Acta Limnologica Brasiliensia

Publication of the Brazilian Association of Limnology

Original Article
Acta Limnologica Brasiliensia, 2023, vol. 35, e11
https://doi.org/10.1590/S2179-975X6822
ISSN 2179-975X on-line version

Reproduction of the invasive largemouth bass *Micropterus salmoides* (Lacepède, 1802) in a Neotropical reservoir with suggestions to management and control

Reprodução do black-bass invasor *Micropterus salmoides* (Lacepède, 1802) em um reservatório neotropical, com sugestões para manejo e controle

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Cite as: Freitas, M.O., et al. Reproduction of the invasive largemouth bass *Micropterus salmoides* (Lacepède, 1802) in a Neotropical reservoir with suggestions to management and control. *Acta Limnologica Brasiliensia*, 2023, vol. 35, e11.

Abstract: Aim: The largemouth bass *Micropterus salmoides* was introduced in more than 50 countries for the purpose of sport fishing. In Brazil, records date from 1922, and nowadays many populations are known to occur in the southern region. Here we investigated the reproductive biology of this species in a small reservoir in southern Brazil, to understand the role of the reproductive strategy in the invasion process and assist in its management. We conclude with suggestions for the management in local scale, as this species can bring relevant problems to the public supply through trophic cascade and other negative effects. **Methods:** Samples were performed with different methods, between May 2011 and April 2012. All fishes were measured, and the reproductive season was determined through the relative frequency of reproductive stages and the gonadosomatic index. Sex ratio was calculated monthly and for each total length (TL) class and compared with the estimates presented in the literature. Total length at maturity (L50, length at which 50% of the individuals were mature) was estimated for females, males and the entire study population. **Results:** A total of 565 individuals of *M. salmoides* were analyzed. Overall sex-ratio did not differ significantly. The monthly distribution of the gonadosomatic index for females and males showed higher values between August (late winter) and November 2011 (austral spring). β-Binomial modelling of sex-ratio data of native (n=1) and introduced populations (n=7) showed a lower proportion of females in its native range. All individuals > 21.5 cm TL were mature (L95), and L_{50} was calculated at 18.7 cm TL. After the reproductive period, an increase in the abundance of young individuals (≤ 7.0 cm TL) was registered, corresponding to 50% of all individuals collected. The relative frequency of mature individuals and the variation in the gonadosomatic index indicate a prolonged spawning period during the austral spring, coinciding with the increase in temperature. **Conclusions:** Results suggested that some changes in life history traits, such as the early sexual maturation and the long reproductive period, coupled

with a remarkable adaptability, may have facilitated the establishment of *M. salmoides* populations in this reservoir. The illegal and intentional stocking by anglers plays a central role in the introduction and dispersal of *M. salmoides* in Brazil. We encourage studies on the sanitary quality of this fish for human consumption, so that massive campaigns of education the population to encourage capture and consumption are implemented by the competent authorities as a control measure in local scale, combined with targeted samplings with artificial baits during the reproductive period, identification, and removal of nests and fingerlings using electric fishing or trap nets during the recruitment period.

Keywords: exotic species; Centrarchidae; reproductive period; predator; size at maturity.

