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Research Article

Reproductive Aspects of *Moenkhausia intermedia* Eigenmann, 1908 (Pisces, Characidae) in the Upper Paraná River Basin, Brazil

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The aim of this work was to determine the reproductive tactic of the population of *Moenkhausia intermedia*, inhabiting the Capivara Reservoir, lower Paranapanema River. Four different stretches of the reservoir were selected, and sampling was carried out quarterly, in the period from 2001 to 2004. Fish were captured utilizing gill nets, cast nets, and seine nets. Later, fish were identified and measured and had their sex identified. Here we describe sex proportion, standard length at first maturation (L_{50}), site and period of spawning, fecundity and type of spawning. The population showed an effective reproductive activity in the area, with the number of females higher than males and L_{50} corresponding to 6,1 cm and 5,5 cm, respectively. A greater intensity of spawning occurred in the warmest months. Reproductive activity was identified in all stretches studied, and the absolute fecundity average was 9611 oocytes per gonad, and the mean relative fecundity corresponded to 1065 oocytes/grams.

1. Introduction

Continuous anthropogenic activity alters natural environments and cause substantial modifications in their biota. Among them is the hydroelectric utilization of the rivers, which significantly alters the natural characteristics of the fluvial regime due to the construction of power plants and damming [1]. According to Agostinho and Gomes [2] and Agostinho et al. [3], reservoirs are sequence of impacted environments, which cause drastic changes in the hydrological regime, alterations in the composition, and diminution of species richness. In addition, Luiz et al. [4] report that the fish fauna inhabiting reservoirs is subjected to impacts from various anthropogenic activities, at local and regional scales, such as extensive ranching, agriculture using chemical products, poor soil conservation practices (causing siltation), deforestation, introduction of nonnative species, and disorderly fishing. All these are reported for the Paranapanema river basin, where 10 hydroelectric dams were installed along its main channel [5].

The small fish *Moenkhausia intermedia* Eigenmann, 1908 is widely distributed in the basin of the upper Rio Parana and is known commonly as piqui, pequi, or viuvinha [6], but it is given another popular designation in the region of the Rio Paranapanema (lambari corintiano). These authors believe that as it is a small-sized species, it is an important component of the food chain, especially for the other species of fish, birds and other carnivores that coexist with this population in the environment. According to Shibatta et al. [7], it has a silvery coloration with dark spots at the end of the caudal fin, prefers calm waters and lives close to the surface. Hoffman et al. [5] and Orsi and Sodré [8] consider *M. intermedia* a constant species in the Capivara Reservoir, and Bennemann et al. [9] describe the importance of the species for the ecology in question.

The aim of this work is to describe the reproductive tactics involved in its survival and, thereby, broaden our knowledge of the biology of the group to support future management plans in the reservoir. Despite the reservoir being considered old (1974) and theoretically closer to being stable, it still shows constant anthropic impacts which make it somewhat unstable from an environmental and biological point of view with constant introduction of nonnative

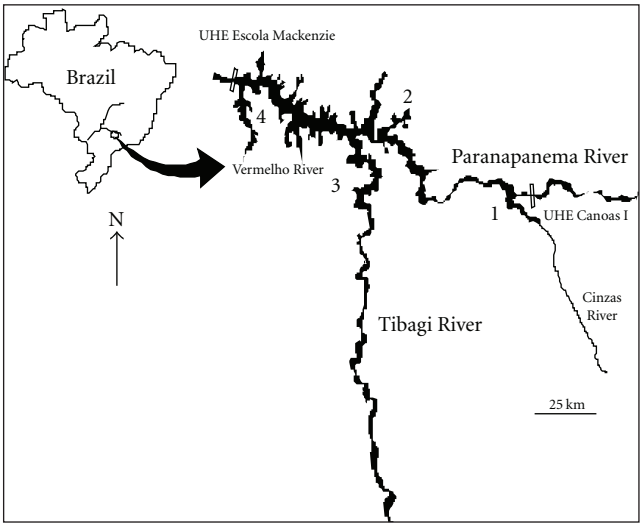

Figure 1: Localization of the sampling stretches in the Capivara Reservoir, Rio Paranapanema (Scale 1 : 15000), with numbers indicating, from right to left, the four stretches, located between downstream of the UHE Canoas I and the dam of UHE Escola Mackenzie: 1 = Cinzas; 2 = Cruzália; 3 = Sertanópolis; 4 = Porecatu. UHE, hydroelectric power plant.

