https://doi.org/10.1002/jmor.20738

Campuzano-Caballero, J.C., Uribe, M.C., 2014. Structure of the female gonoduct of the viviparous teleost *Poecilia reticulata* (Poeciliidae) during nongestation and gestation stages. J. Morphol. 275, 247–257. https://doi.org/10.1002/jmor.20200

Chandran, L.R., Prasad, G., 2018. Gonadal development and gametogenesis in the threatened freshwater siluroid fish *Horabagrus brachysoma* (Gunther, 1864): A morpho-histological perspective. Indian J. Fish. 65, 50–58. https://doi.org/10.21077/ijf.2018.65.4.54808-06

Chaves, P.D.T.D.C., Azeredo, F.G., Pinheiro, E., 2017. Fecundidade de peixes e tamanhos máximos de captura: Instrumento auxiliar à gestão de pesca. Bol. do Inst. Pesca 43, 542–556. https://doi.org/10.20950/1678-2305.2017v43n4p542

Conover, D. O., 2007. Net versus nature. Nature. 450, 179-180.

Costello, C., Ovando, D., Hilborn, R., Gaines, S.D., Deschenes, O., Lester, S.E., 2009. Reports Status and Solutions for the World ' s Unassessed Fisheries. Sci. Express 1–4.
David Gomes, I., Gerson Araújo, F., 2014. Reproductive biology of two marine catfishes (Siluriformes, Ariidae) in the Sepetiba Bay, Brazil. Rev. Biol. Trop. 52, 143. https://doi.org/10.15517/rbt.v52i1.14763

De Mitcheson, Y. S. 2016. Mainstreaming Fish Spawning Aggregations into Fishery

Management Calls for a Precautionary Approach. Bio Science 66, 295-306.

Espeland, S. H., Olsen, E. M., Knutsen, H., Gjøsæter, J., Danielssen, D., Stenseth, N.C. 2008. New perspectives on fish movement: kernel and GAM smoothers applied to a century of tagging data on coastal Atlantic cod. Mar. Ecol. Prog. Ser. 372, 231–241

FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

Fávaro, L.F., Frehse, F. de A., Oliveira, R.N. de, Schwarz Júnior, R., 2005. Reprodução do bagre amarelo, *Cathorops spixii* (Agassiz) (Siluriformes, Ariidae), da Baía de Pinheiros, região estuarina do litoral do Paraná, Brasil. Rev. Bras. Zool. 22, 1022–1029. https://doi.org/10.1590/s0101-81752005000400030

Fischer, L.G., Pereira, L.E.D., Vieira, J.P., 2011. Peixes Estuarinos Costeiros, 2nd ed. Rio Grande.

Fitzhugh, G.R., Shertzer, K.W., Todd Kellison, G., Wyanski, D.M., 2012. Review of size- and age-dependence in batch spawning: Implications for stock assessment of fish species exhibiting indeterminate fecundity. Fish. Bull. 110, 413–425.

Fontoura, N.F., Schulz, U.H., Alves, T.P., Silveira, T.C.L., Pereira, J.J., Antonetti, D.A., 2019. How far upstream: A review of estuary-fresh water fish movements in a large neotropical basin. Front. Mar. Sci. 6, 1–12. https://doi.org/10.3389/fmars.2019.00039

Froese, R., 2004. Keep it simple: Three indicators to deal with overfishing. Fish Fish. 5, 86–91. https://doi.org/10.1111/j.1467-2979.2004.00144.x

Giora, J., Fialho, C.B., 2009. Reproductive biology of weakly electric fish *Eigenmannia trilineata* López and Castello, 1966 (Teleostei, Sternopygidae). Brazilian Arch. Biol. Technol. 52, 617–628. https://doi.org/10.1590/S1516-89132009000300014

Gomes, I.D., Araújo, F.G., Azevêdo, M.C.C. de, Pessanha, A.L.M., 1999. Biologia reprodutiva dos bagres marinhos *Genidens genidens* (Valenciennes) e *Cathorops spixii* (Agassiz) (Siluriformes, Ariidae), na Baía de Sepetiba, Rio de Janeiro, Brasil. Rev. Bras. Zool. 16, 171–180. https://doi.org/10.1590/s0101-81751999000600017

Grier, H. J., Uribe, M. C., Patino, R., 2009. The ovary, folluculogenesis, and oogenesis in teleosts. In Jamieson B. G. M., Reproductive biology, and phylogeny of fishes: Agnathans and bony fishes. 8A, 25–84. Enfield, New Hampshire: Science Publishers.

Haimovici, M., Cardoso, L.G., 2017. Long-term changes in the fisheries in the Patos Lagoon estuary and adjacent coastal waters in Southern Brazil. Mar. Biol. Res. 13, 135–150. https://doi.org/10.1080/17451000.2016.1228978
He, X., Field, J.C., Beyer, S.G., Sogard, S.M., 2015. Effects of size-dependent relative fecundity specifications in fishery stock assessments. Fish. Res. 165, 54–62. https://doi.org/10.1016/j.fishres.2014.12.023

Hilborn, R., Walters, C.J. 1992. Quantitative fisheries stock assessment. New York: Chapman and Hall, Inc.

Hughes, M. J., Meadows N. M., Stewart, J., Booth, D. J., Fowler, A. M. 2022. Movement patterns of an iconic recreational fish species, mulloway (Argyrosomus japonicus), revealed by cooperative citizen-science tagging programs in coastal eastern Australia. 2022. Fisheries Research 247.

Hunter, J.D., 2007. Matplotlib: A 2D graphics environment. Comput. Sci. Eng. 9, 99–104. https://doi.org/10.1109/MCSE.2007.55

Hunter, J.R., Macewicz, B.J., 1985. Measurement of spawning frequency in multiple spawning fishes. NOAA Tech. Rep. NMFS 79–94.

Hunter, J.R., Macewicz, B.J., Chyan-Huei Lo, N., Kimbrell, C.A., 1992. Fecundity, spawning, and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. Fish. Bull. 90, 101–128.

IBAMA, 1999. Desembarque de pescados no Rio Grande do Sul. Annual Report, Centro de Pesquisa do Rio Grande, RS, Brasil.

Instituto Chico Mendes de Conservação da Biodiversidade, 2018. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção. Brasília: ICMBio. 4162 p.

JFRS - Justiça Federal Seção Judiciária do Rio Grande do Sul, 2018. JFRS autoriza projeto de monitoramento para pesca do Bagre no Rio Tramandaí. Retrieved from: https://www2.jfrs.jus.br/noticias/jfrs-autoriza-projeto-de-monitoramento-para-pesca-do-bagre-no-rio-tramandai/

King, M.G., 2007. Fisheries biology, assessment, and management, 2nd ed. Blackwell Publishing Ltd, Oxford.

Kjesbu, O., Hunter, J., Witthames, P., 2003. Report of the working group on modern approaches to assess maturity and fecundity of warm- and coldwater fish and squids. S. 2003–12.

López, R. B., Bellisio, N. B., 1965. Contribución al conocimiento del *Tachysurus barbus* (Lacépède), bagre del mar argentino. (Pisces, Ariidae). Anuario del II Congreso Latinoamericano Zool. Vol. 2, p.145–153.

Lowerre-Barbieri, S., 2009. Reproduction in Relation to Conservation and Exploitation of Marine Fishes. Reprod. Biol. Phylogeny Fishes, Vol 8B 371–394. https://doi.org/10.1201/b10257-11

Lowerre-Barbieri, S.K., Brown-Peterson, N.J., Murua, H., Tomkiewicz, J., Wyanski, D.M., Saborido-Rey, F., 2011. Emerging issues and methodological advances in fisheries reproductive biology. Mar. Coast. Fish. 3, 32–51. https://doi.org/10.1080/19425120.2011.555725

Lowerre-Barbieri, S. K., Burnsed, S. L. W., Bickford, J. W. 2016. Assessing reproductive behavior important to fisheries management: a case study with red drum, Sciaenops ocellatus. Ecological Applications 26 (4), 979-995.

Machado, R., Ott, P.H., Sucunza, F., Marceniuk, A.P., 2012. Ocorrência do bagre marinho *Genidens machadoi* (Siluriformes, Ariidae) na laguna Tramandaí, sul do Brasil. Neotrop. Biol. Conserv. 7, 214–219. https://doi.org/10.4013/nbc.2012.73.08

Marceniuk, Alexandre P., 2005a. Chave para identificação das espécies de bagres marinhos (Siluriformes, Ariidae) da costa brasileira. B. Inst. Pesca 31, 89–101.

Marceniuk, Alexandre P., 2005b. Redescrição de *Genidens Barbus* (Lacépède, 1803) e *Genidens Machadoi* (Miranda-Ribeiro, 1918), Bagres Marinhos (Siluriformes, Ariidae) do Atlântico Sul Ocidental. Pap. Avulsos Zool. 45, 111–125.

Marceniuk, A.P., Menezes, N.A., 2007. Systematics of the family Ariidae (Ostariophysi, Siluriformes), with a redefinition of the genera. Zootaxa. Vol. 1416, p. 1–126.

Marceniuk, A.P., Menezes, N.A., Britto, M.R., 2012 Phylogenetic analysis of the family Ariidae (Ostariophysi: Siluriformes), with a hypothesis on the monophyly and relationships of the genera. Zoological Journal of the Linnean Society. Vol. 165, p. 534–669.

