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Environmental predictors of the life history of the flag tetra *Hyphessobrycon heterorhabdus* (Characiformes: Characidae) in streams of the Eastern Amazon

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This study aimed to characterize the reproductive biology of *Hyphessobrycon heterorhabdus*, and its response to environmental variations in the Eastern Amazon streams. We sampled specimens every two months, between March 2019 and January 2020. The population was evaluated for sex ratio, reproductive activity, growth pattern, condition factor, size at the first sexual maturation, spawning type, and fecundity. We analyzed 180 specimens, which showed a sex ratio of 1.6 males for each female across the whole period, with 2.3 males for each female during the period of greatest reproductive activity. The peak of reproductive activity coincided with higher precipitation periods and was partially predicted by factors such as water temperature, stream discharge, dissolved oxygen, substrate complexity, and electrical conductivity. The length where 50% and 100% of population to reach sexual maturity was 18.0 and 22.0 mm for males and 19.7 and 27.0 mm for females. The oocyte diameters showed a bimodal frequency, with at least two batches of oocytes. The average fecundity of 197 oocytes. The results indicate that this species presents an opportunistic strategy, and the tactics that make up this strategy depend on variations in both the physical structure of the habitat and physicochemical aspects of the water.

Keywords: Fecundity, Rainfall, Reproductive strategy, Sexual maturation, Spawning.

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Este estudo teve como objetivo caracterizar a biologia reprodutiva de *Hyphessobrycon heterorhabdus* e sua resposta às variações ambientais em riachos da Amazônia Oriental. Os espécimes foram amostrados bimestralmente, entre março de 2019 e janeiro de 2020. A população foi avaliada quanto à razão sexual, atividade reprodutiva, padrão de crescimento, fator de condição, tamanho na primeira maturação sexual, tipo de desova e fecundidade. Foram analisados 180 exemplares, que apresentaram proporção sexual de 1,6 machos para cada fêmea durante todo o período, com 2,3 machos para cada fêmea no período de maior atividade reprodutiva. O pico da atividade reprodutiva coincidiu com períodos de maior precipitação sendo parcialmente predito por fatores como temperatura da água, vazão, oxigênio dissolvido, complexidade do substrato e condutividade elétrica. O comprimento onde 50% e 100% da população atingiram a maturidade sexual foi de 18,0 e 22,0 mm para os machos e 19,7 e 27,0 mm para fêmeas. Os diâmetros dos oócitos apresentaram frequência bimodal, com pelo menos dois lotes. A fecundidade média da espécie foi de 197 oócitos. Os resultados indicam que a espécie possui uma estratégia oportunista, onde as táticas que compõem esta estratégia dependem tanto de variações na estrutura física do habitat quanto de aspectos físico-químicos da água.

Palavras-chave: Desova, Estratégia reprodutiva, Fecundidade, Maturação sexual, Precipitação.

INTRODUCTION

Fish stand out among vertebrates as the group with the most remarkable diversity of reproductive strategies (Helfman *et al*., 2009), resulting mainly from the heterogeneity of secondary sexual characters, oocyte morphology, parental care, and the variety of courtship behaviors (Vazzoler, 1996). Their reproductive tactics tend to vary according to the intensities of intra and interspecific interactions or even in response to environmental variations, which may be natural or resulting from anthropic activities (Wootton, 1992; Souto *et al*., 2017).

Reproductive strategies and tactics are among the most important aspects of fish autecology and are directly related to the maintenance of their populations in each environment (Nikolsky, 1969; Braga, 2006; Monaco *et al*., 2014). In this sense, knowing these strategies and tactics contributes to the development of management plans for these biological populations, directing actions aimed at minimizing possible damage resulting from threats such as predatory fishing (Chapman *et al*., 1998; Ribeiro *et al*., 2008) and anthropic disturbances that affect the dynamics of aquatic ecosystems (Souto *et al*., 2017).

Conducting this type of study also fills a significant knowledge gap about the life history of tropical fish species (Raunkiaeran Gap) (Hortal *et al*., 2015). This is even more relevant for the Amazon basin, which has a high environmental heterogeneity (Sioli, 1984) and is home to the most extraordinary diversity of freshwater fish in the world, with a large part of this diversity present in creek ecosystems (Oberdorff *et al*., 2019).

In the Amazon basin, hydrological variations are considered the main predictor of natural seasonal variations in the environmental conditions of aquatic ecosystems (Espírito-Santo *et al*., 2013). In large rivers, this directly contributes to flooding pulses, which are predictable variations directly related to the reproductive activities of Amazonian fish (Junk *et al*., 1989). However, in upland streams, this seasonal variation of environmental conditions is much more unpredictable and is more strongly associated with the rainfall regime along the catchment (Kramer, 1978; Tomasella *et al*., 2008). This local rainfall is considered the primary regulator of the environmental conditions of the streams and, consequently, the main predictor of the reproductive tactics of the fish living in this environment (Kramer, 1978).

Hyphessobrycon heterorhabdus (Ulrey, 1894) is a small characid that is widely distributed in the lower Amazon region (Faria, 2020), standing out among the most abundant species in streams of the Eastern Amazon (Benone *et al*., 2017; Ferreira *et al*., 2018; Prudente *et al*., 2018; Santos *et al*., 2019). This species inhabits small tributaries and forms schools of five to 30 individuals that occupy backwater areas close to the banks, where they feed on items available both in the water column and associated with the substrate of these banks (Brejão *et al*., 2013).

Like other Amazonian characids, *H. heterorhabdus* is regionally known as “piaba” and economically distinct for being on the list of ornamental species popularly known as flag tetra (IBAMA, 2012). However, information about their reproductive ecology is still lacking.

In this sense, the present study aimed (i) characterize the reproductive biology of *H. heterorhabdus* considering the trilateral continuum model of Winemiller, Rose (1992), based on sex ratio, gonadal maturation stages, reproductive activity, growth pattern, allometric condition factor (K), spawning type, fecundity, and length where 50% and 100% of population to reach sexual maturity; and (ii) understand how this reproductive activity responds to temporal variation in environmental conditions in the upland streams of the Eastern Amazon. We hypothesized that the species would have reproductive traits corresponding to an opportunistic strategy, such as a prolonged period of reproductive activity that is positively related to hydrological variations such as precipitation and variation in depth and width of the channel, with a smaller influence of physicochemical characteristics of the water.

MATERIAL AND METHODS

Study area. This study occurred in a catchment of 1,240 ha on the left margin of the Guamá River, in the municipality of Capitão Poço, state of Pará, Eastern Brazilian Amazon (Fig. 1). The local vegetation is classified as equatorial sub-perennial forest (IBGE, 1992). However, the catchment landscape is currently dominated by farmland and cattle pasture (Pacheco, Bastos, 2001), with small remnants of secondary (Silva *et al*., 1999) and riparian forests, which are considered areas of permanent preservation under article 30 of the Brazilian Forest Code, federal law number 12,651/12. The region has a humid tropical climate, subtype *Af* in the Köppen classification adapted by Peel *et al.* (2007). The mean annual temperature is 26.9 °C, which varies only slightly over the year. The mean annual rainfall is 2,370 mm, with a rainy season between January and May, and a dry season between August and November (Pacheco, Bastos, 2001; INMET, 2021).

We sampled three low-order streams (1st to 2nd order *sensu* Strahler, 1957) with similar physical habitat characteristics. In each stream, we defined a 50 m stretch divided by six cross sections, resulting in five 10 m longitudinal sections where we measured the local environmental condition and sampled the fish species. The streams were assessed every two months between March 2019 and January 2020.