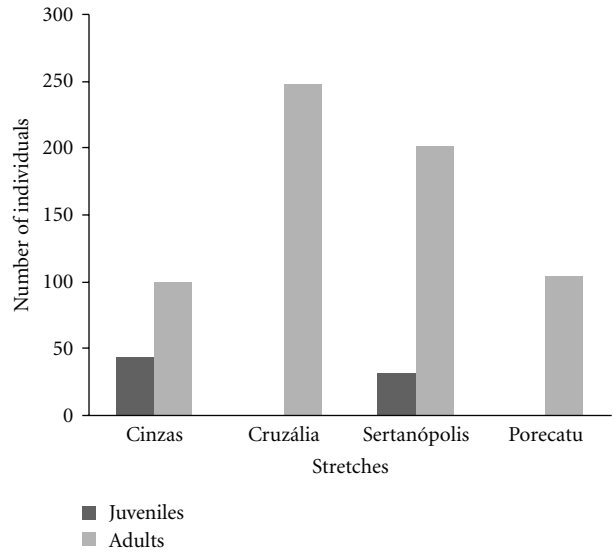

Figure 2: Distribution of the number of juveniles and adults captured in the 4 stretches sampled.

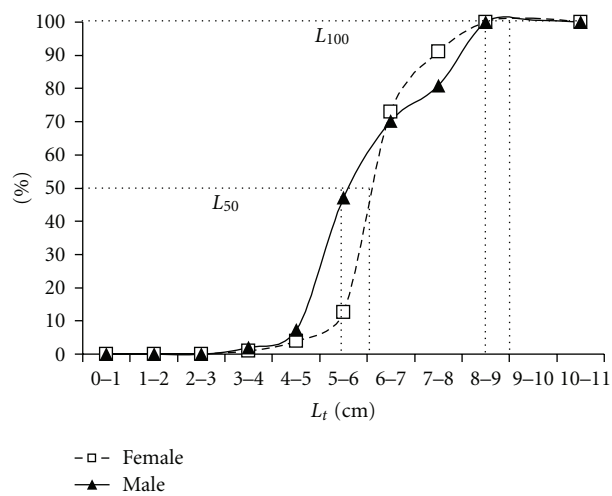

Figure 3: Female and Male maturation curve obtained through standard length with indication if the L_{50} and L_{100} for both sexes.

species such as *Cichla monoculus* (Spix and Agassiz 1831) and *Plagioscion squamosissimus* (Heckel, 1840).

The study was conducted in the portion of the lower Paranapanema River, relative to the dam Escola Mackenzie Power Plant (Capivara Dam). Over all its area, it shows a distinct gradient of preservation of its banks and water flow (lotic to semilotic).

In this study, four stretches were selected, according to their fluvial conditions of upstream to downstream, including different environmental characteristics. These sampling points were called Cinzas 1 (fluvial stretch), Cruzália 2 (lacustrine stretch), Sertanópolis 3 (transition stretch), and Porecatu 4 (lacustrine stretch, close to dam). These sampling locations are shown in Figure 1.

2. Material and Methods

2.1. Samplings. Studies of the fish community in reservoirs are important for evaluating the impacts of these impoundments and proposing measures to reduce or impede their construction through alternative forms of energy production. According to Garutti [10], the most important aspects connected with the knowledge of the ichthyofauna are in regard to reproductive biology, especially the form of reproduction, the reproductive period, and the sites of spawning, constituting basic support in the preservation of species.

The samplings were carried out from March 2001 to July 2004, where all the seasons of the year were included. As noted by Orsi et al. [11] and Orsi and Sodré [8], there are fewer males and sexual dimorphism is distinct in this species. Thus, in order to reduce the selectivity of capture, fish were captured with the sequence of pairs of nets for each mesh size (1,0–6,0 cm between opposite knots), with a total of 1827 m^2 covering deeper areas and the banks of the reservoir (Figure 1). The fish were caught with the sequence of pairs of nets for each mesh size (1,0–6,0 cm between opposite knots), with a total of 1827 m^2, in place for total of 24 hours with two revisions of the net in this period. Others gears, such as drag nets, cast nets, and seine nets covering 85 m^2 were used for two hours in an area of approximately 200 m^2, in a standardized fashion to complement the sampling. After capture, the fish were fixed in 10% formalin and transported to the laboratory to record biological data.

