



## Reproductive aspects of an isolated population of *Psalidodon aff. fasciatus* (Teleostei: Characidae) from southern Brazil

Aspectos reprodutivos em uma população isolada de *Psalidodon aff. fasciatus*  
(Teleostei: Characidae) do sul do Brasil

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**Abstract: Aim:** To investigate reproductive characteristics in a small population of *Psalidodon aff. fasciatus* living in isolation in an environment with a unique geological configuration, a doline-shaped depression (known as *Furna 2*), with a mean diameter of 80 m and water depth of 50 m, a natural heritage of the Vila Velha State Park (VVSP). **Methods:** The fish were collected between February 2019 and January 2020 using nets and pots; standard length and total weight were measured. The gonads were also removed for histological analysis. Estimates were made for the weight-length ratio, the relative condition factor, the sex ratio, and the size at first maturity (L50 and L95) for males and females. **Results:** The five gonadal stages that were characterized (resting, early mature, mature, partially empty, and totally empty) were consistent with what was expected for the species. The results obtained here indicate that these *lambaris* have an opportunistic (r) life strategy. Compared to previous studies, we discovered that fish of this population start maturing at smaller sizes. Indicating negative allometric growth, the length-weight ratio was  $WT = 0.0002 * LT^{2.3039}$ , and the condition index ( $Kn = 0.95$ ) showed no significant gender differences. When compared to other non-isolated populations of the same species, the population's sex ratio showed an unexpectedly significant male predominance (1M: 0.6 F). **Conclusions:** Our results show an imbalance in the sex ratio, contrary to what would be expected for non-isolated populations. This imbalance may be related to the complete isolation of this environment, suggesting that females may be affected by some evolutionary force, such as inbreeding. However, other factors cannot be ruled out, as the underlying genetic mechanisms remain unknown, and only future investigations can reveal a more complete understanding of reproduction in isolated populations.

**Keywords:** lambari; reproduction; sex ratio; gonads; inbreeding.



**Resumo: Objetivo:** Investigar caracteres reprodutivos em uma pequena população natural de *Psalidodon* aff. *fasciatus* que está isolada em um ambiente com rara configuração geológica, uma depressão em forma de dolina (conhecida como *Furna 2*), com diâmetro médio de 80 m e lâmina d'água de 50 m, patrimônio natural do Parque Estadual de Vila Velha. **Métodos:** Os peixes foram coletados entre fevereiro de 2019 e janeiro de 2020 com redes e covos; foram determinados o comprimento padrão e o peso total, além da remoção das gônadas para análises histológicas. Também, foram estimadas a relação peso-comprimento, o fator de condição relativo, a proporção sexual e o tamanho da primeira maturação (L50 e L95) para machos e fêmeas. **Resultados:** Caracterizamos cinco estádios de desenvolvimento gonadal: repouso, maduro inicial, maduro, parcialmente vazio e totalmente vazio, não diferindo do esperado para a espécie. Os resultados aqui obtidos indicam que esses lambaris possuem estratégia de vida do tipo oportunística (r). Comparado a estudos anteriores, descobrimos que os peixes desta população começam a amadurecer em tamanhos menores. Indicando crescimento alométrico negativo, a relação peso-comprimento foi  $WT = 0.0002 * LT^{2.3039}$ , e o fator de condição ( $K_n = 0.95$ ) não diferiu significativamente entre os sexos. A proporção sexual revelou uma predominância significativa e inesperada de machos (1M: 0,6 F) dentro da população, e também quando comparada com outras populações não isoladas da mesma espécie. **Conclusões:** Nossos resultados revelam um desequilíbrio na proporção sexual, contrastando com o esperado para a espécie em condições de não isolamento. Esse desequilíbrio pode estar relacionado ao isolamento completo desse ambiente, sugerindo que as fêmeas podem estar sendo afetadas por alguma força evolutiva, como a endogamia. No entanto, não podem ser descartados outros fatores, pois, os mecanismos genéticos subjacentes permanecem ainda desconhecidos, somente investigações futuras poderão revelar uma compreensão mais completa da reprodução em populações isoladas.