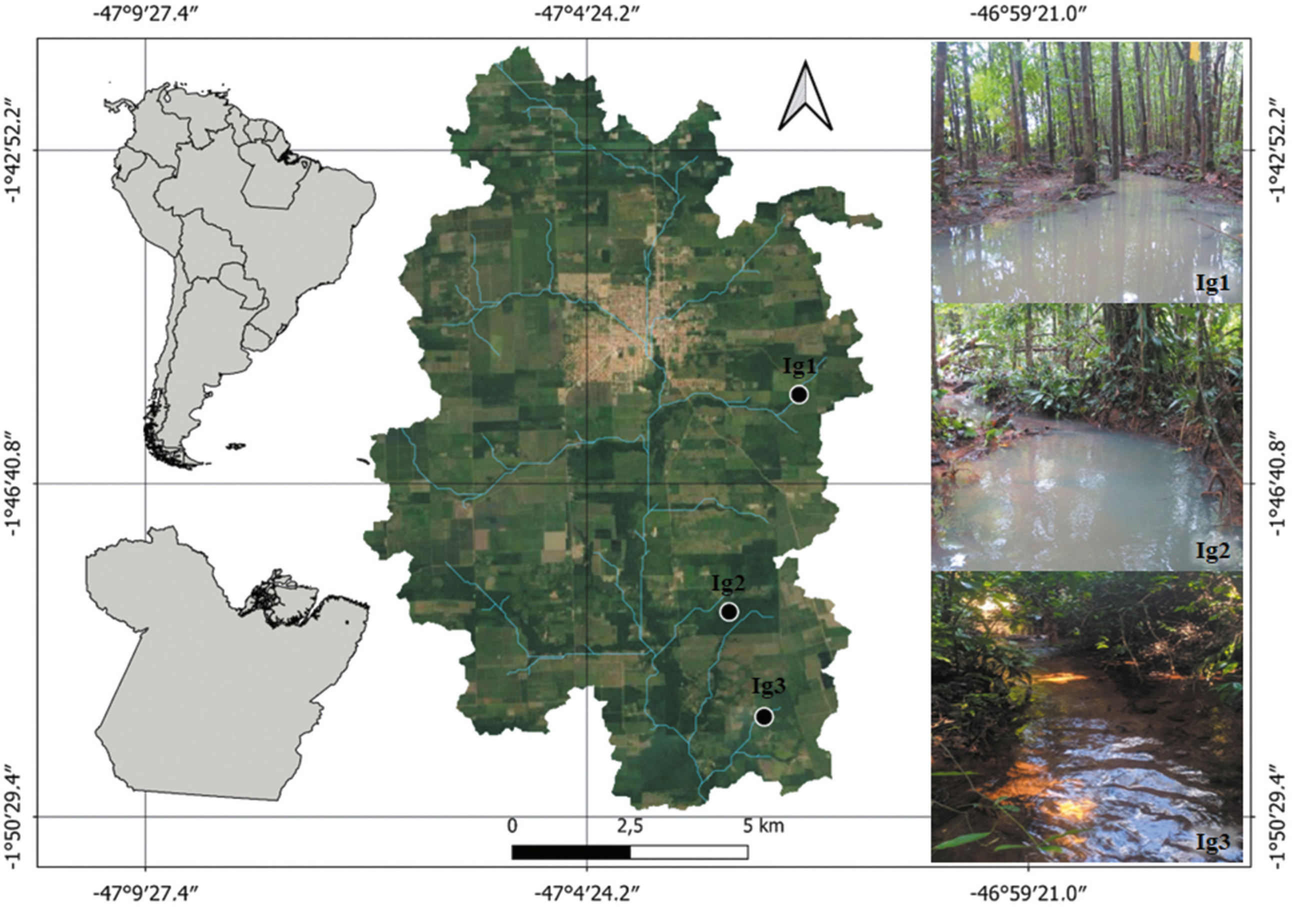

FIGURE 1 | Location of the streams (black circles) in the Guamá River basin, Eastern Amazon, State of Pará, Brazil, where the specimens of *Hyphessobrycon heterorhabdus* were sampled between March 2019 and January 2020.

Data sampling. Before the fish collection, we measured four physicochemical characteristics of the water in each stream, such as Dissolved Oxygen (%), Electrical Conductivity (μS/cm^{-1}), pH, and Temperature (°C), using a Horiba U-50 multiparameter device. In each cross-section established at each stream, we measured the wetted width (WW – m), the thalweg depth (TD – cm) and the percentage of structured substrate (SS – %). We determined the WW (transversal distance between stream-flooded margins) and the TD using a ruled pole. The SS was obtained by summing the percentages of leaf litter, woody fragments and roots visually estimated at five equidistant points within each cross-section.

We recorded the flow speed (FS – m/s) of the stream at three equidistant points along the channel by measuring the time taken by a floating object to move a known distance. This information was used to calculate the stream discharge (D – m3/s) based on the equation, $Q = A * Vm$, where Q is the discharge, Vm the mean flow speed, and A the mean transect area. The mean transect area was calculated by $= \Sigma A_n$, where A is the area of the transect, which is given by the sum of

$$\left[\frac{(Z_1 + Z_2)}{2}\right]^{*} w + \left[\frac{(Z_2 + Z_3)}{2}\right]^{*} w + \ldots \left[\frac{(Z_n + Z_n + 1)}{2}\right]^{*} w$$

where, Zn is the the measured depth of each segment, and w is the width of each segment. Accumulated monthly rainfall data were provided by the meteorological station of the Instituto Nacional de Meteorologia (INMET 2021 – Station A248), located in the municipality of Capitão Poço (Fig. **S1**). We used the average monthly values of the environmental variables obtained for each stream for the statistical analysis.

The specimens of *Hyphessobrycon heterorhabdus* were collected from each 10 m longitudinal section for 12 min by three people using rectangular sieves (80 cm x 60 cm) with a 2 mm mesh. The individuals captured were euthanized with an overdose of Eugenol (6 ml / 3 L of water) and fixed in a 4% formalin solution in the field and preserved in 70% ethanol solution under vouchers numbers GEA.ICT 12101–12106 in the ichthyological collection of Grupo de Ecologia Aquática (GEA), Universidade Federal do Pará (UFPA), Belém, State of Pará, Brazil.

In the laboratory, ten individuals were randomly selected from each stream every two months, resulting in 30 individuals per month and 180 individuals in total. The selected specimens were measured for their total weight (W_t) using an analytical balance with a precision of 0.0001g, and for their standard length (L_s) in millimeters using a caliper with 0.01 mm precision. The specimens were eviscerated through a longitudinal incision in the ventral region to remove the gonads, which were also weighed (W_g) with a precision of 0.0001 g and conditioned in 70% alcohol. Afterward, the specimens were checked for their eviscerated weight (W_{evi}) in grams.

The gonads of the 180 individuals were assessed macroscopically to a previous definition of sex and gonadal maturation stage, following Vazzoler (1996), and subsequently, subjected to routine histological analysis, following Prophet *et al.* (1995). Considering that mature females are easily identifiable macroscopically because of the size of the gonad and the large number of oocytes, only three gonads from females at this stage were subjected to histological analysis to corroborate the previously identification of the stage. The microscopic definition of the sex and gonadal maturation stages was based on the presence and frequency of different types of cells of oogenic and spermatogenic lineages based on the classification proposed by Núñez, Duponchelle (2009).

Males were classified into four stages:1) immature, characterized by the presence of undifferentiated germ cells and spermatogonia surrounded by a large amount of connective tissue; 2) maturing, characterized by the presence of spermatogonia located along the seminiferous tubules, spermatocytes, spermatids, and a small amount of spermatozoids; 3) mature, characterized by the presence of a large number of spermatozoids in the lumen of the tubule; and 4) spent, characterized by the presence of practically empty seminiferous tubules and a few residual spermatozoids.

The females were classified into five gonadal stages:1) immature, characterized by the predominance of previtellogenic oocytes (stage I), which present a basophilic homogenous ooplasm, large central nuclei with central or sub-central nucleoli, and a high nucleoplasmic ratio; 2) maturing: characterized by the presence of previtellogenic oocytes (stage I), with a predominance of oocyte in early vitellogenesis (stage II). The latter is distinguished from stage I oocytes by the presence of cortical alveoli. Oocytes in advanced vitellogenesis (stage III) can also be observed at this stage of gonadal maturation, which is characterized by the presence of the chorion being clearly visible, folicular cells and the theca generally well developed; the nucleus or germinal vesicle is still visible and located in a central position; 3) mature: characterized by the predominance of stage IV oocytes, which are characterized by cytoplasm filled with large yolk globules. Previtelogenic (stage I) and early vitellogenic (stage II) oocytes were also observed at a low frequency during this stage of gonadal maturation; 4) spawned: characterized by the presence of previtellogenic oocytes (Stage I) and new batches of vitellogenic oocytes (stage II and III), which makes the ovary partially filled. Post-ovulatory follicles and some atretic oocytes were also found in this gonadal maturation stage; 5) resting: It presents characteristics similar to those of an immature female, with a predominance of previtellogenic oocytes (stage I), distinguishing it from this gonadal stage by the presence of a thicker ovarian wall. Some atretic follicles were also observed in this gonadal stage.

Reproductive traits. The proportion of males and females in the population was evaluated for the whole study period and each month. Differences in this sex ratio were tested using the Chi-square test (χ^2) of adherence, considering as the null hypothesis that the sex ratio of this population does not differ from 1:1, as proposed by Vazzoler (1996).

The period of reproductive activity of the population of *Hyphessobrycon heterorhabdus* was evaluated through the Gonadosomatic Index (GSI) and the variation in the frequency of maturation stages over the sampled period. The GSI was obtained through the equation, $GSI = Wg/Wt * 100$, where Wg is the gonad weight and Wt is the individual's total weight. The GSI values were verified for the statistical assumptions of normality and homoscedasticity and tested for their variation between the studied months using the non-parametric Kruskal Wallis test followed by a Wilcoxon multiple comparison test. This test was performed separately for males and females and disregarded immature specimens.

The growth pattern of *Hyphessobrycon heterorhabdus* was evaluated through the weight-length relationships of the individuals and followed the model proposed by Järvi (1920) (see Froese, 2006), $Wt = a * L_S{}^b$, where W_t is the individual total weight; L_S the individual standard length; *a* is the proportionality coefficient, and *b* the allometric coefficient. To verify possible differences in the growth pattern between the sexes, the residuals of this relationship were evaluated according to the statistical assumptions of normality and homoscedasticity and tested between males and females using a Mann-Whitney U test. In case of significant differences, the weight-length relationship was evaluated separately for males and females, and the residues of this relationship were tested using a linear regression model to verify a possible non-random pattern of variation. In the case of non-random patterns, the weight-length relationship was adjusted through a polyphasic model proposed by Bervian *et al.* (2006).

The polyphasic model consists of using parameters *a* and *b* of the Huxley equation (1924),

$$y = f(a) * x^{f(b)},$$

where $$f(a) = a_1 + \frac{(a_2 + a_1)}{1} + e^{RSCa*(Ls - LSC)}$$ and

$$f(b) = b_1 + \frac{(b_2 + b_1)}{1} + e^{RSCb*(Ls-LSC)}.$$

The parameters a_1 and b_1 represent the coefficients of proportionality and allometry determined in the first growth phase, and a_2 and b_2 are the same coefficients determined in the second growth phase. *RSC* (*a*/*b*) is the rate of change of the coefficients between the first and the second growth phase, and *LSC* is the standard length when the change in the growth pattern occurs.