Resumo: Objetivo: O black-bass *Micropterus salmoides* foi introduzido em mais de 50 países para fins de pesca esportiva. No Brasil, os primeiros registros datam de 1922 e atualmente muitas populações são conhecidas na região sul. Neste trabalho investigamos a biologia reprodutiva desta espécie em um pequeno reservatório no sul do Brasil, para entender o papel da estratégia reprodutiva no processo de invasão e auxiliar no manejo. Concluímos com sugestões para o manejo em escala local, pois esta espécie pode trazer problemas relevantes ao abastecimento público através da cascata trófica e outros efeitos negativos. **Métodos:** As amostragens foram realizadas com diferentes métodos, entre maio de 2011 e abril de 2012. Todos os peixes foram medidos, e a época reprodutiva foi determinada através da frequência relativa dos estádios reprodutivos e do índice gonadossomático. A razão sexual foi calculada mensalmente para cada classe de comprimento total (CT) e comparada com as estimativas apresentadas na literatura. O comprimento total de primeira maturidade (L50, comprimento em que 50% dos indivíduos estavam maduros) foi estimado para fêmeas, machos e toda a população de estudo. **Resultados:** Um total de 565 indivíduos de *M. salmoides* foi analisado. A razão geral entre os sexos não foi significativa. A distribuição mensal do índice gonadossomático para fêmeas e machos apresentou maiores valores entre agosto (final do inverno) e novembro de 2011 (primavera austral). A modelagem β-binomial dos dados de proporção sexual de populações nativas (n=1) e introduzidas (n=7) mostrou uma menor proporção de fêmeas em sua área nativa. Todos os indivíduos > 21,5 cm CT eram maduros (L95), e o L50 calculado foi de 18,7 cm TL. Após o período reprodutivo, foi registrado um aumento na abundância de indivíduos jovens (≤ 7,0 cm CP), correspondendo a 50% de todos os indivíduos coletados. A frequência relativa de indivíduos maduros e a variação do índice gonadossomático indicam um período prolongado de desova durante a primavera austral, coincidindo com o aumento da temperatura. **Conclusões:** Os resultados sugerem que algumas mudanças nas características de vida, como a maturação sexual precoce e o longo período reprodutivo, aliadas a uma notável adaptabilidade, podem ter facilitado o estabelecimento de populações de *M. salmoides* neste reservatório. A estocagem ilegal e intencional por pescadores tem papel central na introdução e dispersão de *M. salmoides* no Brasil. Incentivamos estudos sobre a qualidade sanitária desse pescado para consumo humano, para que campanhas massivas de educação da população para incentivar a captura e consumo sejam implementadas pelos órgãos competentes como medida de controle em escala local, combinada com amostragens direcionadas com iscas artificiais durante o período reprodutivo, identificação e retirada de ninhos e alevinos por meio de pesca elétrica ou rede-armadilha durante o período de recrutamento.

Palavras-chave: espécie exótica; Centrarchidae; período reprodutivo; predador; tamanho de primeira maturação.

1. Introduction

The largemouth bass *Micropterus salmoides* is a cool-warm water centrarchid native to the central and eastern United States and northern regions of Mexico (Brown et al., 2009). This top-level predator was introduced in 50 countries for sport fishing purposes (Brown et al., 2009; Pereira & Vitule, 2019), and nowadays it is considered one of the world's worst invasive species (Lowe et al., 2000). The largemouth bass was introduced in Brazil in the early 1920s, and now it is widely recorded in artificial lakes and reservoirs in the southeast and southern Brazil (Pereira & Vitule, 2019). Recreational fishing has played a central role in the introduction and range expansion of *M. salmoides* in hydrologically altered systems in Brazil, which are mainly concentrated in the states of São Paulo, Paraná, Santa Catarina and Rio Grande do Sul.

Cases of ecological disturbances due to high predation pressure and rapid population growth of largemouth bass have been reported in many countries across the world, but with few data in South America (Ribeiro et al., 2015; Pereira & Vitule, 2019). In general, negative effects on native species and ecosystem services occur due to the predatory, competition, and trophic

cascades (Pereira & Vitule, 2019). The spread of largemouth bass negatively affects freshwater food webs (Bezerra et al., 2019), thereby reducing species richness, and their potential for damaging the integrity of aquatic ecosystems has led to widespread discussion (and lack of consensus) regarding the best methods to manage this invasive species.