**Palavras-chave:** lambari; reprodução; razão sexual; gônadas; endogamia.

## 1. Introduction

The most diverse group of vertebrates are fishes, which also display a variety of reproductive strategies that ensure the fitness of a species in a given environment (Vazzoler, 1996; Lassala & Renesto, 2007; Azevedo, 2010). Much remains to be known about the reproductive biology of species in the Neotropical region (Waddell et al., 2019), presumably because this region having the planet's highest variety of freshwater ichthyofauna (Lowe-McConnel, 1999; Reis et al., 2003, 2016; Albert et al., 2020). The Characiformes, along with the Siluriformes, are the largest group in the Neotropical region, with a wide range of morphologies, behaviors, and life histories that reflect their interactions with other species and the ecosystem (Nelson, 2006; Albert et al., 2012; Toussaint et al., 2016; Antonelli et al., 2018). With 1245 species and 142 genera, the family Characidae is the most diverse among the Characiformes (Fricke et al., 2022). They are mostly small (15 cm) fish with a variety of reproductive and feeding strategies (Reis et al., 2016).

*Psalidodon* Eigenmann, 1911 is a genus with 43 species (Fricke et al., 2022) that has recently been revalidated by four molecular and two morphological synapomorphies (Terán et al., 2020). These fish can be found in Argentina, Bolivia, Brazil, Paraguay, and Uruguay, where they live in large rivers, streams, and headwaters (Baumgartner et al., 2012; Reis et al., 2020; Dos Santos & de Britto,

2021). *Psalidodon fasciatus* (Cuvier, 1819), popularly known as lambari, is a species complex that occurs in several Brazilian hydrographic systems. Because the São Francisco population is the type locality, much of the closely related species are likely to be *P.* aff. *fasciatus* (Gavazzoni et al., 2023). Chromosome variation has also been reported in some *P. fasciatus* populations, indicating the presence of cryptic species (Medrado et al., 2015). Most of the differences are found in the amount of satellite DNA (As51) (Medrado et al., 2015; Gavazzoni et al., 2023) and the number of diploid chromosomes ( $2n=46, 48, \text{ and } 50$  chromosomes) (Pansonato-Alves et al., 2013).

External fertilization, opportunistic reproductive strategies, batch-spawning, and no parental care are common among these characids, with some exceptions such as insemination and large investments in fecundity (Menni & Almirón, 1994; Azevedo, 2010; Malabarba & Malabarba, 2020; Quirino et al., 2021). Some researches on the reproductive strategies of the lambaris indicates that they breed throughout the year, with peaks in the warmer months (Abilhoa, 2007; Baumgartner et al., 2016; Silveira et al., 2020; Portella et al., 2021).

In the Vila Velha State Park (VVSP), a protected area located in the upper Tibagi River basin in southern Brazil, a small population of the characin *P.* aff. *fasciatus* (Cuvier, 1819) can be found (Shibatta & Artoni, 2005). This naturally isolated population lives in the VVSP's *Furna 2*, a doline-shaped sandstone depression with an average diameter of

80 meters, a water depth of 50 meters, and walls reaching 110 meters (Campos & Dalcomune, 2011). *Furnas* are among the most prominent landforms in the region, and they represent a unique geological heritage in the VVSP (Melo & Giannini, 2007). The *Furnas* Formation, which formed between 395 and 421 million years ago in the Paraná Basin during the Siluro-Devonian period (Guimarães et al., 2007), consists of crater-like depressions formed by the karstification of sandstone rocks (Melo et al., 2011). Because of the environment geological configuration and lack of contact with nearby river drainages, this population of *P. aff. fasciatus* experienced clear isolation and restriction of gene flow (Artoni et al., 2006). Previous research on this population has indicated that a percentage of individuals have malformed vertebrae as a result of isolation and likely inbreeding (Kerniske et al., 2021).

Inbreeding tends to reduce intrapopulation genetic variability by increasing the frequency of deleterious alleles that are normally expressed in recessive homozygosis (Keller & Waller, 2002). Genetic variability may be reduced in populations distributed in distinct areas, this reduces their evolutionary potential and raises their extinction risk (Meffe & Carroll, 1994). Although the underlying mechanisms are poorly understood, the effects on fitness in inbred populations are highly variable, including body size reduction, changes in primary and secondary sexual characters, and morphological

traits (Gilpin & Soulé, 1986; Keller & Waller, 2002; Mariette et al., 2006; Ala-Honkola et al., 2009; Fitzpatrick & Evans, 2009; Losdat et al., 2014).