Melo, R.M.C., Arantes, F.P., Sato, Y., dos Santos, J.E., Rizzo, E., Bazzoli, N., 2011. Comparative morphology of the gonadal structure related to reproductive strategies in six species of neotropical catfishes (Teleostei: Siluriformes). J. Morphol. 272, 525–535. https://doi.org/10.1002/jmor.10931

Mendonça, J.T., Balanin, S., Garrone-Neto, D., 2020. The marine catfish *Genidens barbus* (Ariidae) fisheries in the state of São Paulo, southeastern Brazil: Diagnosis and management suggestions. An. Acad. Bras. Cienc. 92, 1–16. https://doi.org/10.1590/0001-3765202020180450

Menezes, N.A., Buckup, P.A., Figueiredo, J.L. De, Moura, R.L. De, 2003. Catálogo das Espécies de Peixes Marinhos do Brasil, Book.

MINAGRI, 2013. Ministerio de Agricultura y Pesca de la Nación Argentina, Argentina. Disponible in: http://www.minagri.gob.ar/site/pesca/pesca_maritima/02-desembarques/anio.php?anio=2012.

Mishima, M., Tanji, S., 1983. Maturaçao e desova de bagres marinhos (Osteichthyes, Ariidae) do complexo estuarino da cananeia (25°S, 48°W). B. Inst. Pesca.

MMA - Ministério do Meio Ambiente, 2014. Portaria MMA n° 445/2014 (2014) Portarias. No 445 de 17 de dezembro de 2014. Ministério do Meio Ambiente - MMA. Diário Oficial da União-Seção 1, 245, 126; Retrieved from https://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=18/12/2014&jornal=1&pagina=126&totalArquivos=144

MMA - Ministério do Meio Ambiente, 2022. Portaria MMA n° 148/2022 (2022) Portarias. No 148 de 7 de junho de 2022. Ministério do Meio Ambiente - MMA. Diário Oficial da União-Seção 1, edição 108, página 74; Retrieved from:

<https://in.gov.br/en/web/dou/-/portaria-mma-n-148-de-7-de-junho-de-2022-406272733>

Muro-Torres, V.M., Amezcua, F., 2011. Observations on the Reproductive Biology of the Chihuil Sea Catfish in the Southeast Gulf of California: Implications for Management. Conserv. Ecol. Manag. Catfish Second Int. Symp. 1–9.

Murua, H., Saborido-Rey, F., 2003. Female reproductive strategies of marine fish species of the North Atlantic. J. Northwest Atl. Fish. Sci. 33, 23–31. https://doi.org/10.2960/J.v33.a2

Nelson, J.S., Grande, T., Wilson, M.V.H., 2016. Fishes of the World, 5th ed. John Wiley & Sons, Inc, New Jersey.
Palumbi, S.R., 2004. Why mothers matter. Nature 430, 621–622.

Pauly, D., Christensen, V., Guénette, S., Pitcher, T.J., Sumaila, U.R., Walters, C.J., Watson, R., Zeller, D., 2002. Towards sustainability in world fisheries. Nature 418, 689–695. https://doi.org/10.1038/nature01017

Quagio-Grassiotto, I., Wildner, D.D., Ishiba, R., 2013. Gametogênese de peixes: aspectos relevantes para o manejo reprodutivo Gametogenesis in Fish: relevant aspects for the reproductive management 37, 181–191.

Queiroga, F.R., Golzio, J.E., dos Santos, R.B., Martins, T.O., Vendel, A.L., 2012. Reproductive biology of *Sciades herzbergii* (Siluriformes: Ariidae) in a tropical estuary in Brazil. Zoologia 29, 397–404. https://doi.org/10.1590/S1984-46702012000500002

Reis, E.G., 1986a. Reproduction and feeding habits of the marine catfish *Netuma barba* (Siluriformes, Ariidae) in the estuary of Lagoa dos Patos, Brazil. Atlântica 8, 35–55.

Reis, E.G. 1986b. A pesca artesanal de bagres marinhos (Siluriformes, Ariidae) no estuário da Lagoa dos Patos (RS), Brasil: Editora da FURG. Documentos Técnicos, Oceanografia, FURG

Rimmer, M.A., 1985. Reproductive cycle of the fork-tailed catfish *Arius graeffei* kner & steindachner (Pisces: Ariidae) from the clarence river, new south wales. Mar. Freshw. Res. 36, 23–32. https://doi.org/10.1071/MF9850023

Rimmer, M. A., Merrick, J. R., 1982. A review of reproduction and development in the fork-tailed catfishes (Ariidae). Proceedings of the Limnologic Society of New South Wales, 107(1), 41-50.

Ripple, W.J., Wolf, C., Newsome, T.M., Betts, M.G., Ceballos, G., Courchamp, F., Hayward, M.W., Van Valkenburgh, B., Wallach, A.D., Worm, B., 2019. Are we eating the world's megafauna to extinction? Conserv. Lett. 1–10. https://doi.org/10.1111/conl.12627

Rodrigues Maciel, T., Vianna, M., Maichak de Carvalho, B., Miller, N., Avigliano, E., 2021. Integrated use of otolith shape and microchemistry to assess *Genidens barbus* fish stock structure. Estuar. Coast. Shelf Sci. 261. https://doi.org/10.1016/j.ecss.2021.107560

Sang, H.M., Lam, H.S., Hai, T.B., 2019. Reproductive biology of sagor catfish (*Hexanematichthys sagor* Hamilton, 1822) in Can Gio water, Vietnam. Indian J. Geo-Marine Sci. 48, 835–840.

Santos, E. P. dos. 1978. Dinâmica de populações aplicada à pesca e piscicultura. Edusp, São Paulo.

Seeliger, U., Odebrecht, C., Castello, J. P., 1998. Ictiofauna. In: Os ecossistemas costeiros e marinho do extremo Sul do Brasil. Rio Grande: Ecoscientia. 341p.

Soeth, M., Spach, H.L., Ribeiro, G.C., Andrade, V.K., 2014. Variação temporal de peixes em diferentes fases ontogenéticas em uma praia abrigada da Baía Norte, Sul do Brasil. Neotrop. Biol. and Conser. Vol. 9(1), p. 27-41.

Skjæraasen, J.E., Nash, R.D.M., Kennedy, J., Thorsen, A., Nilsen, T., Kjesbu, O.S., 2010. Liver energy, atresia, and oocyte stage influence fecundity regulation in Northeast Arctic cod. Mar. Ecol. Prog. Ser. 404, 173–183. https://doi.org/10.3354/meps08486

Vasconcellos, M., Diegues, A. C., De Sales, R., 2008. Aspectos relevantes relacionados à pesca artesanal costeira nacional. In: COSTA, A. L. (Org.). Nas redes da pesca artesanal costeira. Ibama.

Velasco, G., Oddone, M., 2004. Growth parameters and growth performance indexes for some populations of marine catfishes (Actinopterygii, Siluriformes, Ariidae) 26, 307–313.

Velasco, G., Reis, E.G., 2004. Changes in growth seasonality throughout *Netuma barba* (Lacépède, 1803) (Siluriformes, Ariidae) Ontogeny. Braz. J. Biol. 64, 913–914. https://doi.org/10.1590/S1519-69842004000500024

Velasco, G., Reis, E.G., Vieira, J.P., 2007. Calculating growth parameters of *Genidens barbus* (Siluriformes, Ariidae) using length composition and age data. J. Appl. Ichthyol. 23, 64–69. https://doi.org/10.1111/j.1439-0426.2006.00793.x

Vidal, N., D’Anatro, A., González-Bergonzoni, I., Silva, I., Correa, P., País, J., Tesitore, G., Teixeira de Mello, F., 2021. Río de la Plata voyagers: Deciphering the migration ecology of a vulnerable marine catfish (*Genidens barbus*) in a large subtropical river (lower Uruguay River). Aquat. Conserv. Mar. Freshw. Ecosyst. 31, 1367–1378. https://doi.org/10.1002/aqc.3519

Vontobel, E, D., Beroldt, L., 2019. Análise de um sistema socioecológico pesqueiro, na perspectiva dos recursos de uso comum, em Imbé, Sul do Brasil. Boletim Geográfico do Rio Grande do Sul. 34, 52-77.

Waskom, M., 2021. Seaborn: Statistical Data Visualization. J. Open Source Softw. 6, 3021. https://doi.org/10.21105/joss.03021

West, G., 1990. Methods of assessing ovarian development in fishes:A review. Mar. Freshw. Res. 41, 199–222. https://doi.org/10.1071/MF9900199

Wootton, R.J., Smith, C., 2015. Reproductive Biology of Teleost Fishes. John Wiley & Sons, Ltd., Oxford.

World Bank, F. and W.F., 2012. Hidden harvest: The global contribution of capture fisheries. World Bank. Econ. Sect. Work 92.