Largemouth bass is a lake-dwelling species, preferring structured environments and shallow waters (Hickley et al., 1994). Showing slow growth rates (Lorenzoni et al., 2002) and a reproductive period occurring in the end of winter and spring, females spawn multiple times due to their asynchronous oocyte development (Breder & Rosen, 1966; Beamish et al., 2005; Dadzie & Aloo, 1990; Rodríguez-Sánchez et al., 2009). In Brazil, largemouth bass occurrences are reported associated with reservoirs and population dynamics are only reported by Schulz & Leal (2005); there are no studies on the reproductive biology of the species. Bass maturation could be more dependent on the size than on the age, and the age at maturity (i.e. first reproduction) could vary widely, with fishes reaching sexual maturation at age +2 or +3 (Marinelli et al., 2007; Lorenzoni et al., 2002).

Here, we provide information about the reproduction of *M. salmoides* introduced in a reservoir in southern Brazil (Figure 1). The main objectives of our study were to analyze the reproductive period, sex-ratio, and sexual maturity, providing an opportunity to examine fundamental aspects of *M. salmoides* establishment and persistence. We believe such basic information are fundamental to do the management in local scale, as this species can bring relevant problems to the public supply through trophic cascade and other negative effects in lakes.

Figure 1. Specimen of *Micropterus salmonides* in the Passaúna Reservoir. Credits Raul Rennó Braga.

2. Material and Methods

The samplings were conducted in the Passaúna Reservoir, localized in the Curitiba Metropolitan Region in Southern Brazil (25º31' S, 49º23" W) (Figure 2). This Reservoir was created in 1989 for public water supply. Specimens were obtained monthly between May 2011 and April 2012 by three different types of sampling gear: electrofishing, gill nets and artificial lures. Electrofishing was conducted by two people, operated in rocks and submerged vegetation. Gill nets were composed of simple and trammel nets with mesh sizes ranging from 15 to 150mm knot-to-knot, 5-40 meters long; these nets remained deployed for 24 h. Experienced sport fishermen conducted the angling monthly along the sampling period (Ribeiro et al., 2015).

In the laboratory, total length (TL) and body weight (WT) were measured to the nearest 0.1 cm and 1 g, respectively, for every fish captured. Fresh gonads were removed, weighed (WG) to the nearest 0.001 g, and sex and maturity was initially determined macroscopically according to gonad size, color, vascularization, presence of lateral sperm sinuses and oocytes. After macroscopic classification, gonads were fixed in 10% formalin for 24h, and subsequently stored in 70% ethanol

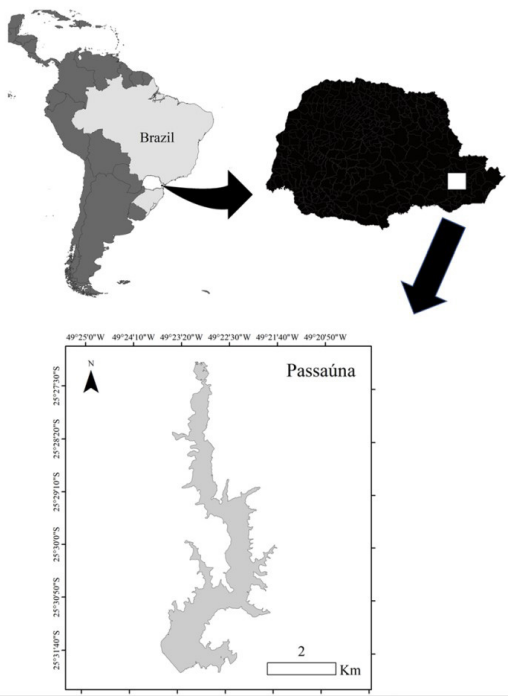

Figure 2. Map of Passaúna Reservoir where individuals of *Micropterus salmonides* were collected between May 2011 and April 2012.

before histological preparation. Histological sections were further examined in the optic microscope and categorized in the five developmental phases as described by Brown-Peterson et al. (2011): Immature (IM), Developing (DV), Spawning Capable (SC), Regressing (RS), and Regenerating (RN). Capture and handling of fish followed regulations and legislation relative to the proposed species (SISBIO 24779-1). For comparison effects, monthly means (mean ± SD) of diurnal air temperature were obtained from the local weather station of the National Institute of Meteorology (www.inmet.gov.br).