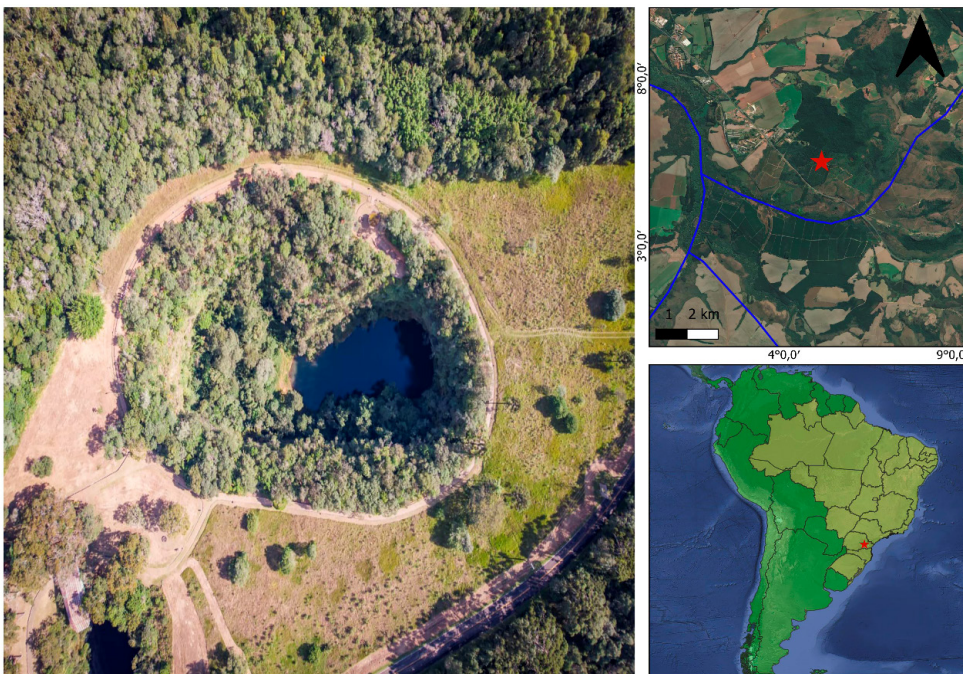
The primary goal of this study was to characterize the reproductive biology of this naturally isolated population of *P. aff. fasciatus*, describing traits such as sex ratio, reproductive activity, sexual maturity, and length-weight ratio, and to determine if isolation could be associated with differences in reproductive tactics and possibly inbreeding (Kerniske et al., 2021).

## 2. Material and Methods

### 2.1. Study area

This research followed the international standards of animal experimentation, approved by the Ethics Committee for Animal Use of the Universidade Tecnológica Federal do Paraná (CEUA process N.º. 2018-025/2018). The collection was authorized by the Ministério do Meio Ambiente (MMA/ICMbio nº 15115-1) and the Instituto Ambiental do Paraná (IAP nº 15.190.528-5; authorization 15.18).

Specimens of *P. aff. fasciatus* were collected in the *Furna* 2 of Vila Velha State Park (VVSP), a protected area located in the upper Tibagi River basin, Ponta Grossa, southern Brazil, at coordinates 25° 15'01 53" S and 50° 00'19 38" E (Figure 1). Precipitation and air temperature data for the time period were obtained from SIMEPAR (Sistema Meteorológico do Paraná; <http://www.simepar.br/>).



**Figure 1.** View of the *Furna* 2 located in the Vila Velha State Park, Brazil.



## 2.2. Sampling methods and data analysis

Except for March 2019, sampling was done monthly using seine nets between February 2019 and January 2020. Specimens of *P. aff. fasciatus* were transported to the UTFPR's Laboratório de Ecologia Aquática, where they were anaesthetised with benzocaine (0.1 g/L) and sacrificed. The presence (male) or absence (female) of bony hooks on the pelvic fin was used to determine sex (Porto-Foresti et al., 2005), which was later confirmed by histological analysis of the gonads.

Each captured individual's standard length (SL) and total weight (TW) were measured in the laboratory to the nearest 0.1 cm and 1 g, respectively. Fresh gonads were removed, and sex and maturity were determined macroscopically based on gonad size, color, vascularity, and the presence of lateral spermathecae or oocytes. Following macroscopic classification, gonads were fixed in Bouin's solution for 48 hours before being stored in 70% ethanol for histological preparation (Vazzoler, 1996; Bazzoli, 2003). Microscopically, histological sections from 69 males and 26 females were examined and classified into five developmental stages: Regenerating (I), Developing (II), Spawning Capable 1 (III), Spawning Capable 2 (IV), and Regressing (V), as adapted from Brown-Peterson et al. (2011).