Wright, P.J., Gibb, F.M., Gibb, I.M., Millar, C.P., 2011. Reproductive investment in the North Sea haddock: Temporal and spatial variation. Mar. Ecol. Prog. Ser. 432, 149–160. https://doi.org/10.3354/meps09168

Yan, H.F., Kyne, P.M., Jabado, R.W., Leeney, R.H., Davidson, L.N.K., Derrick, D.H., Finucci, B., Freckleton, R.P., Fordham, S. V., Dulvy, N.K., 2021. Overfishing and habitat loss drives range contraction of iconic marine fishes to near extinction. Sci. Adv. 7, 1–11. https://doi.org/10.1126/sciadv.abb6026

Zavala-Leal, I., Palacios-Salgado, D., Ruiz-Velazco, M., Nieto-Navarro, J.T., Cadena-Roa, M.A., Domínguez-Ojeda, D., Pacheco-Vega, J.M., Valdez-González, F., 2019. Periodo reproductivo del bagre chihuil *Bagre panamensis* (Siluriformes: Ariidae) en el sureste del Golfo de California. Rev. Biol. Mar. Oceanogr. 54, 21. https://doi.org/10.22370/rbmo.2019.54.1.1459

Tables

Table 1. Absolute numbers of *Genidens barbus* individuals sampled, by sex and sample location. Percentages are indicated in parenthesis.

	Female	Male	Total
Tramandaí Inlet	5 (5.75 %)	14 (11.57 %)	19 (9.13 %)
Passo's Lake	2 (2.30 %)	0 (0.00 %)	2 (0.96 %)
Tramandaí Lagoon	73 (83.91 %)	102 (84.30 %)	175 (84.13 %)
Tramandaí River	7 (8.05 %)	5 (4.13 %)	12 (5.77 %)
Total	87 (41.83%)	121 (58.17 %)	208 (100 %)

Table 2. Reproductive phases of *Genidens barbus* females, based on histological and macroscopical analysis.

Reproductive phase	External macroscopic	Internal macroscopic	Microscopic
Immature	Small, slender, and filiform gonads, occupying less than 5% of the abdominal cavity. Early ovaries on this phase are	Reserve zone without oocytes visible to bare eye. One class diameter oocytes in the ovaries. Thin ovarian wall.	Nests of oogonia and PG oocytes only (Fig. 4a). Reserve zone not developed, without oocytes.

	translucent to whitish, becoming light pink later. Reserve zone already developed. Oocytes not visible to bare eye.		
Early developing	Ovaries small and filiform but more cylindrical than previous phase, occupying from 5 to 10% of the abdominal cavity. Coloration characterized by darker shades of pink, or yellowish and orangish, starting to become opaque; never translucent. Subtle granulation might be seen. Transition between previous and this phase can be indistinguishable due to intermediate features. Elongated reserve zone, representing almost half of gonad length.	Minute oocytes can be observed in the reserve zone. Vitellogenic oocytes and one or more intermediary development class oocytes (Fig. 4a). Thin ovarian wall. Atretic oocytes occur when early developing for a new cycle, but never when early developing for the first time.	PG, PV (Fig. 5c) and vitellogenic oocytes (Fig. 3d). Reserve zone showing PG and chromatin nuclear PV oocytes (Fig. 5e).
Late developing	Ovaries much larger than previous phase. Lobular, cylindrical, and robust, occupying up to half of the abdominal cavity. Well-developed vitellogenic oocytes, visible to bare eye. A smaller second oocyte diameter class often observable. Visible blood vessels. Pinkish-yellow coloration. Well-developed reserve zone, representing up to 1/3 of the gonad length.	Reserve zone occupied by lamellas/fringes full of minute oocytes. Ovaries replete with hydrated vitellogenic oocytes and at least two intermediary oocyte diameter classes (Fig. 4b). Thin ovarian wall.	Reserve zone containing vitellogenic oocytes. PG, PV and vitellogenic oocytes observed in the ovaries.
Spawning capable	Ovarian format similar to the previous phase, but larger and occupying up to 70% of the abdominal cavity. Oocytes fully developed, with higher diameter than before, commonly making the ovaries turgid and sometimes reddish. Well-developed reserve zone, representing up to 1/3 of the gonad length.	Reserve zone occupied by lamellas/fringes full of minute oocytes. Ovaries replete with hydrated vitellogenic oocytes and at least two intermediary oocyte diameter classes (Fig. 5c). Thin ovarian wall. .	Reserve zone containing vitellogenic oocytes (Fig. 5f). PG, PV and vitellogenic oocytes observed in the ovaries (Fig 5d).

Regressing	Ovaries shorter and less voluminous than last phase, but still cylindrical and occupying up to 30% of the abdominal cavity. Small, stored oocytes can be seen, and often a few fully developed vitellogenic remaining from the last spawn. Atretic oocytes often observable. Yellow, pinkish, and purple colours might be seen. Visible blood vessels. Reserve zone representing up to more than half of the gonads length.	Reserve zone still full of lamellas/fringes full o minute oocytes. Ovaries full of vitellogenic oocytes and at least two intermediary oocyte diameter classes, visible recent POFs (more vascularized and fresher than older POFs) and scarce vitellogenic and hydrated vitellogenic oocytes, remaining from last spawn (Fig. 4e). No obvious vascularization. Thick ovarian wall.	Reserve zone containing only PG and PV oocytes, but no vitellogenic. PG, PV, vitellogenic oocytes and POFs observed in the ovaries.
Regenerating	Ovaries can be externally undifferentiated from the previous phase, but less cylindrical and voluminous, more elongated, and flaccid. Yellow, pinkish, orangish and purple colours might be seen.	Reserve zone can still be "invading" ovaries, as the last shrunk. Transiting to "Early developing", the reserve zone elongates again, full of lamellas/fringes, more separated from the ovaries than previous but with part still inside it. Ovaries similar to "Regressing" phase but with older POFs (as recent POFs are more vascularized) and few vitellogenic oocytes. Thick ovarian wall. At least another two intermediary oocyte diameter classes, vascularization more visible than previous (Fig. 4f).	Reserve zone containing only PG and PV oocytes, but no vitellogenic. PG, PV, vitellogenic oocytes, and POFs observed in the ovaries.

Notes: PG = Primary Growth; PV = Previtellogenic; POF = Post-ovulatory follicle.

Table 3. Reproductive phases of *Genidens barbus* males based on histological and macroscopical analysis.

Testis' analysis		
Reproductive phase	Macroscopic	Microscopic
Immature	Testis small, thin, smooth, and filiform. Translucent to pinkish coloration can be observed.	Large amount of Sg present, no cystic organization observed. Connective and interstitial tissue more visible (Fig. 7a)
Early developing	Testis similar to previous phase, making macroscopic determination imprecise. Gonads can be slightly longer, rougher, and less translucent, appearing more pinkish or opaque, and firmer than previous phase.	Sg and first appearance of Sc. Initial development of cysts (Fig. 7b). Less interstitial tissue than previous phase. Continuous GE.
Mid developing	Testis similar to previous phase, making macroscopic determination imprecise. Gonads can be more robust and firmer and show external vascularization and fringes through testis.	Greater abundance of Sc. Fully developed cystic organization and continuous GE (Fig. 7c). Formation of early lumen and often with few St. No Sz observed.
Late developing	Testis robust, thicker than previous phases, usually with fringes, and with opaque coloration varying from light to darker shades of pink.	Presence of Sg and Sc, along with St and lumens with few Sz (Fig. 7d). Fully developed cystic organization and continuous GE.
Spawning capable	Testis with external features similar to previous phases but may become flatter and less thick than “Late developing”.	All germ cells present. Greater amount of St than “Late developing”, lumens fulfilled of Sz (Fig. 7e). Discontinuous GE.
Regressing	Testis flat and with fringes. Might be very similar to "Spawning capable" phase.	Lumens empty or with residual Sz (notably less than "Spawning capable" phase). Tubules mostly occupied by Sg, few Sc, and rare St. Differential organization of GE, with recovering of continuality where secondary Sg are proliferating, and discontinuities are observed near empty lumens and the liberation duct (Fig. 7f).

Notes: Sg = spermatogonia; Sc = spermatocyte; St = spermatid; Sz = spermatozoa; GE = Germinal epithelium.