The reproductive season was determined through the relative frequency of reproductive stages and the gonadosomatic index (GSI), obtained by expressing gonad mass (WG) as a proportion of the whole fish weight (WT): GSI = [WG/(WT-WG)] × 100. The mean monthly GSI was calculated separately for females and males.

Sex ratio was calculated monthly and for each TL class (Sturge´s rule), with significant differences determined by χ^2-test. Sex ratio was compared with the estimates presented in the literature (searched on google scholar using "*Micropterus salmoides* reproduction" as search terms) through a Bayesian method using the total number of females (f) and the number of females plus males (n). We used a conjugate beta-binomial model to estimate the proportion of females with uninformative Beta (1,1) as a prior distribution and Beta (f + 1, n – f + 1) as posterior distribution (Gelman et al., 2004).

Total length at maturity (L50, length at which 50% of the individuals were mature) was estimated for females, males and the entire study population. For the L50 analysis, DV, SC, RS and RN were considered sexually mature (adults). The following logistic regression model was used: $PM = 1[1+e^{(a+\beta L)}]^{-1}$, where PM is the proportion of mature specimens in length class L, a is a constant and $L50 = -a\beta^{-1}$ (King, 1995). To predict the probability that an individual was mature at a given length, binary maturity observations (0=immature, 1=mature) and TL were fitted to binary logistic models (logit function) to construct maturity ogives (maturity-at-length probability plots) based on logistic regression (Hazelton, 2007). A bootstrap method with 1000 interactions was applied to estimate 95% credibility interval (CI). Statistical procedures were conducted in R, FSA Package (Ogle et al., 2023). Additionally, we present a comparison of L50 values available in the literature (Table 1).

3. Results

A total of 565 individuals of *M. salmoides* were captured in the Passaúna Reservoir, ranging from 2.2 to 52.0 cm TL (mean = 20.6 ± SD 11.7 cm TL). Females (N = 248 – 43.9%) ranged from 6.1 to 52.0 cm TL (mean = 23.5 ± SD 10.2 cm TL) and males (N = 223 – 39.5%) from 5.7 to 51.0 cm TL (mean = 23.3 ± SD 10.2 cm TL). Ninety-four small individuals (<8.9 cm TL) with unsexed gonads were recorded.

Although overall sex-ratio did not differ significantly ($\chi^2 = 0.66$, $p = 0.41$), the proportion of females was higher than males in June 2011 ($\chi^2 = 6.66$, $p = 0.01$), and in the 36.6 – 40.8 cm TL size-class ($\chi^2 = 3.97$, $p < 0.04$, 4.66 females: 1 male). β-Binomial modelling of sex-ratio data of native (n=1) and introduced populations (n=7) showed a lower proportion of females in its native range [data from Bennett & Gibbons (1975)] (Figure 3).

The monthly distribution of the gonadosomatic index for females and males showed higher values between August (late winter) and November 2011 (austral spring) (Figure 4). Gonads in DV

Table 1. Comparative estimates of the size at first maturity (L50) for *Micropterus salmoides* according to sex and geographic distribution.

Author	Geographic distribution	L50	Sex	Country	Ecosystem
Chew (1974)	Native	25.0	Grouped	US	Lake
Beltrán-Álvarez et al. (2012)	Nonnative	33.0	Grouped	Mexico	Reservoir
Lorenzoni et al. (2002)	Nonnative	22.0	Males	Italy	Lake
Lorenzoni et al. (2002)	Nonnative	30.0	Females	Italy	Lake
Marinelli et al. (2007)	Nonnative	20.0	Males	Italy	Lake
Marinelli et al. (2007)	Nonnative	19.0	Females	Italy	Lake
Dadzie & Aloo (1990)	Nonnative	27.4	Males	Kenya	Lake
Dadzie & Aloo (1990)	Nonnative	32.4	Females	Kenya	Lake
Weyl & Hecht (1999)	Nonnative	30.0	Grouped	South Africa	River
This study	Nonnative	18.5	Females	Brazil	Reservoir
This study	Nonnative	18.9	Males	Brazil	Reservoir