For the entire study population, the total length at maturity (L50, the length in which 50% of individuals were mature; and L95, the length in which most of the individuals reached sexual maturity) was estimated. The L50 and L95 analysis took into account sexually mature individuals (adults). The logistic regression model shown below was used:  $PM = [1 + e^{-(a + \beta L)}]^{-1}$ , where PM denotes the proportion of mature individuals in length class L, a denotes a constant, and  $L50 = -a/\beta - 1$ . Binary maturity observations (0=immature, 1=mature) and total length were fitted to binary logistic models (logit function) to construct maturity ogives (maturity-at-length probability plots) based on logistic regression to predict the probability that an individual was mature at a given length (Hazelton, 2007). The 95% confidence interval was estimated using a bootstrap method with 1000 interactions. The R package was used for statistical procedures (R Core Team, 2019).

The reproductive season was determined by the relative frequency of reproductive stages, and the sex ratio was calculated for each month, with the  $\chi^2$  test determining significant differences ( $df = 1; p = 0.05; \chi^2 < 3.84$ ). To compare the sex ratio,

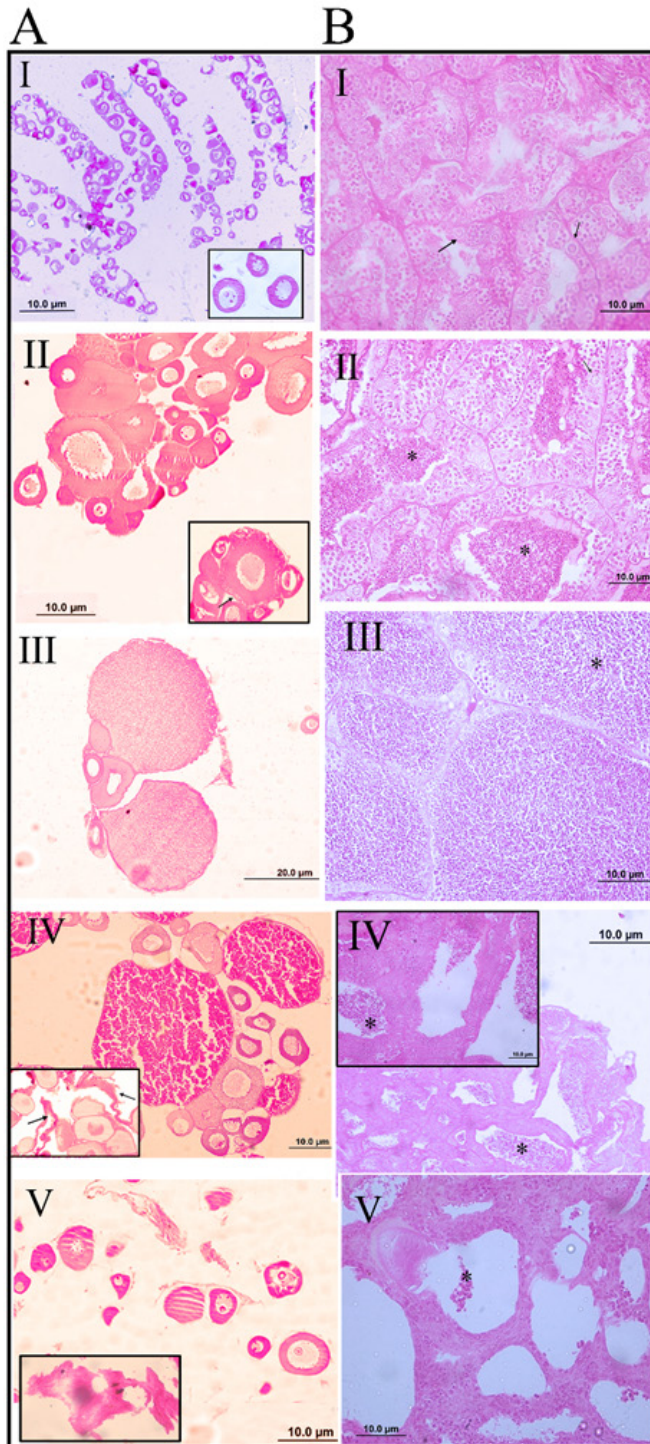
we employed the Bayesian approach, estimating the proportion of females in comparison to external populations by applying a conjugate beta-binomial statistical model. Within this model, we established an uninformative beta distribution (1,1) as the prior, signifying the absence of prior knowledge about the female proportion within the population. The posterior distribution was then modeled using the beta distribution ( $f + 1, n - f + 1$ ), where "f" denotes the number of observed females and "n" represents the total sample size, encompassing both females and males. Statistical inferences concerning the female proportion were subsequently derived from the resulting posterior beta distribution (Gelman et al., 2013).