The allometric condition factor (K) of each specimen, which reflects the balance between energy acquired through feeding and spent on activities such as reproduction and ecological interactions (Braga, 1986), was estimated through the equation $K = Wevi/\ L_S{}^b$, where *Wevi* is the eviscerated body weight obtained from the total mass of the specimen subtracting the weight of the gonad (W_g) and stomach (W_e); *Ls* is the standard length and *b* the allometric coefficient obtained through the weight-length relationship. K values were obtained separately for males and females, evaluated according to the statistical assumptions of normality and homoscedasticity and submitted to a Kruskal-Wallis test to verify its variation between the months sampled, followed by a Wilcoxon multiple comparisons test. In the case of polyphasic growth, the individuals had their allometric condition factor (K) calculated based on the allometric coefficient of their respective lengths.

The mean length where 50% (L_{50}) and 100% (L_{100}) of population to reach sexual maturity was obtained separately for males and females through the logistic equations

$$P = A * (1 + e^{r*(Ls-(L50)})^{-1} \text{ and } P = A * (1 + e^{r*(Ls - L100)})^{-1}$$

respectively, where *P* is the proportion of adult individuals, *A* is the proportionality coefficient, *r* is the phase change rate parameter (from juveniles to adults), L_s is the standard length in mm, and L_{50} and L_{100}, are the mean length at sexual maturation of 50% and 100% of the population respectively. A length class with an amplitude of 2 mm for this analysis was defined. Both equations were adjusted using the Solver routine of the Microsoft Office Excel 2016® software.

The spawning type and fecundity of *Hyphessobrycon heterorhabdus* were defined by considering ten randomly selected mature gonads. The spawning type was defined based on the frequency distribution of oocyte diameters, macroscopically measured, and corroborated by the assessment of the frequency of occurrence of different phases of oocyte development in the histological sections. The oocytes were manually dissociated and subsequently photographed using a Motic stereomicroscope, model BA310E (zoom 40x), equipped with a camera. The diameter of the oocytes was measured using ImageJ® software with a spherical object of known diameter as a reference. The fecundity of *H. heterorhabdus* was estimated considering the number of vitellogenic oocytes, defined as oocytes in stage IV of maturation (Núñez, Duponchelle, 2009). Macroscopically, vitellogenic oocytes were distinguished from the other oocytes because they were larger, fuller, and yellowish. In addition, only oocytes with a diameter greater than the smallest microscopically identified vitellogenic oocyte (stage IV) were used for fecundity estimation. Oocyte count was also performed under a stereomicroscope, 40x zoom.

Effects of environment variables. To verify the predictive capacity of the physicochemical variables of water and the physical habitat in the reproductive activity of *H. heterorhabdus*, the variables was submitted to a Multiple Linear Regression Model (MLR), with GSI as the response variable. The selection of the variables with the highest predictor capacity of the GSI variation was validated through the Akaike selection criterion (AIC) (Zuur *et al*., 2009). The best model, according to AIC criteria, was used to interpret the relationships between reproductive activity and environmental variables. The analysis was run separately for males and females, considering a significance level of 5%, with R 4.1.1 software, using the MuMIn (Bartoń, 2020), Car (Fox, Weisberg, 2019), and Vegan (Oksanen *et al*., 2020) packages.

RESULTS

Considering the whole sample period, the streams presented an average value of 60.31% of dissolved oxygen, an average Electrical Conductivity of 22.60 $\mu S/cm^{-1}$, pH of 5.60 and temperature of 26.28 °C. Concerning the channel morphology, the streams presented an average wetted width of 2.68 m, thalweg depth of 38.05 cm, and flow speed of 0.12 m/s. The average percentage of structured substrate was 67.62%. The variation of these environmental characteristics in the different sampling months can be seen in the Fig. **S2.**

Of the 180 analyzed specimens, 111 were male and 69 females. Males presented a mean standard length (Ls) of 20.65 mm (varying from 11.63 mm to 26.88 mm), while the females presented a mean standard length of 23.01 mm (varying from 12.68 mm to 32.57 mm). The population showed a difference in the sexual proportion between males and females for the sampled period ($\chi^2 = 8.88$; df = 2; $p < 0.05$), with 1.6 males for each female. In the assessment by month, only January showed a difference in the sexual proportion ($\chi^2 = 4.8$; df = 2; $p < 0.05$), with 2.3 males for each female (Fig. 2).

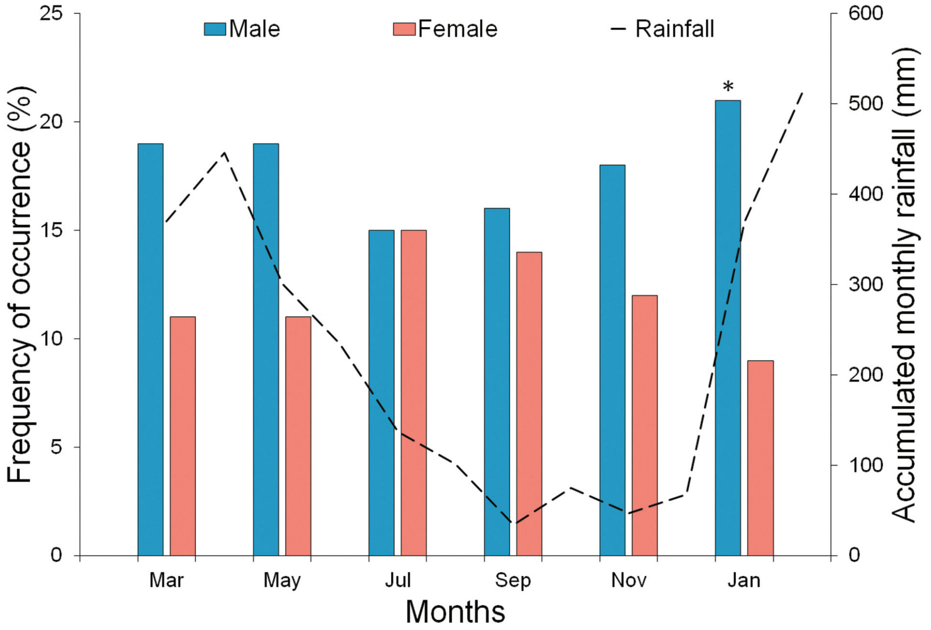

FIGURE 2 | Sex ratio of *Hyphessobrycon heterorhabdus* sampled between March 2019 and January 2020 in the Guamá River basin, Eastern Amazon, State of Pará, Brazil. Asterisk represents significant differences in sex ratio and the dashed line represents the accumulated monthly rainfall.

Gonadal maturation activity, based on Gonadosomatic Index (GSI) values, differed for males ($H_{(5;75)} = 25.913$; $p < 0.05$) and females ($H_{(5;50)} = 21.65$; $p < 0.05$) of *H. heterorhabdus* between the sampled months. The specimens recorded the highest gonadal maturation activity between March and May 2019, with a decrease in gonadal maturation activity from July 2019 on and reaching their lowest average values between September and November 2019. A resumption of gonadal maturation was observed in January 2020. According to the multiple comparison test, for males, the months of the period with the highest intensity of rainfall (March and May) differed from the two months with the lowest intensity of rainfall (September and November). Therefore, differences were also evidenced between January and March (Fig. 3A). For females, the values of GSI of the period with the highest intensity of rainfall (March and May) also differed from the two months with the lowest intensity of rainfall (September and November). Nevertheless, January also differed from September (Fig. 3B).

Variations in the frequency of the stages of gonadal maturation evidenced, for both sexes, a predominance of mature individuals in March 2019 (males = 63.15%; females = 63.63%), a higher frequency of immature between May (males = 35%; females = 30%) and July (males = 37.5%; females = 21.40%) and a higher frequency of maturing individuals in September (males = 43.7%; females = 71.40%). Spent males were recorded throughout the entire period studied, with a lower frequency in March 2019 (5.20%) and a higher frequency in January 2020 (38.00%) (Fig. 3C). Females of *H. heterorhabdus* also showed a higher predominance of gonads in the spawned stage in January (77.7%). Finally, resting females were observed only in September (14.28%) (Fig. 3D).