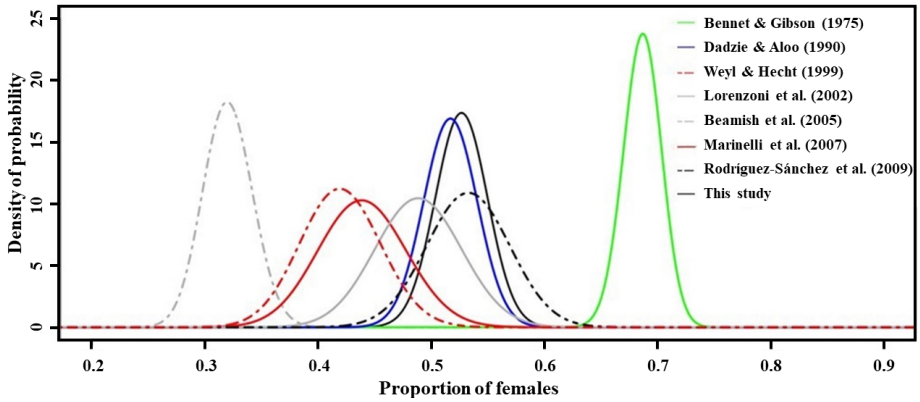

Figure 3. Posterior distribution of the proportion of females in the population, estimated through the β-binomial model using the overall sex ratio of *M. salmoides* captured between May 2011 and April 2012 in Southern Brazil, compared with the estimates from the literature.

stage were firstly observed in June (winter), and they increased in frequency until October (Figure 5). Spawning Capable individuals (presence of tertiary vitellogenic oocyte and germinal vesicle breakdown) were observed from August to December.

Females' L50 was estimated at 18.5 cm, and all females > 21.8 cm TL were mature (L95). For males, L50 was estimated at 18.9 cm, and all males > 21.2 cm TL were mature (L95). Of the entire sample population, L50 and L95 were estimated at 18.7 and 21.5 cm TL, respectively (Figure 6).

4. Discussion

Considering the gonad maturation stages and GSI used to determine the reproductive period, spawning took place in end of winter and spring (September to January), following the same pattern reported in the literature for temperate zones (Lorenzoni et al., 2002; Bennett & Gibbons, 1975). Warmer seasons (spring and summer) can increase the reproductive success of the species, by increasing the chances of survival and ensuring higher growth rates for young individuals, where food resources are most abundant (Parkos & Wahl, 2002; Beamish et al., 2005). This could be due to a direct metabolic effect, or through indirect factors (e.g., greater resource in warmer seasons due to increased productivity) (Bertolini et al., 2021). Environmental factors are described as capable of controlling the beginning and duration of reproduction in fish. For *M. salmoides,* the reproductive period coincides with the increase in temperature (Gross et al., 2002), photoperiod (Dadzie & Aloo, 1990; Beamish et al., 2005) and rainfall (Sammons et al., 1999; Waters & Noble, 2004).

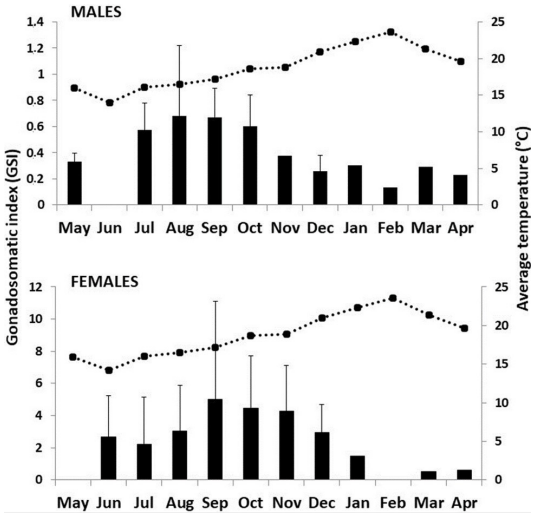

Figure 4. Mean gonadosomatic index (black bars ± SD) by month of males and females of *M. salmoides* captured between May 2011 and April 2012 in Southern Brazil and the average monthly values of air temperature (dashed line) for the sampled period. Temperature data were obtained from the National Institute of Meteorology (INMET).