The length-weight ratios were calculated using the least-squares fit to log-transformed data function:  $WT = aLT^b$ , where WT is the total weight, LT is the total length, a is a body shape coefficient, and b is an exponent indicating isometric growth when equal to 3 (Le Cren, 1951; Lima-Junior et al., 2002). The mean value of the relative condition factor (Kn), which is the quotient of the observed weight and the theoretically expected weight for a given length ( $Kn = Wt/We$ ), was then estimated (Le Cren, 1951). Using the Student's t-test ( $p < 0.05$ ), the values obtained for males and females were statistically compared with the standard  $Kn = 1.00$ .

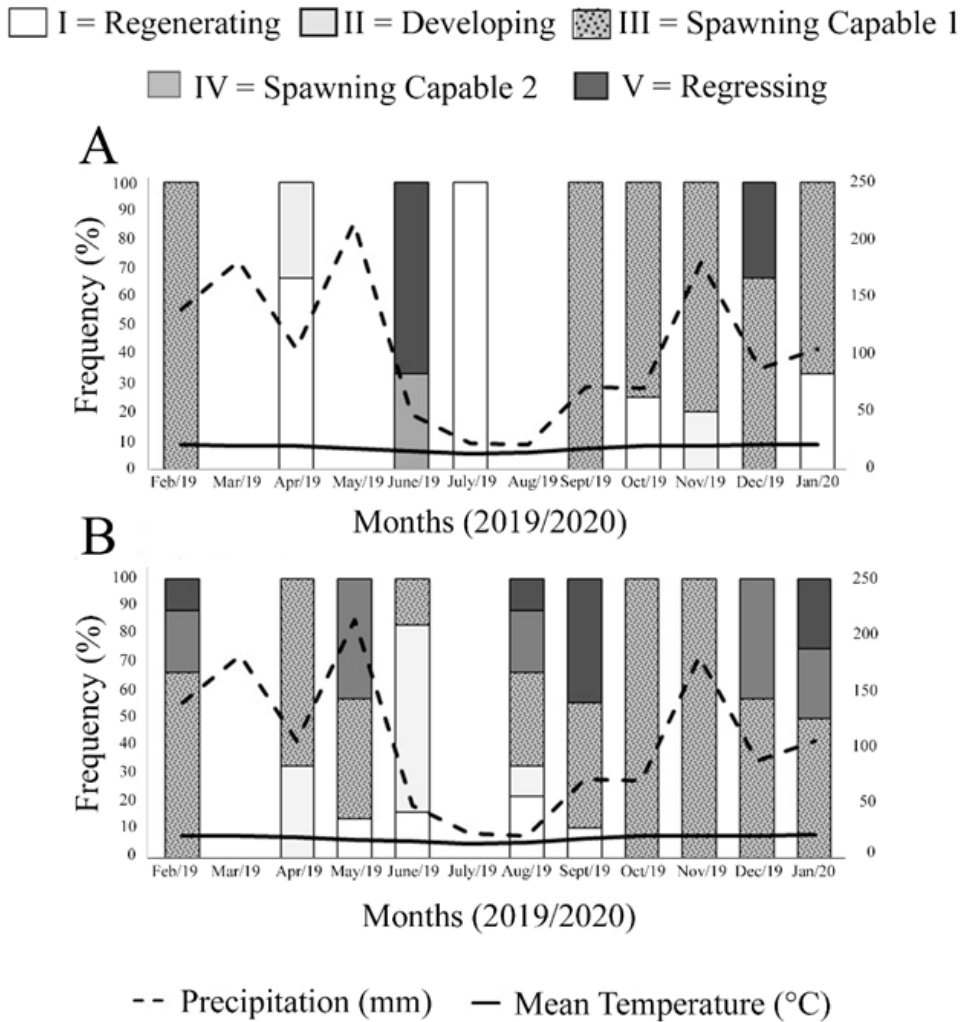
## 3. Results

A total of 254 *P. aff. fasciatus* individuals were collected and analyzed, with the presence of males (N=154; 60.6%) and females (N=100; 39.4%). During the sampling period, the average temperature values were highest from October to March (spring-summer), the months from June to October (winter) were the driest of the year, and the month of May (autumn) had the highest precipitation index.