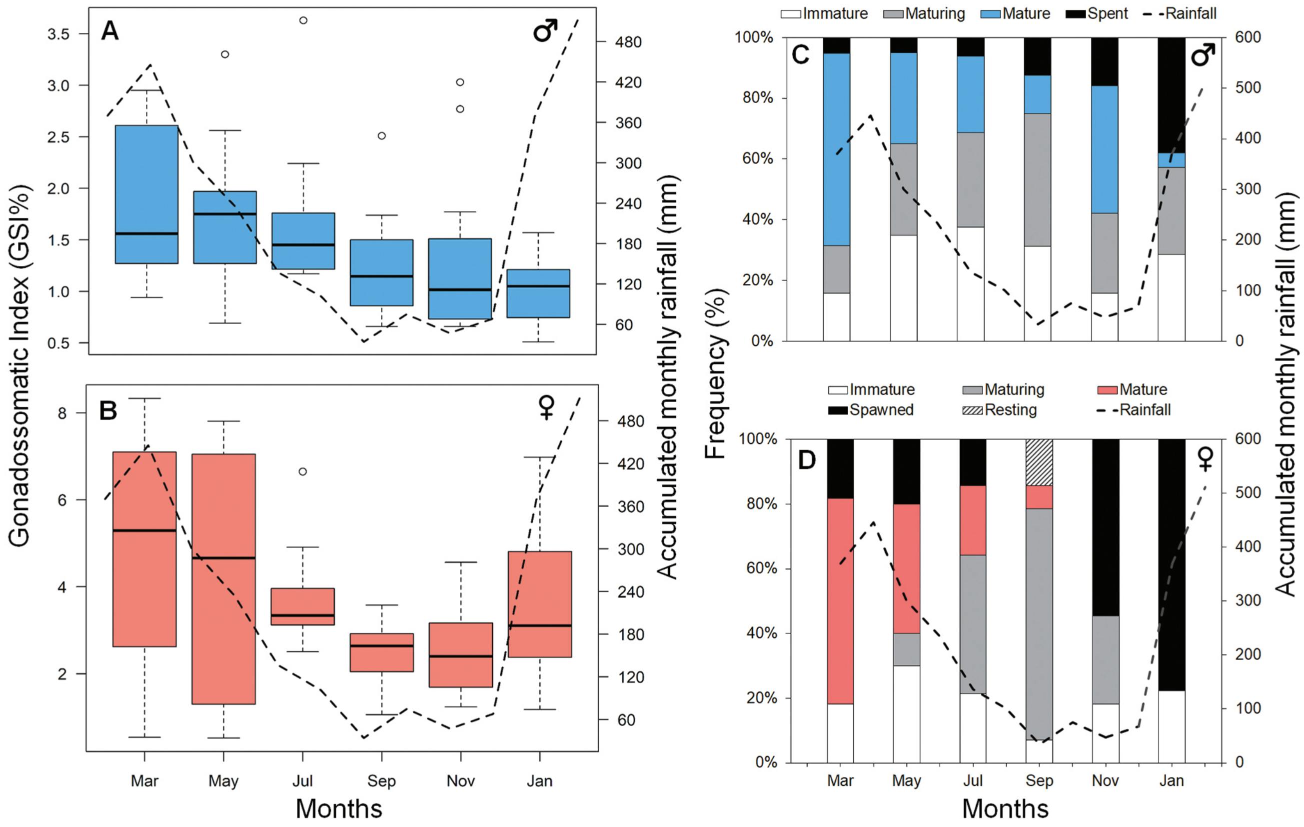

FIGURE 3 | Gonadosomatic Index Variation (GSI) of males (**A**) and females (**B**) and gonadal maturation stage of males (**C**) and females (**D**) of *Hyphessobrycon heterorhabdus* sampled between March 2019 and January 2020 in the Guamá River basin, Eastern Amazon, State of Pará, Brazil. The dashed line represents the accumulated monthly rainfall.

Hyphessobrycon heterorhabdus showed differences in growth patterns between males and females (W = 4723; df = 108; $p < 0.05$). Males showed positive allometric growth in a single phase represented by the model $Wt = 0.0000126 * L_S^{3.12}$ ($R^2 = 0.95$) (Fig. **S3**), while females showed negative allometric growth in two phases (Fig. **S3**), with the first phase represented by model $Wt = 0.0001350 * L_S^{2.29}$ ($R^2 = 0.93$), up to 20.71 mm, and the second phase represented by the model $Wt = 0.0000817 * L_S^{2.58}$ ($R^2 = 0.93$).

The allometric condition factor (K) varied between the months sampled both for males ($H_{(5; 75)} = 14$, $p < 0.05$) and females ($H_{(5; 50)} = 12.482$; $p < 0.05$). In both sexes, an increase in this parameter was observed between July and September, followed by a decrease in November, which was more marked in females, and a new rise in January. According to the multiple comparison test, for males, it was evidenced that the months with the highest rainfall (March and May) differed from the month with the lowest rainfall (September) and the month in which rainfall stars to increase (January). However, differences were also observed between March and July (Fig. 4A). For females, the K values of the period with higher rainfall (March–May) also differed from the month with lower rainfall (September). However, January also differed from March (Fig. 4B).

The lengths where 50% (L_{50}) and 100% (L_{100}) of the population reached sexual maturity for males of *H. heterorhabdus* were 18.0 mm and 22.0 mm, respectively (Fig. 5A). For females, L_{50} was 19.7 mm, which was 1.7 mm higher than that of males, with a L_{100} of 27.0 mm, which was 5 mm higher than that of males (Fig. 5B).

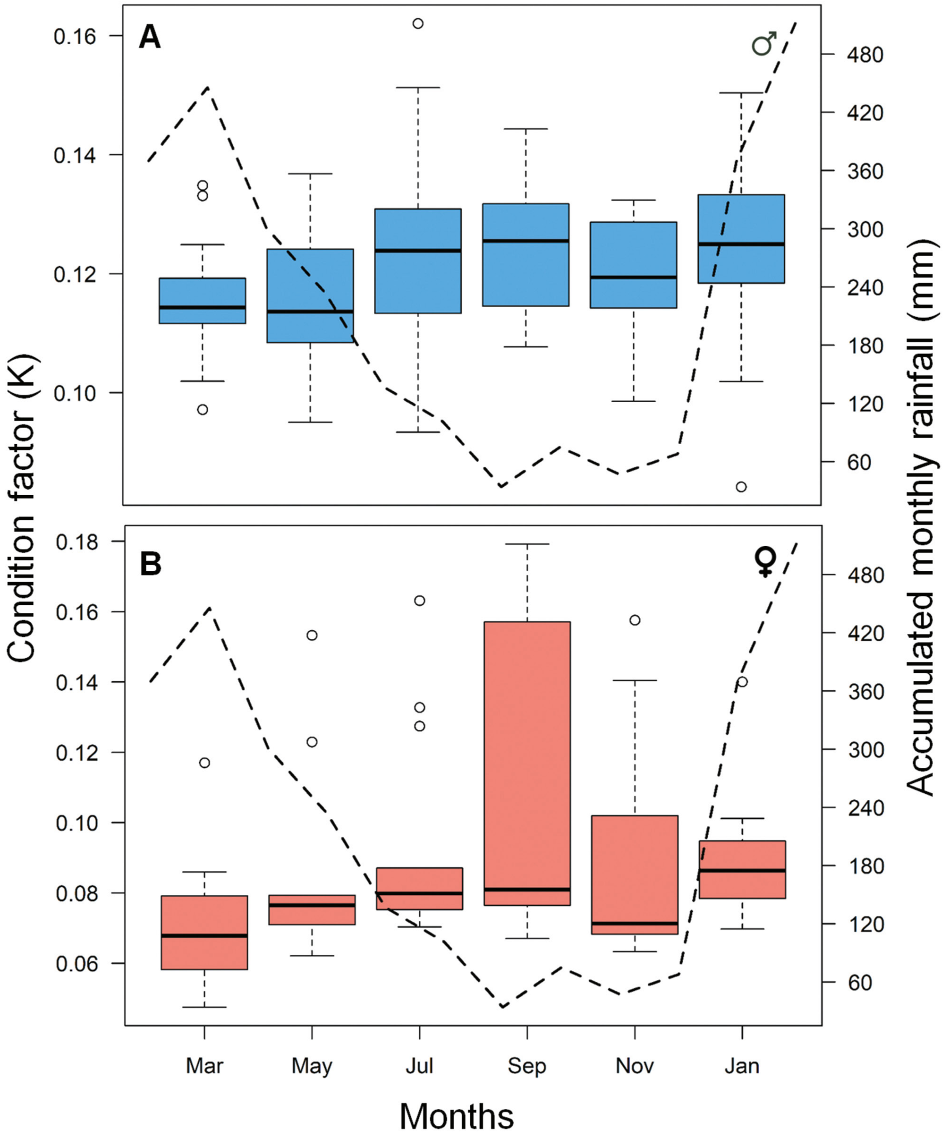

FIGURE 4 | Variation of Condition Factor (K) for males (**A**) and females (**B**) of *Hyphessobrycon heterorhabdus* sampled between March 2019 and January 2020 in the Guamá River basin, Eastern Amazon, State of Pará, Brazil. The dashed line represents the accumulated monthly rainfall.