Results showed that the reproductive strategies of *M. salmoides* in a reservoir in southern Brazil are a cause of concern. Individuals of *M. salmoides* introduced in the reservoir reached sexual maturity at a smaller size than those reported from a native and other introduced region (Table 1). This species was introduced in Brazil in the 20's. This precocious maturity registered here may be indicative of an intra-specific selective pressure (genetic introgression) or may even arise due to disordered fishing selection. The higher proportion

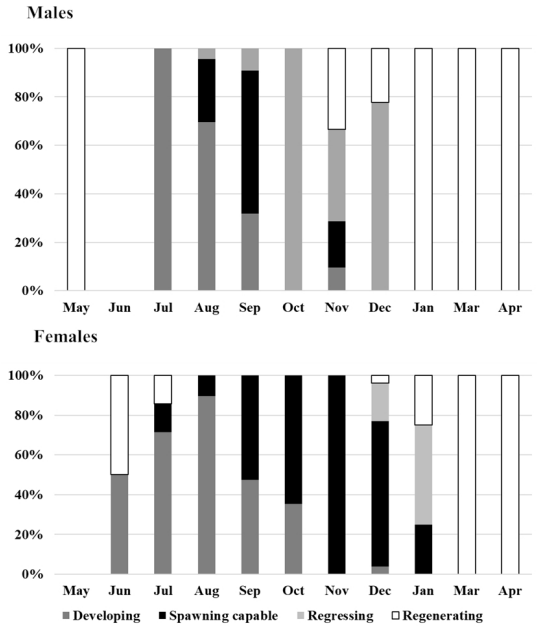

Figure 5. Relative frequency of reproductive stages for males and females by month of *M. salmoides* captured between May 2011 and April 2012 in Southern Brazil.

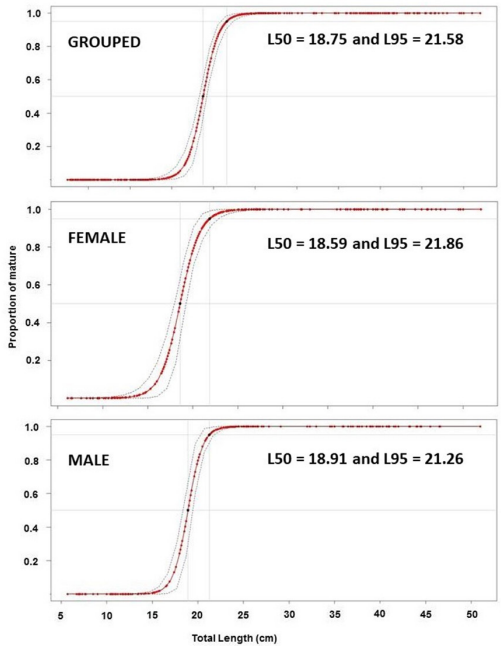

Figure 6. Length at maturity (L50 and L95) with observed (red dots) and fitted logistic (red lines) regression model of the entire study population, females and males of *M. salmoides* captured between May 2011 and April 2012 in Southern Brazil. Dash lines indicate the 95% of confidence interval.

of females found in the reservoir in comparison with its native range can be attributed to differential growth and longevity (Beamish et al., 2005). In fact, the capture of *M. salmoides* is method/dependent [as evidenced in Ribeiro et al. (2015)], so different sampling methods between studies can also explain these unequal proportions.