The stages of gonadal development in females and males were identified (Figure 2), and the relative frequency in relation to the months of the year was calculated (Figure 3). Histological examination of reproductively active ovaries revealed the presence of oocytes at various stages of maturation at the same time, indicating multiple spawning patterns. Individuals in the capable of spawning 1 stage (III) were recorded between September 2019 and February 2020, implying that spawning activity peaks occur between spring and summer. In June 2019, females capable of spawning capable 2 stage (IV), with partially empty gonads, post-ovulatory follicles, and regressing (V) were observed.



**Figure 2.** Histological sections of ovaries (A) and testes (B) of *P. aff. fasciatus*. (A) I - Regenerating stage containing early and advanced perinuclear oocytes with basophilic cytoplasm and several nucleoli in the nucleus (highlighted), II - Developing stage besides perinuclear oocytes presents pre-vitellogenic oocytes with characteristic cortical alveoli (highlighted - arrow), III - Spawning Capable 1stage predominantly vitellogenic oocytes filled with yolk granules, IV - Spawning Capable 2 stage with oocytes in all growth stages and also post-ovulatory follicles (highlighted - arrow), V - Regressing stage containing perinuclear oocytes, post-ovulatory follicle and atresic oocyte (highlighted); (B) Testes of *P. aff. fasciatus* stained with HE. I - Regenerating stage organized in cysts containing spermatogonia in the seminiferous tubule and occluded lumen, II - Developing stage with presence of spermatozoa in small proportion within the lumen and spermatogenic cell cysts in the seminiferous tubule, III - Spawning Capable 1 stage exhibiting lumen filled with sperm embedded in acidophilic secretion and some spermatogenic cell cysts, IV - Spawning Capable 2 stage containing few sperm, V - Regressing stage with only residual sperm. Arrow = spermatogonia, \* = spermatozoa.



**Figure 3.** Relative frequency distribution of gonadal maturation stages for females (A) and males (B) of the naturally isolated population of *P. aff. fasciatus* collected between Feb/2019 - Jan/2020 in Furna 2 (n=95). Monthly average of air temperature and precipitation for the city of Ponta Grossa from 02/2019 to 01/2020.

In July, the total number of individuals collected was represented by regenerating females (I).

Males, on the other hand, had spermatozoa-filled testes throughout the sampling period, but the frequency of stage (III) increased in October (spring) and decreased in June (winter). In September, individuals in stage (V) were more frequently observed, with gonads containing only residual spermatozoa.

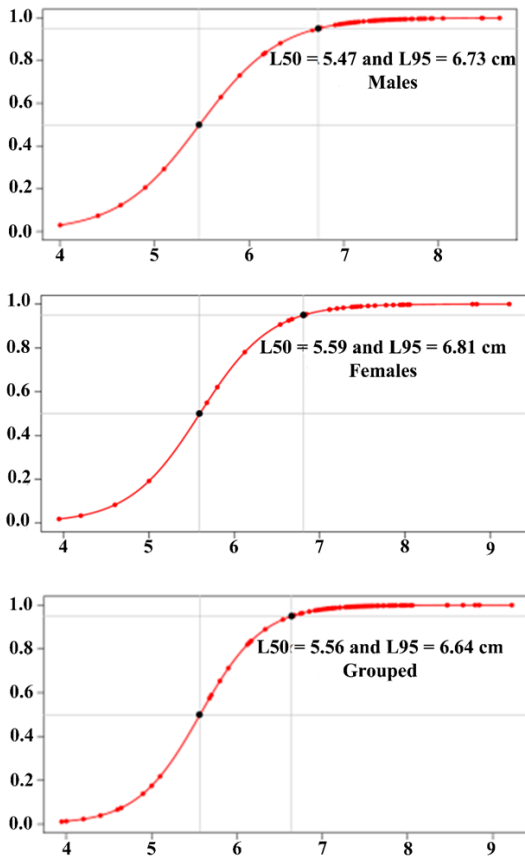
Females had an L50 of 5.59 cm, and all females over 6.81 cm LT were mature (L95). L50 was estimated to be 5.47 cm for males, and all males > 6.73 cm LT were mature (L95). L50 and L95 were estimated to be 5.56 and 6.64 cm LT for the entire population, respectively (Figure 4).