Table 4. Total length (TL), Total weight (TW), Absolute fecundity (AF) and Relative fecundity (RF) of *Genidens barbus* females utilized for fecundity analysis

TL (mm)	TW (kg)	AF	RF
745	4.88	104	21.31
995	12.28	272	22.15
866	9.49	213	22.44
926	10.68	248	23.22
737	4.62	115	24.89
760	6.05	156	25.79
761	4.65	122	26.24
799	5.93	169	28.50
681	3.89	122	31.36
807	6.94	169	25.10

Figures

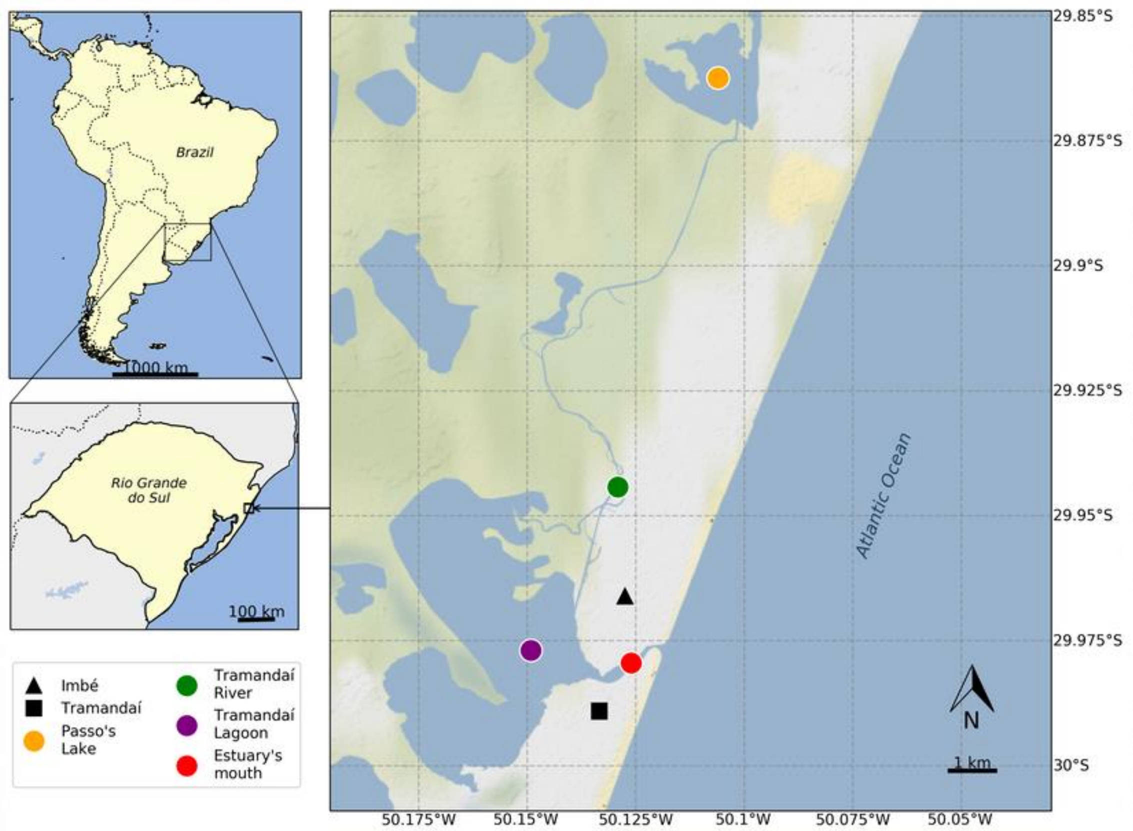

Figure 1. Study area. Tramandaí Inlet divides the municipalities of Tramandaí and Imbé, RS, Brazil. Sampling sites are indicated by circles.

Figure 2. Specimen of *Genidens barbus* sampled through professional fishery capture during MOPERT activities.

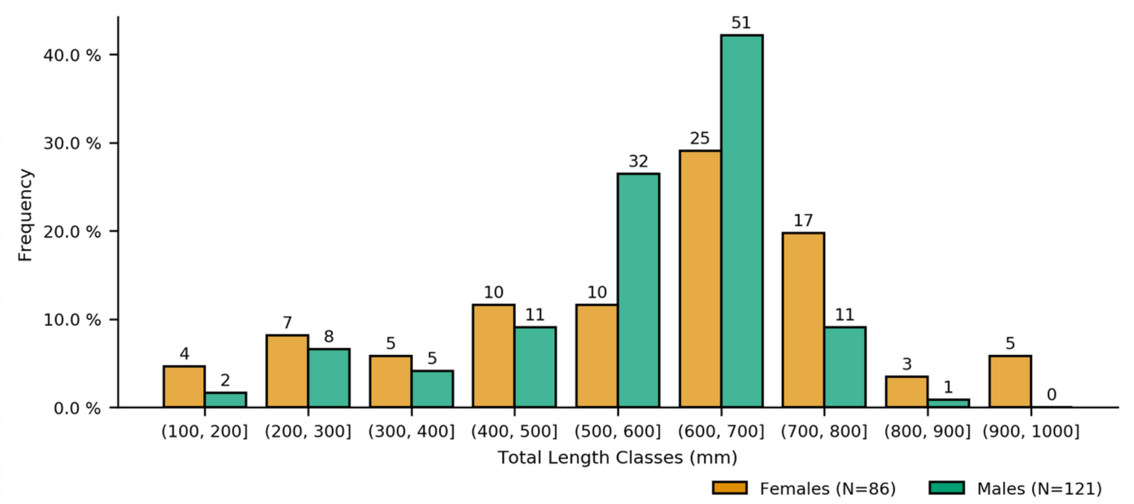

Figure 3. Frequencies of size classes (Total Length) of sampled *Genidens barbus* individuals during the research. Bars demonstrate the proportion of individuals that fall under the length classes per sex; the numbers above the bars indicate the absolute number of individuals that belong to the category

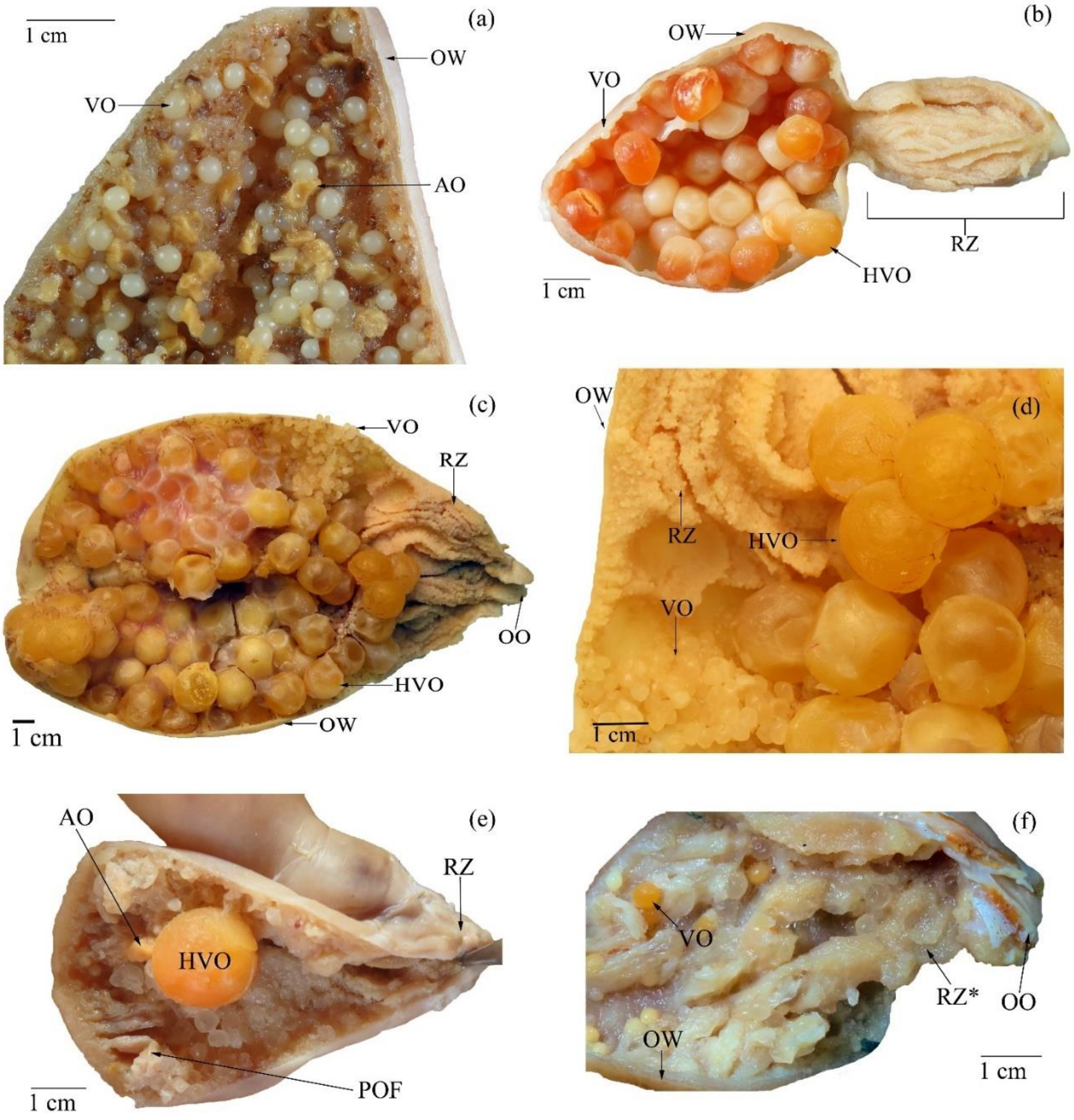

Figure 4. Internal macroscopic images of *Genidens barbus* ovaries. Early developing (a), Late developing (b), Spawning capable (c), Spawning capable reserve zone detail (d), Regressing (e) and Regenerating (f) reproductive phases. OW = Ovarian Wall; AO = Atretic Oocyte; HVO = Hydrated Vitellogenic Oocyte; RZ = Reserve zone; OO = Ovary's orifice; POF = Post Ovulatory Follicle; VO = Vitellogenic Oocyte; RZ * = Smooth reserve zone.