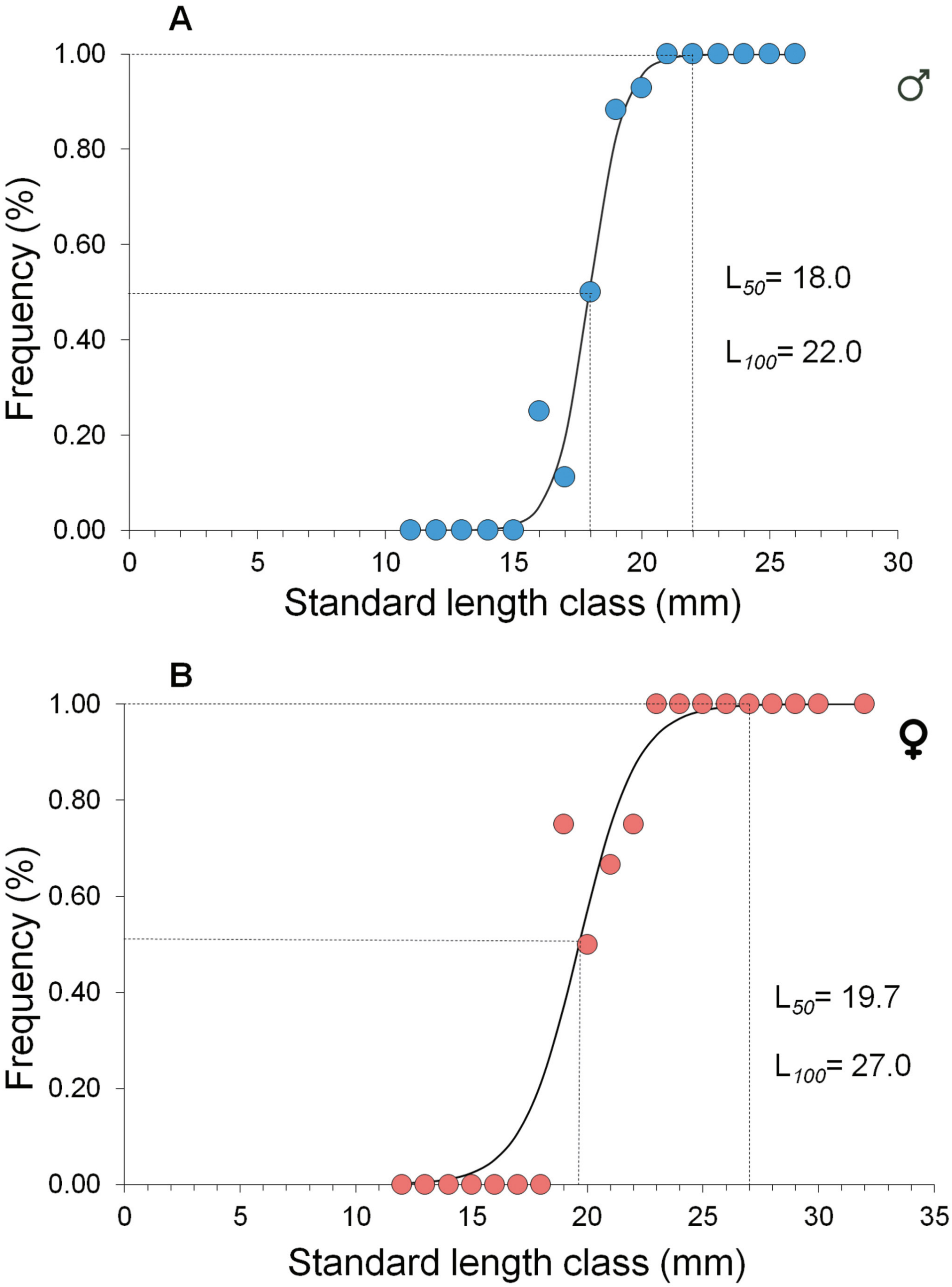

FIGURE 5 | Estimated standard length where 50% (L_{50}) and 100% (L_{100}) of the population reached sexual maturity for males (**A**) and females (**B**) of *Hyphessobrycon heterorhabdus* sampled between March 2019 and January 2020 in streams of the Guamá River basin, Eastern Amazon, State of Pará, Brazil.

The total number of oocytes at different developmental stages in mature females ranged from 176 to 655 (324 ±161; n = 10), with a diameter between 0.120 mm and 0.650 mm. A bimodal distribution of oocyte diameter frequency was detected with at least two batches of oocytes at different stages of development (Fig. 6A). The presence of oocytes at different developmental stages of maturation can also be visualized through macroscopic analysis of the mature ovaries of *H. heterorhabdus* (Fig. 6B). Histological sections revealed oocytes at all stages of maturation, indicating a continuous release of oocytes after reaching sexual maturity, reinforcing the idea of batch spawning in this species. The mean fecundity was 197 vitellogenic oocytes (179 ± 110) with a range between 108 to 420 oocytes, whose diameter varied from 0.242 mm to 0.650 mm.

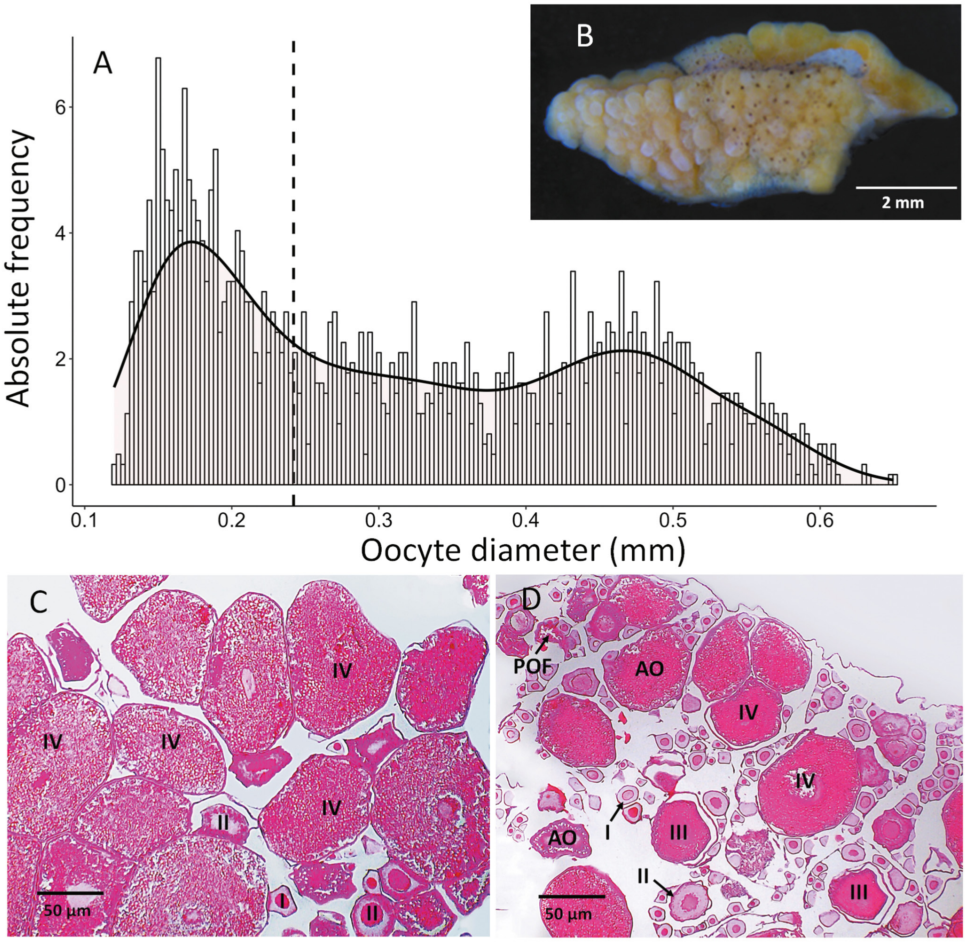

FIGURE 6 | Variation in the oocyte diameter of *Hyphessobrycon heterorhabdus* sampled between March 2019 and January 2020 in streams of the Guamá River basin, Eastern Amazon, State of Pará, Brazil (**A**). The dashed black line indicates the minimum diameter of the vitellogenic oocytes. Mature ovary with oocytes in different stages of maturation (**B**). Photomicrograph of mature (**C**) and spawned (**D**) ovary with oocytes in different stages of maturation: I, stage I oocyte; II, stage II oocyte; III, stage III oocyte; IV, stage IV oocyte; AO, atretic oocyte; POF, post ovulatory follicle.

Considering the global Multiple Linear Regression Model (MLR) (Tab. **S4**), followed by the Akaike Information Criterion (AIC), the variables with the greatest predictive capacity of variation in the GSI of males were water temperature, electrical conductivity, and stream discharge (Tab. **S5**). According to the selected model, these variables explained 10.74% of the variation in the GSI ($p < 0.05$) (Tab. 1). For females, the MLR highlighted the percentage of structured substrate and dissolved oxygen as the variables with the best predictive capacity for GSI variation (Tab. **S6**). According to the selected model, these variables explained 29.47% of the variation in the GSI of females ($p < 0.05$) (Tab. 1).

TABLE 1 | Multiple Linear Regression Model showing the effects of the environmental variables in the Gonadosomatic Index variation (GSI) of male and female *Hyphessobrycon heterorhabdus* sampled between March 2019 and January 2020 in streams of the Guamá River basin, Eastern Amazon, State of Pará, Brazil.

Response Variable	Multiple regression	Environmental variables	*ß*	SE of *ß*	*t*	*p*
Males GSI	R^2=0.1074; $F_{(3,90)}$= 4.73, p<0.01	Water temperature	0.5925	0.1592	3.721	0.00034
		Electrical Conductivity	-0.0820	0.0383	-2.141	0.03495
		Stream Discharge	-3.3458	1.7555	-1.906	0.05985
Females GSI	R^2=0.2947; $F_{(2,60)}$ = 13.95, p<0.01	Structural complexity of the substrate	-0.0561	0.0233	-2.408	0.01915
		Dissolved Oxygen	0.0357	0.0078	4.602	0.00002

DISCUSSION

The flag tetra *Hyphessobrycon heterorhabdus* showed reproductive activity throughout the studied period, with a pronounced activity of gonadal maturation between March and May 2019. The GSI values of females were positively associated with the percentage of structured substrate and mean values of dissolved oxygen. For males, the GSI values were positively associated with water temperature, electrical conductivity and stream discharge. The response to environmental variations was more evident for females than males.

The sex ratio between males and females provides basic information about the reproductive dynamics of a population (Vazzoler, 1996; Wootton, 1999; Vicentini, Araújo, 2003). Although a 1:1 male to female ratio is expected in a natural environment, this can vary throughout the species' life cycle due to factors such as mortality, growth rate, and behavior (Raposo, Gurgel, 2001; Vicentini, Araújo, 2003; Fagundes *et al*., 2020). In the present study, the higher proportion of males in periods of higher reproductive activity indicates the possibility of oocytes from a single female to be fertilized by spermatozoa from more than one male, thus increasing the genetic variability of this population, as suggested by Wootton *et al*. (1978).