The overall monthly sex ratio differed significantly ( $\chi^2 = 21.885$ ,  $p = 0.025$ ). Females outnumbered males in July 2019 ( $\chi^2 = 8.00$ ,

$p = 0.01$ ), but males outnumbered females in August 2019 ( $\chi^2 = 6.4$ ,  $p = 0.04$ ) (Figure 5A). Beta-Binomial modeling of sex ratio data revealed that the proportion of females in the naturally isolated population of *P. aff. fasciatus* was lower than estimates reported in the literature (Figure 5B).

The weight-length relationship was estimated for all individuals grouped, and the equation obtained was  $WT = 0.0002 * LT^{2.3039}$ , indicating that the parameter  $b$  of 2.3. Also, the weight-length relationship was estimated for females ( $b = 2.3$ ) and males ( $b = 2.2$ ) indicating negative allometric growth ( $b < 3$ ); it means more energy expenditure in length than in mass. There was no statistically significant difference in body condition between genders (Figure 6), with both presenting an average value of  $Kn = 0.95$ .

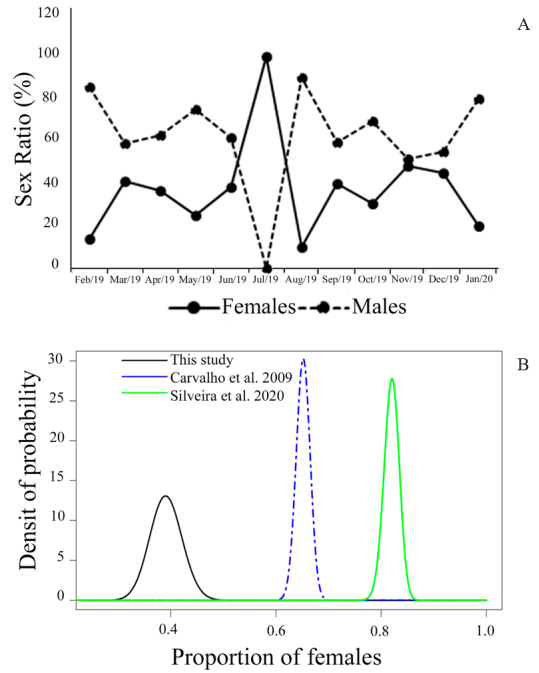




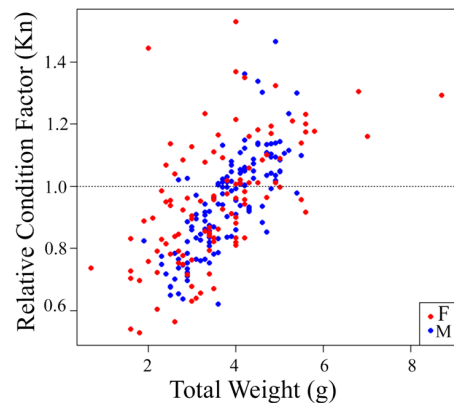
**Figure 4.** Length at maturity (L50 and L95) with fitted logistic regression model of males, females and the entire study population of the naturally-isolated population of *P. aff. fasciatus* captured between February/2019 and January/2020 in *Furna 2*.

#### 4. Discussion

Except for the sex ratio, which seems unbalanced and unexpectedly favorable to males, the general pattern observed in this study of the reproductive period and sexual maturity of a naturally isolated population of *P. aff. fasciatus* from southern Brazil is similar to others small characins (e.g., Hojo et al., 2004; Carvalho et al., 2009; Portella et al., 2021). This species can spawn throughout the year, with peak spawning activity in spring and summer, with an increase in air temperature associated with precipitation, as evidenced by numerous studies (Baroiller et al., 2009; Bartolini et al., 2015; Firkus et al., 2018; Quirino et al., 2021). This characin's reproductive characteristics indicate an opportunistic (*r*) strategy, which is common in small-sized species, particularly Characiformes, with no or little parental care and a long reproductive period (Winemiller, 1989; Vazzoler, 1996; Quirino et al., 2021).



**Figure 5.** (A) Relative frequency distribution of males and females of *P. aff. fasciatus* collected in *Furna 2* of the VVSP in the period 2019-2020; (B) Posterior distribution of the proportion of females in the naturally-isolated population of *P. aff. fasciatus*, estimated through the Beta-binomial model using the overall sex ratio captured between February/2019 and January/2020 collected in *Furna 2* of VVSP compared with the estimates from the literature (Carvalho et al., 2009; Silveira et al., 2020).