The extended spawning period (austral spring) and the early sexual maturation, together with the parental care, a common behavior among Centrarchidae (Cooke et al., 2002), can result in high population densities and increased impacts on abundance and diversity of the native community. Taking into consideration that the establishment stage of the invasion process requires survival and reproductive success of the species in its new environment (Blackburn et al., 2011), the largemouth bass can be considered established in this aquatic ecosystem.

Predicting the success of an invader can be tricky, and sometimes it is not recognized until the species is fully established. Established populations can become invasive by reaching high population densities and high growth rates (Britton et al., 2010), which may increase predation and competition effects on the invaded community (Gratwicke & Marshall, 2001; Takamura, 2007; Weyl et al., 2010). In Brazil, Schulz & Leal (2005) demonstrated that *M. salmoides* grows at higher rates than North American populations, indicating a high potential for establishment and propagation in natural watercourses. In fact, besides *M. salmoides* has already successfully invaded many environments in Brazil and South America, basic ecological data are scant (Pereira & Vitule, 2019). This raises a serious concern that the negative effects of these establishments can be at high levels and with serious consequences for the Neotropical biodiversity.

We demonstrated the reproductive success of *M. salmoides* in southern Brazil, as well as its establishment, highlighting the record of early sexual development and extended spawning period, which can help this species to reach high population densities and increased impacts on the native community. It is noteworthy that the Passaúna reservoir is located in a region considered a priority for conservation, and the establishment of a fish species listed among the "100 worst invasive species in the world" (Lowe et al., 2000) should be considered an urgent issue to implement control plans in the region (Ribeiro et al., 2015).

The illegal and intentional stocking by anglers has played a central role in the introduction and dispersal of *M. salmoides* in hydrologically altered systems in Brazil. Given these data and historic of

arguments we encourage solid and integrative (i.e. including all local society, legislators, researchers, fishers, public) management, particularly in local scale, as this species can bring relevant problems to the public supply through trophic cascade and other negative effects. We highlight that education and citizen science in local level are essentials. Public policies offer easy roads to success, which emphasize the need for continuous technical support to evaluate environmental, social and economic risks associated with *M. salmoides*. Local society must have access to the best knowledge available in particular the biological and ecological aspects of invasion processes of powerful predators such as *M. salmoides*, so negative and positive outcomes can be better balanced among local stakeholders and other instances related in other tangential scales. Potential solutions to address these require continuous technical assistance and innovative approaches in a very local scale, in community and ecosystem levels. Some promising avenues include increased collaboration and cooperation among local stakeholders, state agencies and scientists, the valuation of local human communities, indigenous and traditional groups, participatory monitoring and research, and more education and research about Non-Native Species (Azevedo-Santos et al., 2021; Maasri et al., 2022).

Finally, we suggest that our results could be incorporated into monitoring schedules together with other biological groups and the water quality in the reservoir and put energy of society in specific and focal appropriate management (Pereira & Vitule, 2019; Ribeiro et al., 2015). Only with this can we assess the positive and negative effects of the management. First, we suggest sanitary studies of the quality of this invasive species as fish meat for human consumption. Second, start massive campaigns to encourage capture and consumption by the competent authorities with ample participation of all society. Such intensive, massive sampling and targeted tournaments with artificial baits should be during the reproductive period (September to December). Also, the identification and removal of nests using electric fishing during the recruitment period (November to January) are crucial. The nests in this period are relatively easy to find, mainly because they are being focally cared for by the males that are still in very shallow waters close to them, or the young nestlings. Combining all majeures above would provide efficient intentional overfishing effects, with potential to reduce the populations of *M. salmoides* in reservoirs in southern Brazil and its negative effects.

Acknowledgements

JRSV is thankful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the constant research productivity grants provided to JRSV (PQ Process Numbers: 302367/2018-7 and 303776/2015-3). MOF is grateful to Meros do Brazil Project, sponsored by Petrobras. We thank IBAMA/ICMBio for research permits.

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Received: 19 October 2022
Accepted: 12 May 2023

Associate Editor: Fernando Mayer Pelicice.