Regarding gonadal maturation, *Hyphessobrycon heterorhabdus* showed reproductive activity throughout the sampling cycle, with greater intensity between March and May. According to Azevedo (2010), 20% of small characids have a long reproductive period that can last up to six months, which is strongly associated with an opportunistic strategy (Winemiller, 1989; Winemiller, Rose, 1992). Fish with this strategy are also characterized by having reduced body size, early sexual maturation size, multiple spawning, and low fecundity (Winemiller, 1989) and are common in stream ecosystems (Kramer, 1978). Long reproductive periods also contribute to the increase in population density (Winemiller *et al*., 2008), corroborating the fact that this species is among the most abundant in streams of the Eastern Amazon, even in areas that are affected by anthropogenic activities (Ferreira *et al*., 2018; Prudente *et al*., 2018; Santos *et al*., 2019).

About L_{50}, males showed a smaller size at first sexual maturation than females. This pattern is typical in Neotropical characids, such as *Astyanax scabripinnis* (Jenyns, 1842) (Veregue, Orsi, 2003) and *A. lacustris* (Lütken, 1875) (Súarez *et al*., 2017), and even for other small fish, such as *Characidium lauroi* Travassos, 1949 (Braga, 2006) and *Aspidoras fuscoguttatus* Nijssen & Isbrücker, 1976 (Araujo, Garutti, 2003). This smaller size may be related to the fact that females demand higher energy for gonadal maturation (Pereira-Filho *et al*., 2011) since the energy investment for oocyte maturation seems to be greater than for sperm maturation (Vazzoler, 1996; Wootton, 1999). Therefore, females would require a larger body size (Bromley, 2003). Individuals with larger body sizes also have a greater capacity to absorb oxygen, which is fundamental to biochemical processes involved in gonadal maturation (Pauly, Cheung, 2017).

The growth pattern of *H. heterorhabdus* also appears to reflect the energy demand of reproductive activity and the significant female reproduction investment. Although females maintain a negative allometric growth throughout their development, the increase in the value of the allometric coefficient, after 20.71 mm, is an important indicator decrease in investment in growth when analyzed proportionally to weight. Considering that this change occurs at a length where more than 50% of the females are sexually mature, it is likely that this change is related to a greater investment in reproductive activities, such as gonadal maturation.

The bimodal distribution of oocyte diameters defines the spawning type of *H. heterorhabdus* as group-synchronous, characterized by two populations of oocytes recognized in the ovary throughout the reproductive season (Wallace, Selman, 1981; Lubzens *et al*., 2010). This pattern was evidenced in congeneric species, *Hyphessobrycon santae* (Eigenmann, 1907) (Sales *et al*., 2015) and *Hyphessobrycon anisitsi* (= *Psalidodon anisitsi* (Eigenmann, 1907)) (Gonçalves *et al*., 2013) in reservoirs of the state of Minas Gerais and upper Paraná River basin, respectively, and in *Hyphessobrycon eques* (Steindachner, 1882) (Santana *et al*., 2019) in floodplains of the Brazilian Pantanal and the Paraguay and Amonguijá rivers. On the other hand, the spawning type of *H. heterorhabdus* differs from the congeneric species *Hyphessobrycon bifasciatus* Ellis, 1911 and *H. eques* in a lake of the upper Paraná River basin (Gonçalves *et al*., 2013). Seasonal fluctuation of environmental parameters is considered the primary selective pressure of this biological strategy (Winemiller, 2005). In seasonal environments, fish species tend to present a total spawning, which generally occurs during the high-water periods, when better environmental conditions benefit the offspring's survival (Gonçalves *et al*., 2013). In environments with less seasonal variation, batch spawning would minimize intraspecific competition and enhances the use of the microhabitats, thus contributing to the viability of the offspring (Barbieri, 1992). According to Winemiller (1989), batch spawning is frequently observed in fish with an opportunistic reproductive strategy.

The average fecundity of *H. heterorhabdus* was lower (324 ± 161), considering congeneric species that inhabit reduced seasonal variation environments in the Brazilian Southeast, such as *H. bifasciatus*, which presents an average of 482 (± 221) mature oocytes, and *H. eques*, with an average of 486 (± 159) mature oocytes, as well as those inhabiting environments with greater seasonal variation in the same basin, such as *H. anisitsi* with 931 (± 521) mature oocytes (Gonçalves *et al*., 2013). In contrast, *H. heterorhabdus* showed a higher fecundity than *H. eques* (average = 191.9 mature/vitellogenic; min = 67; max = 624 oocytes) sampled in the floodplains of the Brazilian Pantanal and the Paraguay and Amonguijá rivers (Santana *et al*., 2018). As suggested by Gonçalves *et al*. (2013), environmental conditions can be important predictors of fish fecundity. According to these authors, marked seasonal variations in environmental conditions restrict periods of favorable conditions for reproduction, resulting in lower fecundity in places with more stable environmental conditions. However, according to Wootton (1992) fish fecundity can also be affected by body size, where in species with a larger body size, females have a larger coelomic cavity, being able to house a greater number of mature oocytes.

The batch spawning of *H. heterorhabdus* reinforces the idea of low seasonal fluctuation of the environmental condition of the Amazonian streams concerning Brazilian subtropical streams, such as savanna and Atlantic Forest streams (Veregue, Orsi, 2003; Gonçalves *et al*., 2013) and large amazon rivers with a marked flood pulse regime (Junk *et al*., 1989). However, this reproductive tactic can also be related to the habitat use by *H. heterorhabdus*, which inhabits the water column in backwater areas close to the banks and occupies microhabitats in the stream during high and low rainfall. The batch spawning also matches with the low fecundity recorded in *H. heterorhabdus*, which does not require a high production of oocytes to be released in a short time when environmental conditions are more favorable.

The predictive effect of environmental variables in the reproductive activity of *H. heterorhabdus* was more evident for females (29.47% of explanation; $p < 0.05$) than for males (10.74%; $p < 0.05$), which may be associated with the fact that oocyte production demands more energy (Pereira-Filho *et al*., 2011) than the production and maturation of spermatozoa (Vazzoler, 1996; Wootton, 1999) (Tab. 1). The elevated GSI values observed in male individuals can be partially attributed to the augmentation of mean water temperature, thereby reinforcing the notion of its significance in the reproductive biology of fish (Larsen *et al*., 2011). High water temperatures allow an increase in the metabolic rates of fish, affecting their reproductive performance (Vagner *et al*., 2019). The relationship between fish reproductive activity and temperature is frequently reported for fish from temperate regions (Migaud *et al*., 2010), while for tropical stream fish, mainly from the Amazon, reproductive activity is often associated with the local rainfall regime (Carvalho *et al*., 2021; López-Rodríguez *et al*., 2021). However, experimental evidence has demonstrated that increased water temperature leads to a reduction in the duration of spermatogenesis and an increase in cell proliferation in Neotropical characids, resulting in heightened GSI values in these fish (Quirino *et al*., 2021).

The increase in the reproductive activity of females, expressed by the increase in the GSI values, coincided with the increase in the average values of dissolved oxygen, corroborating the idea that this physicochemical parameter of the water is an essential factor for the reproduction of tropical fish (Pauly, Cheung, 2017). The positive relationship between the GSI and the percentage of structured substrate may indicate the importance of transporting organic matter from adjacent regions to the stream bed. The cumulative organic matter allows for the formation of favorable environments for spawning, shelter, and feeding of juvenile fish (Kramer, 1978; Casatti, 2005), which are essential to supply the energy invested in reproduction (Castillo-Rivera *et al*., 1994). This process would be essential since *H. heterorhabdus* has a diet predominantly composed of items of allochthonous origin (Benone *et al*., 2020). However, studies focused on the relationship between fish feeding and reproduction are scarce (Ballesteros *et al*., 2009) and non-existent for stream ecosystems.

In this sense, we believe that females may have a greater ability to adjust their physiological conditions to the temporal variation of the stream's environmental conditions. On the other hand, we think that gonadal maturation in males would be less affected by the measured environmental variables because the time for gonadal maturation is shorter and has a lower energetic cost than in females. Another possibility is that the gonadal maturation of males is also influenced by behavioral or even hormonal stimuli resulting from female gonadal maturation. However, behavioral and physiological studies involving these issues are still insufficient (Nakatsuru, Kramer, 1982; Bhat *et al*., 2021).

The results obtained in the present study demonstrate that *H. heterorhabdus* presents a reproductive activity synchronized with environmental conditions related to the rainfall regime. However, a portion of the reproductive activity responds to other factors not yet clarified in this study, which may be better evaluated in experimental studies focused on triggers of gonadal maturation and spawning in this species. Even so, reduced body size and multiple spawning in an extended reproductive activity period tend to fit the species in an opportunistic strategy according to the classification proposed by

Winemiller (1989) and Winemiller, Rose (1992). The presence of *H. heterorhabdus* in the list of species authorized by the Brazilian government to be exploited for ornamental or aquarium purposes (Interministerial Regulatory Instruction nº 01/2012 of extinct Ministry of Fisheries and Aquaculture of Brazil) makes the information herein compiled of paramount importance for elaborating strategies aimed at conservation and sustainable exploitation practices, such as period and minimal size of capture.