**Figure 6.** Individual values for the relative condition factor (Kn) considering the standard value (Kn=1.0) in males (M) and females (F).

Growth was negatively allometric ( $b = 2.3$ ), which means that more energy was expended on length than on weight. This could be due to inbreeding, as body weight is affected in inbred individuals (Fessehaye et al., 2007). Beyond that, the maximum observed total length was 9.2 cm,

which is less than that reported for the species (13.2 cm) (Vazzoler, 1996). Despite their smaller sizes, females had the highest values recorded, which could be related to fecundity, because the size of the peritoneal cavity allows for a greater or lesser number of oocytes (Vazzoler, 1996; Araújo et al., 2019). Studies with Characiformes suggest that detrimental effects on Kn may reflect seasonal changes or even be a consequence of parasitism (Gomiero et al., 2012; Ito, 2013; Antunes et al., 2022). Nonetheless, the mean value of Kn = 0.95 was not significant in our study, both individually and when the genders were combined, indicating that these lambaris have a high body condition. This could be due to the intra and interspecific isolation of this population (Shibatta & Artoni, 2005; Artoni et al., 2006; Matoso et al., 2013), which reflects the lack of competition among different species, and, as a result, the abundance of available food in Furna 2 (Mayer et al., in preparation).

The standard length at which 50% of the population reached maturity ( $L_{50}=5.56$  cm SL) was 60.3% of the maximum recorded length (9.22 cm). The length at first maturity was estimated to be 5.47 cm for males and 5.59 cm for females, which is lower than the values reported by Carvalho et al. (2009), who reported 6.4 and 6.5 cm for males and females, respectively, and Prado et al. (2011), who reported a total length at maturity of 8.8 cm. According to our findings, the naturally isolated population of *P. aff. fasciatus* reaches sexual maturity at smaller sizes, which is typical of fast-growing fish (Winemiller, 1989).

One of the most important indicators for understanding fish population dynamics is the sex ratio. In natural populations, the number of males is expected to be statistically equal to the number of females (1:1) (Nikolsky, 1969; Vazzoler, 1996). Deviations in the ratio, on the other hand, are relatively common in fish populations and can be caused by factors that affect the genders differently, such as reproductive behavior, birth rates, mortality, and growth rates (Vazzoler, 1996; Araújo et al., 2019). It is important to note that a skewed sex ratio towards one of the genders reduces effective population size and leads to genetic diversity loss (Zajitschek et al., 2009). The higher the number of males in inbred populations, the lower the reproductive success, because the effects of inbreeding are exacerbated when males compete with each other (Fessehaye et al., 2009).

*Psalidodon aff. fasciatus* from Furna 2 showed a male predominance, which contrasts with the

typical *P. aff. fasciatus* female predominance (Carvalho et al., 2009; Silva et al., 2010; Hirt et al., 2011; Silveira et al., 2020), with an exception of Gurgel (2004) who also found a male predominance in a possible oligotrophic environment in the Ceará-Mirim river. The higher number of males found in freshwater fish populations has been linked to oligotrophic environments (Nikolsky, 1969; Gurgel, 2004), which differs from that found in Furna 2 because the environment is stable and the food resource is abundant. On the other hand, reproductive behavior may have an influence that could explain the higher proportion of males during the reproductive peak, since this is a tactic employed by species with external fertilization related to female fecundity, so the higher proportion of males would increase the number of fertilized oocytes (Veregue & Orsi, 2003). But, the sex ratio in all samples cannot be fully explained by the previously stated possible causes, leading us to suspect that other factors may act differently on the genders. These may include inbreeding issues, as male and female survival may be affected differently by inbreeding depression (Lacy et al., 1993). Similarly, Kerniske et al. (2021) discovered spinal malformations in females of this population, with inbreeding as a possible cause. For instance, in an inbred tilapia population, deleterious genes were also linked to a sex ratio imbalance, which resulted in higher female lethality (Shirak et al., 2002).

Concluding, this study provides the first information on the reproductive biology of these lambaris discovered in Vila Velha State Park, Paranas Furna 2. The gonadal development followed the biology of the species, according to the study, and the opportunistic strategy is also common in other small characids, in addition to the expected plasticity of reproductive tactics. However, our findings suggest a sex ratio imbalance that favors males. In an additional way, because this is a small population possibly with limited gene flow, in which the loss of genetic diversity has already been previously reported (Matoso et al., 2004; Matoso et al., 2010), the effects of inbreeding may be amplified, resembling a scenario involving critically endangered individuals.

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