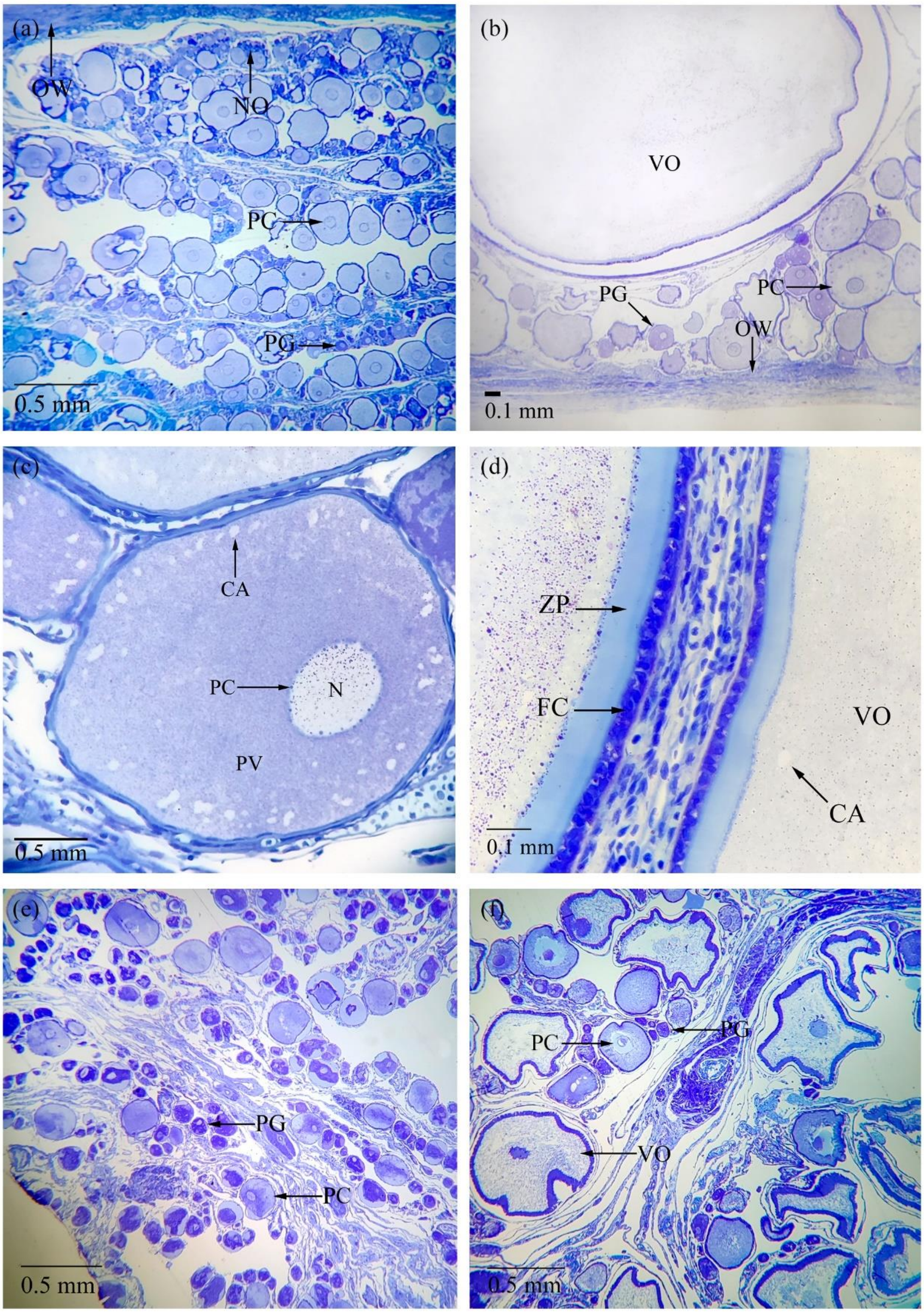

Figure 5. Histological analysis of *Genidens barbus* ovaries. Immature (a) and Early developing (b) reproductive phases, and details of a Previtellogenic oocyte (c) and Vitellogenic oocytes layers (d) in "Early developing" individuals. "Early developing" reserve zone (e) and "Spawning capable" reserve zone (f). OW = Ovarian Wall; NO = Nest of oogonias; PG = Primary growth oocyte; VO = Vitellogenic oocyte; CA = Cortical Alveoli; PV = Previtellogenic Oocyte; PC = Perinucleolar Chromatin; N = Nucleus; ZP = Zona Pellucida; FC = Follicular Cells.

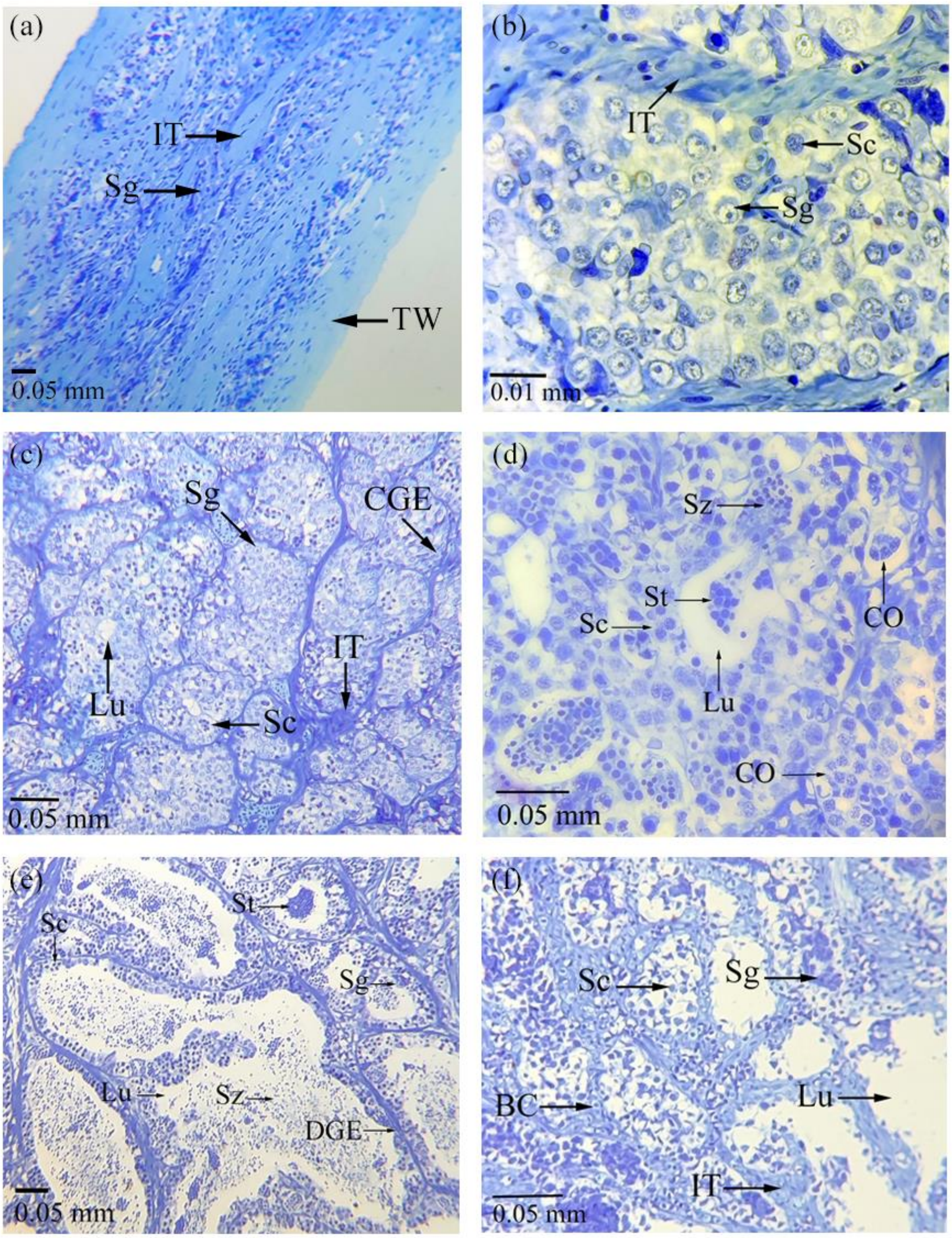

Figure 6. Histological analysis of testis. Immature (a), Early developing (b), Mid developing (c), Late developing (d), Spawning capable (e) and Regressing (f) reproductive phases. IT = Interstitial Tissue; TW = Testis Wall; Sg = Spermatogonia; Sc = Spermatocyte; Lu = Lumen; CGE = Continuous Germinal Epithelium; Sz = Spermatozoa flagella; St = Spermatid; CO = Cystic Organization; DGE = Discontinuous Germinal Epithelium; BC = Blood Cells.