REFERENCES

- **Araujo RB, Garutti V.** Biologia reprodutiva de *Aspidoras fuscoguttatus* (Siluriformes, Callichthyidae) em riacho de cabeceira da bacia do Alto Rio Paraná. Iheringia, Sér Zool. 2002; 92(4):89–98. https://doi.org/10.1590/S0073-47212002000400011
- **Azevedo MA.** Reproductive characteristics of characid fish species (Teleostei, Characiformes) and their relationship with body size and phylogeny. Iheringia, Sér Zool. 2010; 100(4):469–82. https://doi.org/10.1590/S0073-47212010000400020
- **Ballesteros TM, Torres-Mejia M, Ramírez-Pinilla MP.** How does diet influence the reproductive seasonality of tropical freshwater fish? A case study of a characin in a tropical mountain river. Neotrop Ichthyol. 2009; 7(4):693–700. https://doi.org/10.1590/S1679-62252009000400019
- **Barbieri G.** Biologia de *Astyanax scabripinnis* paranae (Characiformes, Characidae) do Ribeirão do Fazzari. São Carlos. Estado de São Paulo. II. Aspectos quantitativos da reprodução. Rev Bras Biol. 1992; 52(4):589–96.
- **Benone NL, Ligeiro R, Juen L, Montag LFA.** Role of environmental and spatial processes structuring fish assemblages in streams of the eastern Amazon. Mar Freshwater Res. 2017; 69(2):243–52. https://doi.org/10.1071/MF17103
- **Benone NL, Lobato CMC, Soares BE, Montag LFA.** Spatial and temporal variation of the diet of the flag tetra *Hyphessobrycon heterorhabdus* (Characiformes: Characidae) in streams of the Eastern Amazon. Neotrop Ichthyol. 2020; 18(4):e200078. https://doi.org/10.1590/1982-0224-2020-0078
- **Bervian G, Fontoura NF, Haimovici M.** Statistical model of variable allometric growth: otolith growth in *Micropogonias furnieri* (Actinopterygii, Sciaenidae). J Fish Biol. 2006; 68(1):196–208. https://doi.org/10.1111/j.0022-1112.2006.00890.x
- **Braga FMS.** Aspectos da reprodução no gênero *Characidium* Reinhardt, 1867 (Crenuchidae, Characidiinae), na microbacia do Ribeirão Grande, serra da Mantiqueira, sudeste do Brasil. Acta Sci Biol Sci. 2006; 28(4):365–71. http://dx.doi.org/10.4025/actascibiolsci.v28i4.170
- **Braga FMS.** Estudo entre fator de condição e relação peso/comprimento para alguns peixes marinhos. Rev Bras Biol. 1986; 46(2):339–46.
- **Brejão GL, Gerhard P, Zuanon J.** Functional trophic composition of the ichthyofauna of forest streams in eastern Brazilian Amazon. Neotrop Ichthyol. 2013; 11(2):361–73. https://doi.org/10.1590/S1679-62252013005000006
- **Bromley PJ.** The use of market sampling to generate maturity ogives and to investigate growth, sexual dimorphism and reproductive strategy in central and south-western North Sea sole (*Solea solea* L.). Ices Mar Sc. 2003; 60(1):52–65. https://doi.org/10.1006/jmsc.2002.1318
- **Bartoń K.** MuMIn: Multi-Model Inference. R package version 1.43.17. 2020. Available from: https://CRAN.R-project.org/package=MuMIn
- **Bhat IA, Dar JY, Ahmad I, Mir IN, Bhat H, Bhat RAH *et al*.** Testicular development and spermatogenesis in fish: insights into molecular aspects and regulation of gene expression by different exogenous factors. Rev Aquac. 13(4):2142–68. https://doi.org/10.1111/raq.12563
- **Casatti L.** Fish assemblage structure in a first order stream, southeastern Brazil: Longitudinal distribution, seasonality, and microhabitat diversity. Biota Neotrop. 2005; 5(1):1–09. https://doi.org/10.1590/S1676-06032005000100009

- **Carvalho IFS, Cantanhêde LG, Diniz ALC, Carvalho-Neta RNF, Almeida ZS.** Reproductive biology of seven fish species of commercial interest at the Ramsar site in the Baixada Maranhense, Legal Amazon, Brazil. Neotrop Ichthyol. 2021; 19(2):e200067. https://doi.org/10.1590/1982-0224-2020-0067
- **Castillo-Rivera M, Moreno G, Iniestra R.** Spatial, seasonal, and diel variation in abundance of the bay anchovy, *Anchoa mitchilli* (Teleostei: Engraulidae), in a tropical coastal lagoon of Mexico. Southwest Nat. 1994; 39(3):263–68. https://doi.org/10.2307/3671591
- **Chapman FA, Colle DE, Rottmann RW, Shireman JV.** Controlled spawning of the neon tetra. Progr Fish Cult. 1998; 60(1):32–37. https://doi.org/10.1577/1548-8640(1998)060%3C0032:CSOTNT%3E2.0.CO;2
- **Espírito-Santo HMV, Rodriguez MA, Zuanon J.** Reproductive strategies of Amazonian stream fishes and their fine-scale use of habitat are ordered along a hydrological gradient. Freshwater Biol. 2013; 58(12):2494–504. https://doi.org/10.1111/fwb.12225
- **Fagundes PC, Dala-Corte RB, Azevedo MA.** Life history traits of a small-sized characid fish (*Diapoma alburnum*) in a subtropical river of Brazil. Pan-Am J Aquat Sci. 2020; 15(1):39–48. Available from: https://panamjas.org/pdf_artigos/PANAMJAS_15(1)_39-48.pdf
- **Faria TC.** Sistemática do grupo *Hyphessobrycon heterorhabdus* (Ulrey) (Characiformes, Characidae). [Master Dissertation]. São Paulo: Universidade Estadual de Campinas; 2020. Available from: https://hdl.handle.net/20.500.12733/3395
- **Ferreira MC, Begot TO, Prudente BS, Juen L, Montag LFA.** Effects of oil palm plantations on habitat structure and fish assemblages in Amazon stream. Environ Biol Fish. 2018; 101(4):547–62. https://doi.org/10.1007/s10641-018-0716-4
- **Fox J, Weisberg S.** An {R} companion to applied regression. Third edition. Thousand Oaks CA: Sage. 2019. Available from: https://socialsciences.mcmaster.ca/jfox/Books/Companion
- **Froese R.** Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. J Appl Ichthyol. 2006; 22(4):241–53. https://doi.org/10.1111/j.1439-0426.2006.00805.x
- **Gonçalves CS, Souza UP, Ferreira FC, Peressin A, Braga FMS.** Life-history strategies associated to reproduction of three *Hyphessobrycon* species (Characidae) in lentic environments of upper Paraná River basin. Acta Limnol Bras. 2013; 25(4):398–405. https://doi.org/10.1590/S2179-975X2013000400005
- **Helfman G, Collette BB, Facey DE, Bowen BW.** The diversity of fishes: biology, evolution, and ecology. John Wiley & Sons; 2009.
- **Hortal J, Bello F, Diniz-Filho JAF, Lewinsohn TM, Lobo JM, Ladle RJ.** Seven shortfalls that beset large-scale knowledge of biodiversity. Annu Rev Ecol Evol S. 2015; 46(1):523–49. https://doi.org/10.1146/annurev-ecolsys-112414-054400
- **Huxley JS.** Constant differential growth-ratios and their significance. Nature. 1924; 114(2877):895–96. https://doi.org/10.1038/114895a0
- **Instituto Brasileiro de Geografia e Estatística (IBGE).** Manual técnico da vegetação brasileira. [Internet]. Rio de Janeiro; 1992. Available from: https://biblioteca.ibge.gov.br/visualizacao/livros/liv23267.pdf
- **Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA).** Instrução Normativa Interministerial. Peixes de captura para fins ornamentais. [Internet]; 2012. Available from: https://www.mprs.mp.br/media/areas/gapp/arquivos/atualizacao_intra/dou/in3_2012.pdf
- **Instituto Nacional de Meteorologia (INMET).** 2021. Available from: http://www.inmet.gov.br/portal/index.php?r=estacoes/estacoesAutomaticas
- **Järvi TH.** Die kleine Maräne (*Coregonus albula* L.) im Keitelesee, eine ökologische und ökonomische studie. Helsinki: Suomalainen Tiedeakatemia; 1920.
- **Junk WJ, Bayley PB, Sparks RE.** The flood pulse concept in river-floodplain systems. In: Dodge DP, editor. Proceedings of the International Large River Symposium (LARS). Can Spec Publ Fish Aquat Sci. 1989; 106:110–27. Available from: https://www.researchgate.net/publication/291143833
- **Kramer DL.** Reproductive seasonality in the fishes of a tropical stream. Ecology. 1978; 59(5):976–85. https://doi.org/10.2307/1938549