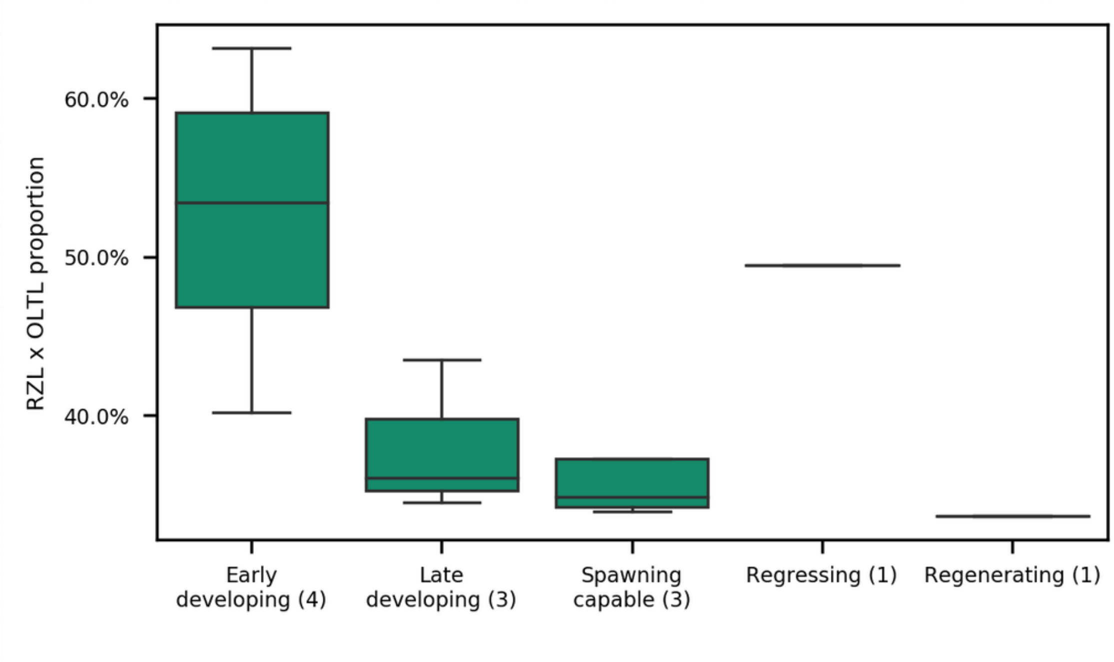

Figure 7. Boxplot of the proportion between Reserve Zone Length (RZL) vs Ovarian Longitudinal Total Length (OLTL), by reproductive phases of *Genidens barbus* females. Sample sizes are in parenthesis. Horizontal lines represent quartiles, and vertical lines represent reproductive phase's sample range.

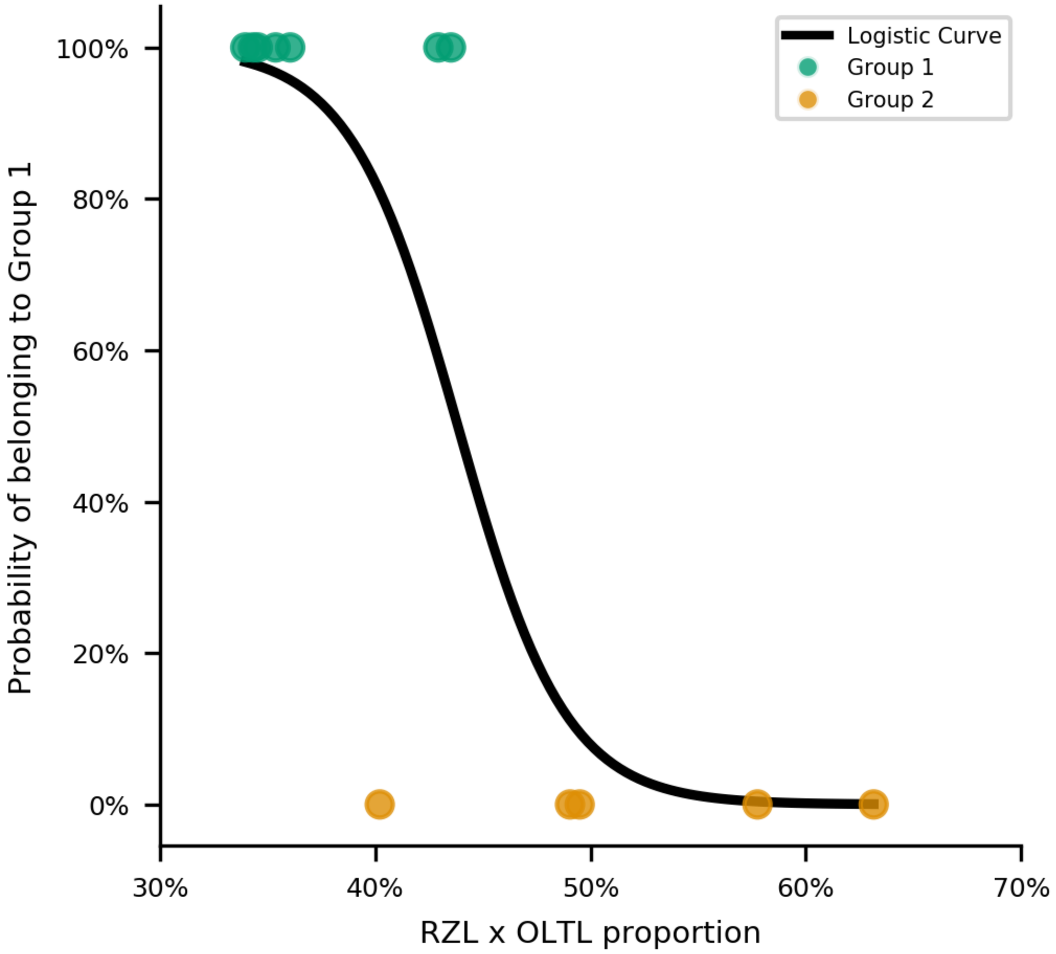

Figure 8. Logistic regression test of *Genidens barbus* reproductive phases assignment, based on Reserve zone length (RZL) vs Ovarian Longitudinal Total Length (OLTL) proportion. Circles represent sampled individuals.

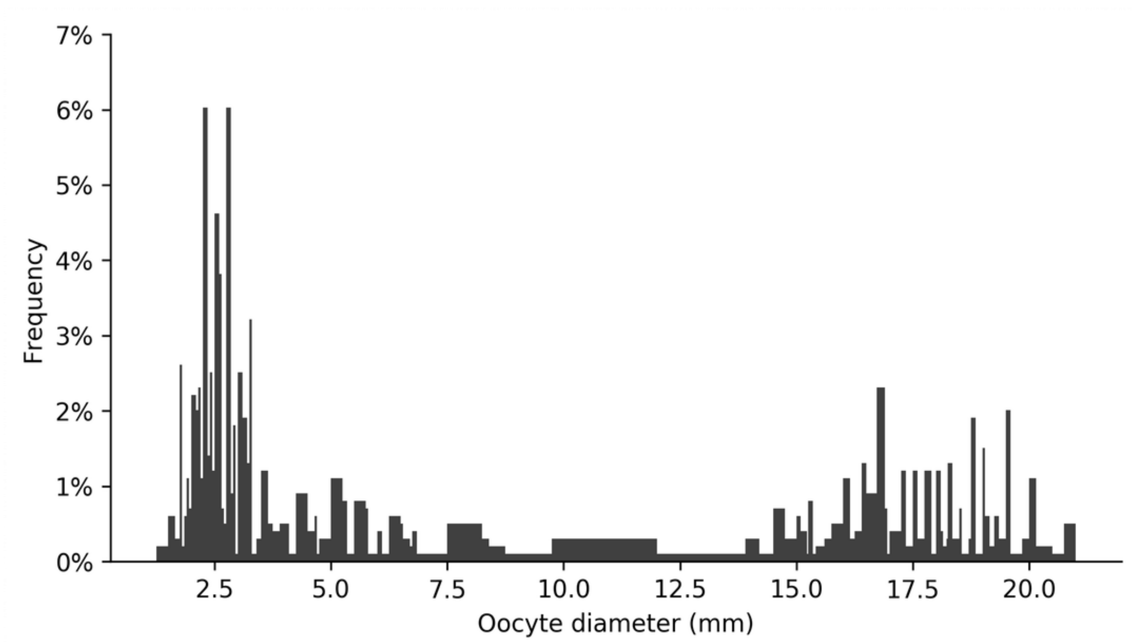

Figure 9. Distribution of absolute frequencies of the subsampled oocytes diameters, of "Spawning capable" *Genidens barbus* females.

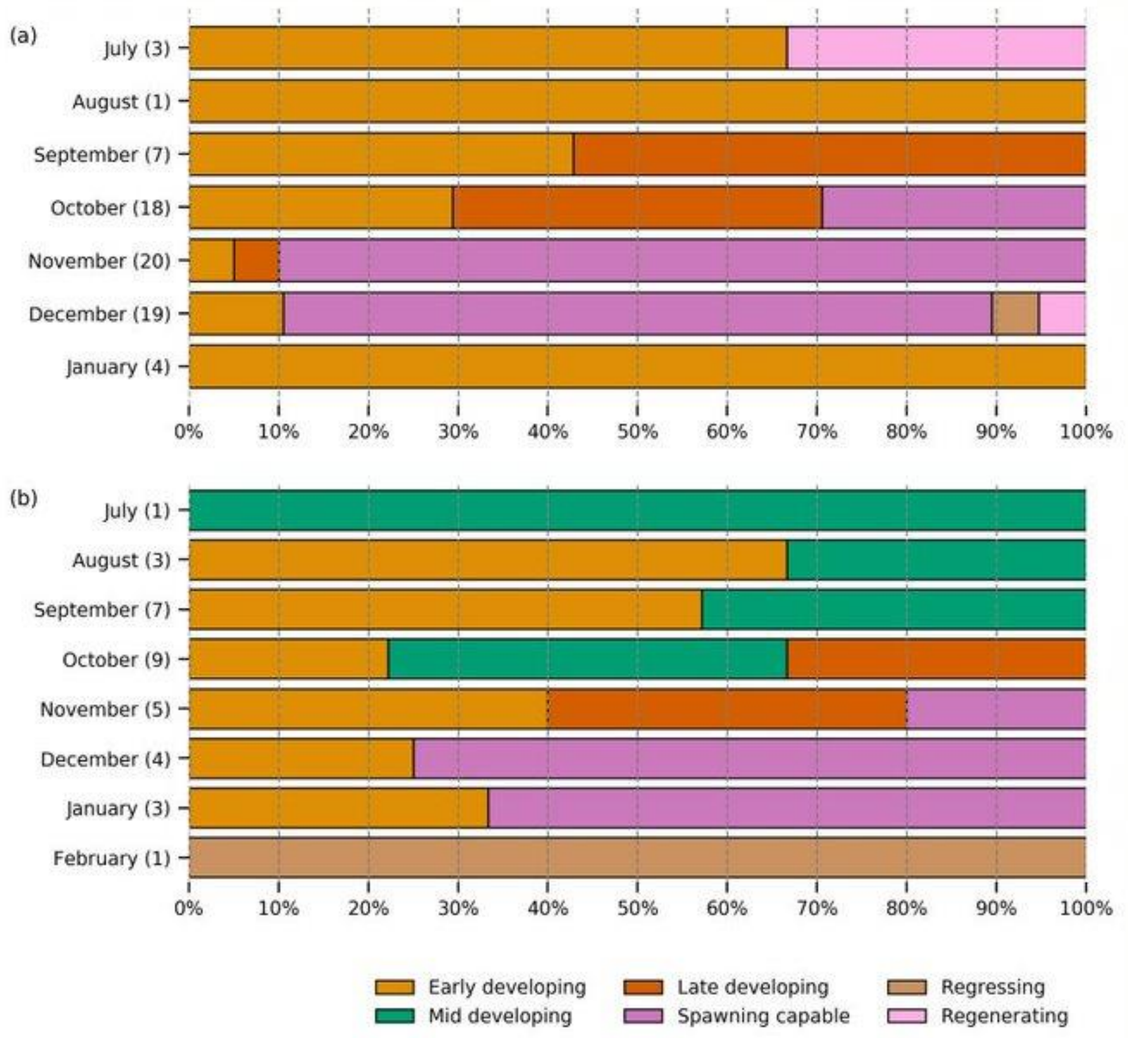

Figure 10. Monthly frequencies of *Genidens barbus* females (a) and males (b) reproductive phases sampled from July/2020 to February/2021. Sample sizes in parenthesis.

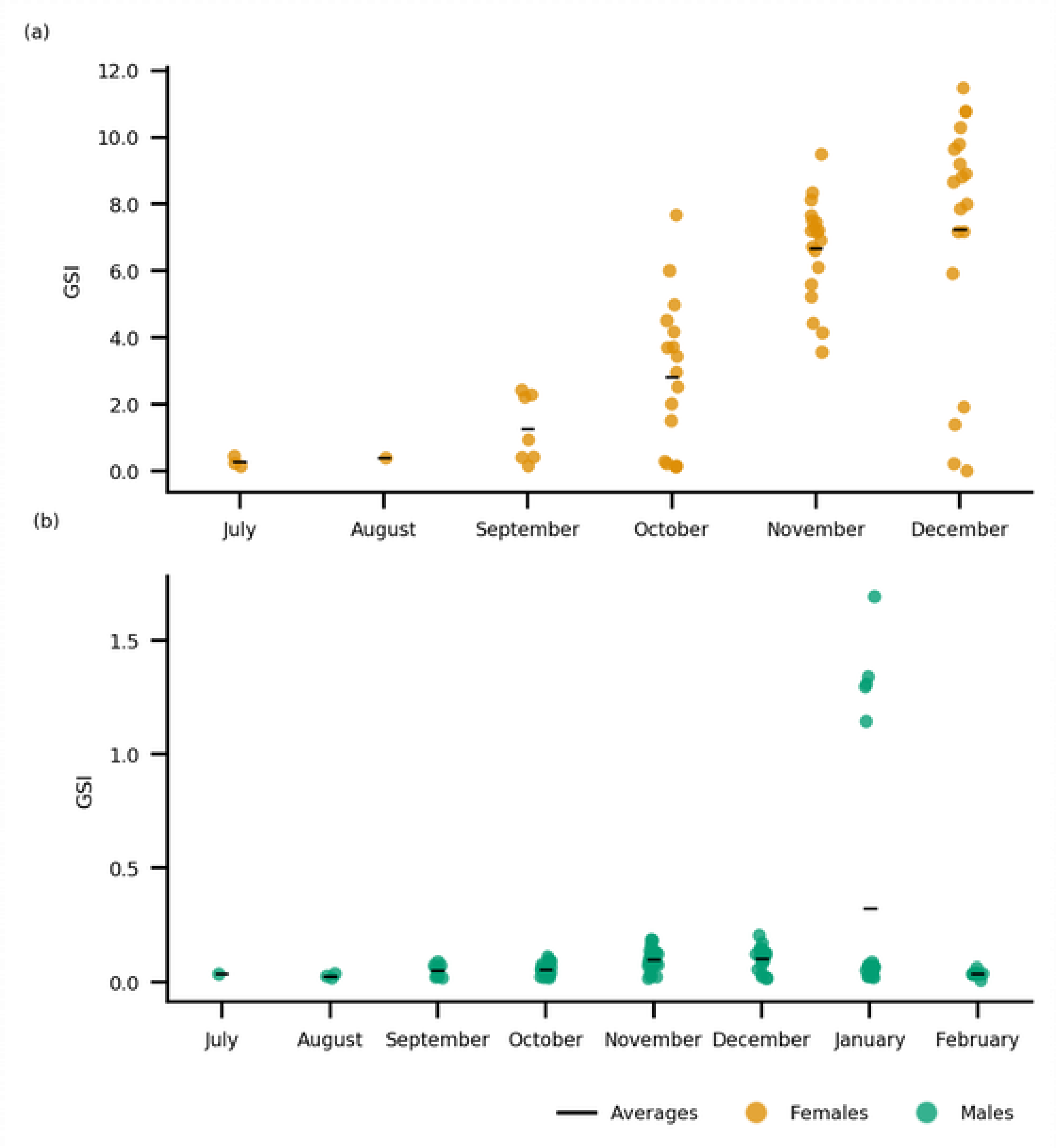

Figure 11. Monthly distribution of gonadosomatic Index (GSI) of *Genidens barbus* females (a) and males (b). Dots represent individuals sampled, and the black traces represent the averages.