- **Larsen SV, Imsland AK, Lohne P, Pittman K, Foss A.** Stepwise temperature regulation and its effect on growth, feeding and muscle growth patterns of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquac Int. 2011; 19:825–37. https://doi.org/10.1007/s10499-010-9402-z
- **López-Rodríguez NC, Leão AH, Rocha RM, Prudente BS, Montag LFA.** Environmental influence on the reproductive strategy of *Helogenes marmoratus* (Siluriformes: Cetopsidae) in the Amazonian streams. Neotrop Ichthyol. 2021; 19(4):e210092. https://doi.org/10.1590/1982-0224-2021-0092
- **Lubzens E, Young G, Bobe J, Cerdà J.** Oogenesis in teleosts: how fish eggs are formed. Gen Comp Endocrinol. 2010; 165(3):367–89. https://doi.org/10.1016/j.ygcen.2009.05.022
- **Migaud H, Davie A, Taylor JF.** Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. J Fish Biol. 2010; 76(1):27–68. https://doi.org/10.1111/j.1095-8649.2009.02500.x
- **Monaco IA, Súarez YR, Lima-Junior SE.** Influence of environmental integrity on feeding, condition and reproduction of *Phalloceros harpagos* Lucinda, 2008 in the Tarumã stream micro basin. Acta Sci Biol Sci. 2014; 36(2):181–88. https://doi.org/10.4025/actascibiolsci.v36i2.21394
- **Nakatsuru KEN, Kramer DL.** Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). Science. 1982; 216(4547):753–55. https://doi.org/10.1126/science.216.4547.753
- **Nikolsky GV.** Theory of fish population dynamics. Edinburgh: Oliver e Boyd; 1969.
- **Núñez J, Duponchelle F.** Towards a universal scale to assess sexual maturation and related life history traits in oviparous teleost fishes. Fish Physiol Biochem. 2009; 35:167–80. https://doi.org/10.1007/s10695-008-9241-2
- **Oberdorff T, Dias MS, Jézéquel C, Albert JS, Arantes CC, Bigorne R *et al*.** Unexpected fish diversity gradients in the Amazon basin. Sci Adv. 2019; 5(9):eaav8681. https://doi.org/10.1126/sciadv.aav8681
- **Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D *et al*.** Vegan: Community ecology package. R package version 2.5-7. 2020. https://CRAN.Rproject.org/package=vegan
- **Pacheco NA, Bastos TX.** Caracterização climática do município de Capitão Poço-PA. Documentos, 79. Belém: Embrapa Amazônia Oriental, 2001. Available from: https://www.infoteca.cnptia.embrapa.br/bitstream/doc/403420/1/OrientalDoc79.pdf
- **Pauly D, Cheung WWL.** Sound physiological knowledge and principles in modeling shrinking of fishes under climate change. Glob Chang Biol. 2017; 24(1):15–26. https://doi.org/10.1111/gcb.13831
- **Peel MC, Finlayson BL, Mcmahon TA.** Updated world map of the Köppen-Geiger climate classification. Hydrol Earth Syst Sci. 2007; 4(2):439–73. https://doi.org/10.5194/hess-11-1633-2007
- **Pereira-Filho HP, Andrade DR, Tonini WCT, Vidal Jr. MV.** Biologia reprodutiva de fêmeas de lambari-prata, *Astyanax scabripinnis* Jenyns, 1842 (Characidae; Tetragonopterinae; Teleostei) em condições de cativeiro. Ci Anim Bras. 2011; 12(4):626–34. Available from: https://revistas.ufg.br/vet/article/view/2202/10027
- **Prophet EB, Milis B, Arrington JB, Sobin LH.** Métodos histotecnológicos. Washington, DC: Instituto de Patología de las Fuerzas Armadas de los Estados Unidos de América (AFIP); 1995.
- **Prudente BS, Pompeu PS, Montag LFA.** Using multimetric indices to assess the effect of reduced impact logging on ecological integrity of Amazonian streams. Ecol Indic. 2018; 91:315–23. https://doi.org/10.1016/j.ecolind.2018.04.020
- **Quirino PP, Rodrigues MS, Cabral EMS, Siqueira-Silva DH, Mori RH, Butzge AJ *et al*.** The influence of increased water temperature on the duration of spermatogenesis in a neotropical fish, *Astyanax altiparanae* (Characiformes, Characidae). Fish Physiol Biochem. 2021; 47(3):747–55. https://doi.org/10.1007/s10695-020-00869-7
- **Raposo RMG, Gurgel HCB.** Estrutura populacional de *Serrasalmus spilopleura* Kner, 1860 (Pisces, Serrasalmidae) da lagoa de Extremoz, Estado do Rio Grande do Norte, Brasil. Acta Sci Biol Sci. 2001; 23(2):409–14.
- **Ribeiro FAS, Carvalho Junior JR, Fernandes JBK, Nakayama L.** Comércio brasileiro de peixes ornamentais. Panor Aquic. 2008; 18(110):54–59.

- **Santana CA, Tondato KK, Súarez YR.** Reproductive biology of *Hyphessobrycon eques* (Characiformes: Characidae) in Southern Pantanal, Brazil. Braz J Biol. 2019; 79(1):70–79. https://doi.org/10.1590/1519-6984.176273
- **Santos LL, Benone NL, Soares BE, Barthem RB, Montag LFA.** Trait–environment relationships in Amazon stream fish assemblages. Ecol Freshw Fish. 2019; 28(3):424–33. https://doi.org/10.1111/eff.12465
- **Sales ML, Braga EPR, Magalhães ALB, Maia BP, Ratton TF.** Biologia reprodutiva do lambari *Hyphessobrycon santae* (Eigenmann, 1907) (Pisces: Characidae) no Parque Urbano Fazenda Lagoa do Nado, bacia do rio São Francisco. Rev Bras Zool. 2015; 16(1–3):55–65. https://periodicos.ufjf.br/index.php/zoociencias/article/view/24596
- **Silva BNR, Silva LGT, Rocha AMA, Sampaio SMN.** Interação biofísica e do uso da terra na dinâmica da paisagem do município de Capitão Poço-PA, em sistema de informação geográfica. Documentos, 10. Belém: Embrapa Amazônia Oriental, 1999. Available from: https://www.infoteca.cnptia.embrapa.br/bitstream/doc/377456/1/OrientalDoc10.pdf
- **Sioli H.** The Amazon and its main affluents: Hydrography, morphology of the river courses, and river types. In: Sioli H, editor. The Amazon: limnology and landscape ecology of a mighty Tropical River and its basin. Dordrech: Dr. W. Junk Publishers; 1984. p.127–65. https://doi.org/10.1007/978-94-009-6542-3_5
- **Souto CN, Faria TM, Oliveira HF, Rosa RM, Silva LAS, Campos MAF.** Visão geral sobrereprodução de peixes teleósteos: da anatomia à sinalização molecular. PUBVET. 2017; 11(11):175-87. https://doi.org/10.22256/PUBVET.V11N11.1175-1187
- **Strahler AN.** Quantitative analysis of watershed geomorphology. Eos Trans AGU. 1957; 38(6):913–20. https://doi.org/10.1029/TR038i006p00913
- **Súarez YR, Silva EA, Viana LF.** Reproductive biology of *Astyanax lacustris* (Characiformes: Characidae) in the southern Pantanal floodplain, upper Paraguay River basin, Brazil. Environ Biol Fish. 2017; 100(7):775–83. https://doi.org/10.1007/s10641-017-0604-3
- **Tomasella J, Hodnett MG, Cuartas LA, Nobre AD, Waterloo MJ, Oliveira SM.** The water balance of an Amazonian micro-catchment: the effect of interannual variability of rainfall on hydrological behaviour. Hydrol Process. 2008; 22(13):2133–47. https://doi.org/10.1002/hyp.6813
- **Vagner M, Zambonino-Infante J-L, Mazurais D.** Fish facing global change: are early stages the lifeline? Mar environ research. 2019; 147:159–78. https://doi.org/10.1016/j.marenvres.2019.04.005
- **Vazzoler AEAM.** Biologia da reprodução de peixes teleósteos: teoria e prática. Maringá: EDUEM; 1996.
- **Veregue AML, Orsi ML.** Biologia reprodutiva de *Astyanax scabripinnis* (Elgenmann) (Ostheichthyes, Characidae), do ribeirão das Marrecas, bacia do rio Tibagi. Rev Bras Zool. 2003; 20(1):97–105. https://doi.org/10.1590/S0101-81752003000100012
- **Vicentini RN, Araújo FG.** Sex ratio and size structure of *Micropogonias furnieri* (Desmarest, 1823) (Perciformes, Sciaenidae) in Sepetiba Bay, Rio de Janeiro, Brazil. Braz J Biol. 2003; 63(4):559–66. https://doi.org/10.1590/S1519-69842003000400003
- **Wallace RA, Selman K.** Cellular and dynamic aspects of oocyte growth in teleosts. Am Zool. 1981; 21(2):325–43. https://doi.org/10.1093/icb/21.2.325
- **Winemiller KO.** Patterns of variation in life history among South American fishes in seasonal environments. Oecologia. 1989; 81(2):255–41. https://doi.org/10.1007/BF00379810
- **Winemiller KO.** Life history strategies, population regulation, and implications for fisheries management. Can J Fish Aquat Sci. 2005; 62(4):872–85. https://doi.org/10.1139/f05-040
- **Winemiller KO, Agostinho AA, Caramaschi EP.** Fish ecology in tropical streams. In: Tropical stream ecology. Academic Press, 2008. p.107–46. https://doi.org/10.1016/B978-012088449-0.50007-8
- **Winemiller KO, Rose KA.** Patterns of life-history diversification in North American fishes: implications for population regulation. Can J Fish Aquat Sci. 1992; 49(10):2196–218. https://doi.org/10.1139/f92-242

- **Wootton RJ.** Fish ecology. New York: Chapman and Hall; 1992.
- **Wootton RJ.** Ecology of teleost fishes. Dordrecht: Kluwer Academic Publishers; 1999.
- **Wootton RJ, Evans GW, Mills L.** Annual cycle in female three-spined sticklebacks (*Gasterosteus aculeatus* L.) from an upland and lowland population. J Fish Biol. 1978; 12(4):331–43. https://doi.org/10.1111/j.1095-8649.1978.tb04178.x
- **Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM.** Things are not always linear; additive modelling. In: Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM, editors. Mixed effects models and extensions in ecology with R. New York: Springer; 2009. p.36–67.

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The author declares no competing interests.

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