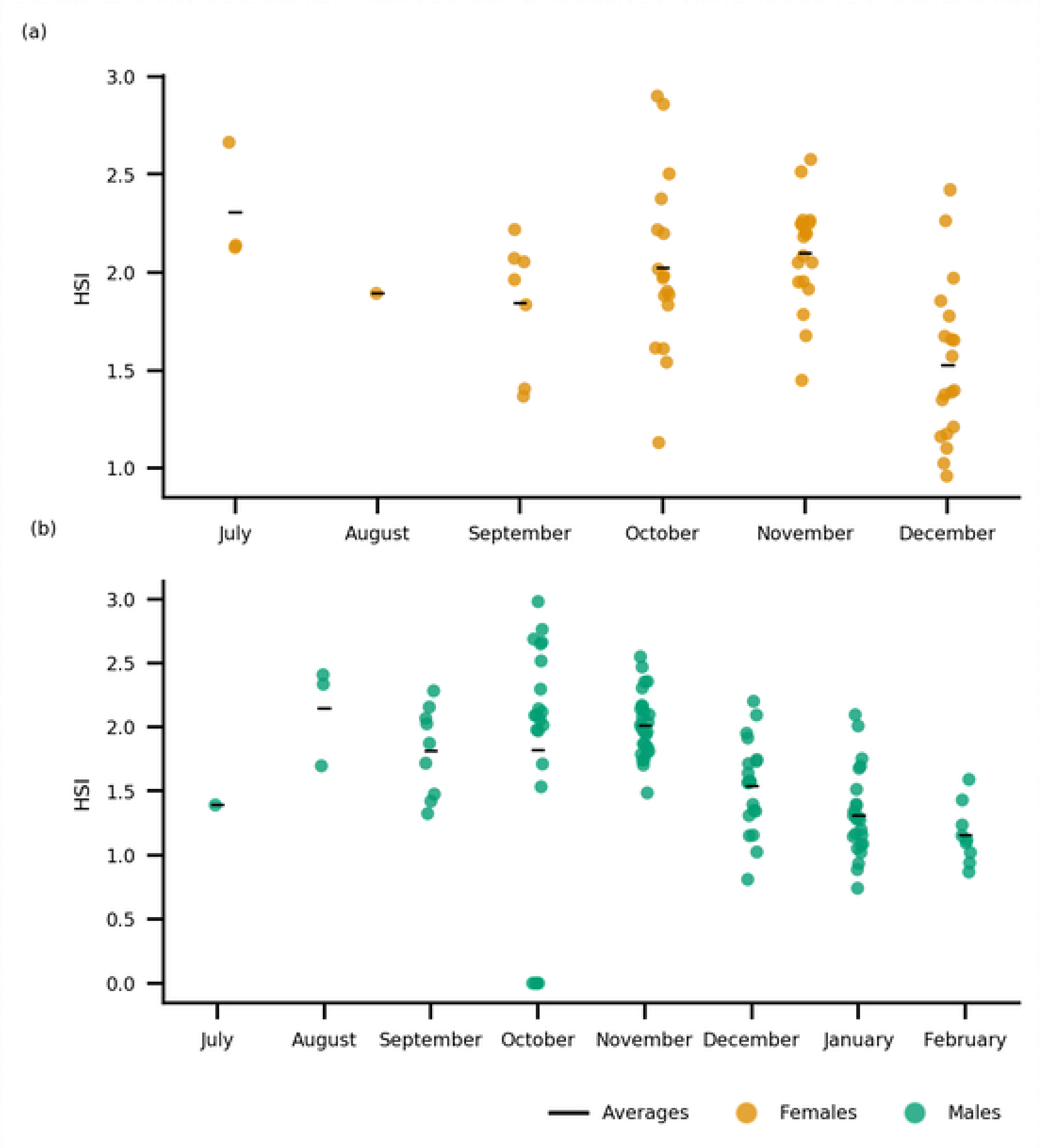

Figure 12. Monthly distribution of Hepatosomatic Index (HSI) of *Genidens barbus* females (a) and males (b). Dots represent individuals sampled, and the black traces represent the averages.

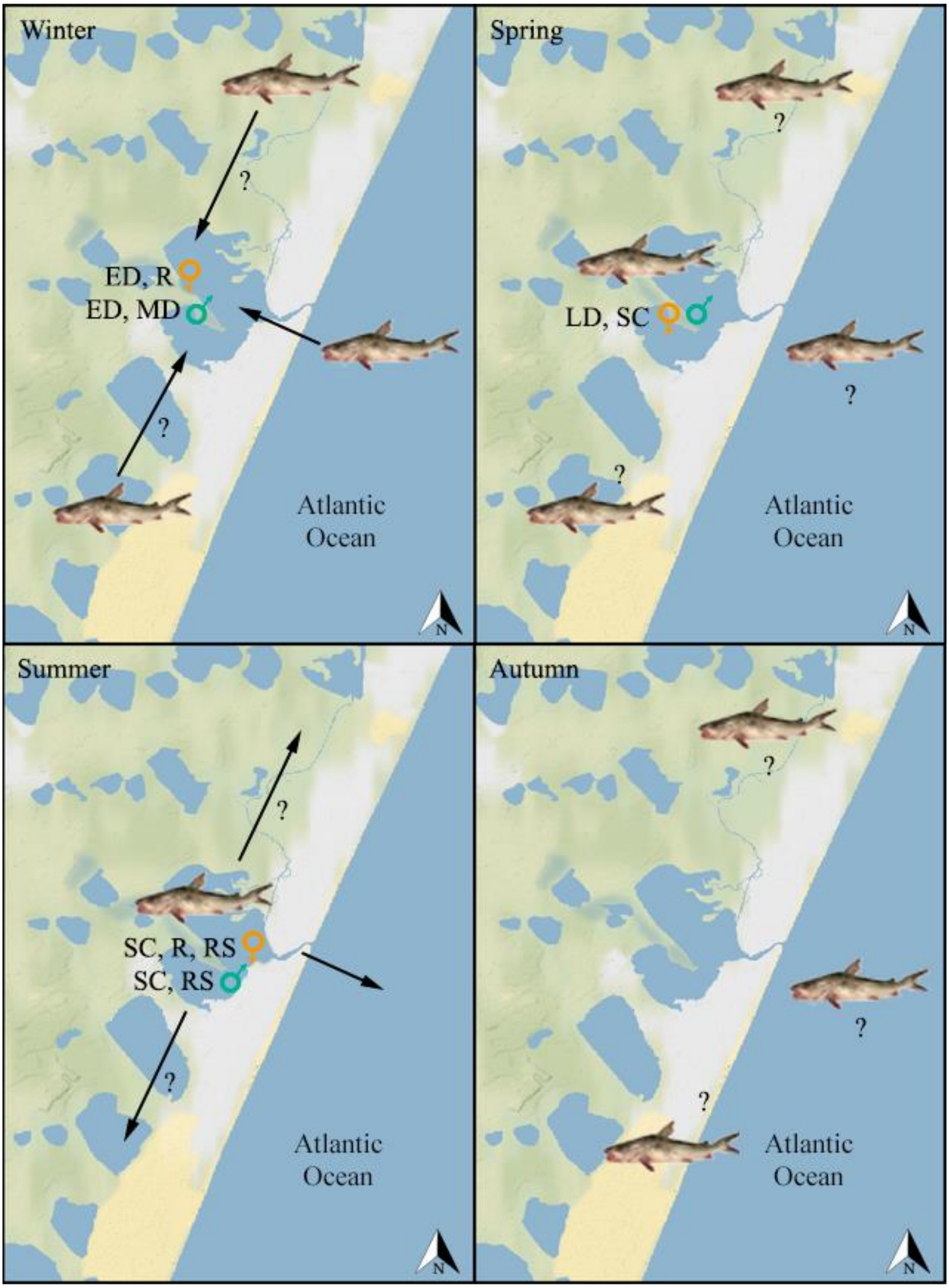

Figure 13. Life cycle possibilities of *Genidens barbus* in the Tramandaí River Estuary region. Austral seasons are indicated at the top-left of each image, and reproductive phases of each sex by abbreviations (Early developing – ED; Mid developing – MD; Late developing – LD; Spawning capable – SC; RS – Regressing; R – Regenerating). Question marks imply hypothesis of migratory movement or reproductive phases. During the winter, individuals (mostly in developing phases) start to aggregate at the Tramandaí lagoon (TL). It is unknown if individuals from other south and northwards Tramandaí River Hydrographic Basin regions join individuals coming from the marine environment. In the spring, individuals are in LD or SC reproductive phases, and spawning occurs, lasting until early summer. In the late summer, R and RS individuals

are observed. Reproductive phases of individuals that do not aggregate at the TL during spring and summer are unknow, as well the destiny of individuals after spawning. In the autumn, *G. barbus* do not aggregate to spawn at the TL.

CAPÍTULO III – Considerações finais

O presente trabalho objetivou a geração de conhecimento de biologia reprodutiva e subsídios para o manejo pesqueiro, de uma espécie históricamente importante para a pesca brasileira, notadamente para o estado do Rio Grande do Sul, e que encontra-se ameaçada de extinção. Além disso, a pesquisa se insere no escopo do Projeto MOPERT (Monitoramento Pesqueiro do Estuário do Rio Tramandaí), do qual a realização surge a partir de uma necessidade de investigação aprofundada sobre a população de bagres marinhos na região do Estuário do Rio Tramandaí, grupo de recursos pesqueiros historicamente importante para o sistema socioeconômico pesqueiro do litoral norte do RS.

Variados aspectos reprodutivos de *Genidens barbus* foram investigados. Uma tabela de descrição de fases reprodutivas detalhada é fornecida, a qual pode ser utilizada em futuras pesquisas e programas de monitoramento pesqueiro que contemplem *G. barbus*, bem como servir de referência para futuras e necessárias pesquisas com outras espécies do gênero. Durante a investigação de fases reprodutivas de fêmeas, foi possível identificar uma característica relevante para a determinação das fases, a qual nunca tinha sido descrita antes pela literatura, cunhada pela presente pesquisa de "zona de reserva", a qual pode apresentar importância ecológica e evolutiva para o grupo, e requer aprofundamento investigativo. As análises de fecundidade e estratégia reprodutiva foram capazes de fornecer informações relevantes sobre a população local da espécie. *Genidens barbus* é uma espécie anádroma, e diversos comportamentos migratórios foram propostos recentemente para a espécie, tornando complexa a análise e descrição de seu ciclo de vida. A partir da análise de desenvolvimento gonadal, a presente pesquisa foi capaz de delimitar a temporada reprodutiva de *G. barbus* na laguna de Tramandaí.

Os resultados encontrados podem subsidiar a revisão da definição do período defeso da espécie na região, de tamanhos mínimos e máximos de captura, e a elaboração de um plano de manejo pesqueiro sustentável, contribuindo com a manutenção da biodiversidade e socioeconomia locais, fatores que encontram-se entre os principais objetivos do Projeto MOPERT. O conhecimento de aspectos de biologia reprodutiva é essencial para o monitoramento e discernimento dos estoques pesqueiros de *G. barbus*, notadamente frente à ameaça de extinção sofrida pela espécie. Espera-se que os resultados encontrados possam ser utilizados ao longo da ampla distribuição da espécie, em programas de monitoramento pesqueiro, pesquisas, e demais possibilidades em que haja interesse na conservação da espécie.

ANEXO I - Normas para publicação

As normas para publicação no periódico “*Canadian Journal of Fisheries and Aquatic Sciencies*” estão disponíveis em: https://cdnsciencepub.com/journal/cjfas/authors#guidelines.