2.2. Methodology of Study. In the laboratory, after taxonomic identification, biometric parameters were obtained: animals sexed, gonads weighed and the gonadal development stage characterized. Mature gonads were removed and stored

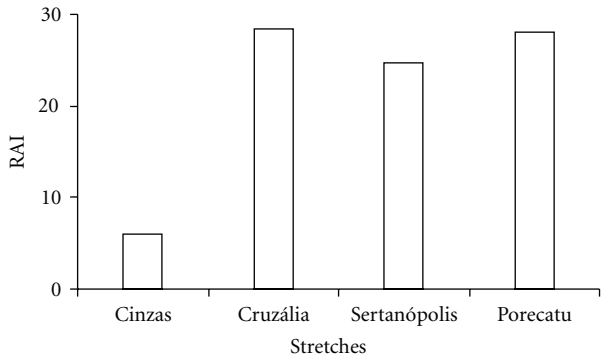

Figure 4: Comparison of the mean reproductive activity per sampling stretch in Capivara Reservoir, based on reproductive activity index (RAI).

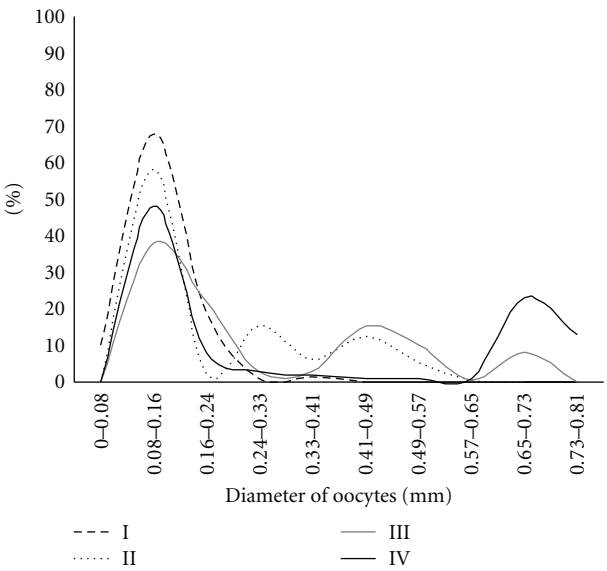

Figure 5: Oocytes distribution by diameter classification in relation to stage of oocytes development (I, II, III and IV).

separately for future microscopic analysis with regard to reproductive dynamics.

The gonads that were removed during sampling activity were preserved in 4% formalin and after in Bouin solution to further utilization of haematoxylin-eosine (HE) technique, and subsequently, their fractions were conserved in 70% alcohol and soaked in paraffin [12].

The sex ratio was determined by the relative frequency of males and females collected in each stretch sampled and by temporal variation, taking into consideration the seasons of the year.

The L_{50} is the mean standard length of the first gonad maturation at which 50% of the individuals are adult, in other words, individuals of the population that have begun the reproductive cycle [12, 13]. The specimens were classified into young, showing immature gonads, and adult, showing any development in the gonads, were separated by sex, and were grouped in classes of 1,0 cm total length. The values of the relative frequency of classes were represented

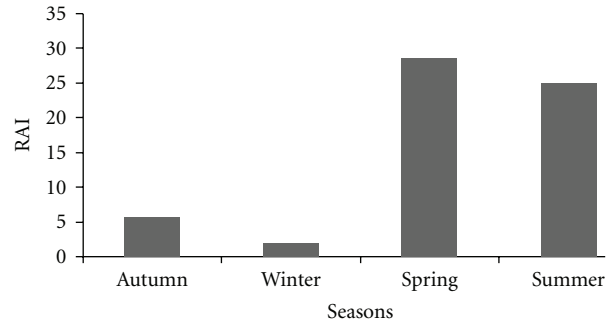

Figure 6: Seasonal variation of the mean values of reproductive activity of *M. intermedia* in the Capivara Reservoir.

by total length. The curve shape was adjusted according to the following mathematical expression: $\mathrm{Rf} = 1 - (e^{-aL_t{}^b})$, where Rf is relative frequency of adult, e is base of natural logarithms, L_t is total length in cm, and a and b are constants related to estimated curve fitting.

Spawning type was evaluated according to the distribution frequencies of the oocyte diameter in the ovaries in different maturation stages along with the analysis of the distribution of relative frequencies of diameters and maturation curve, where histological examination of the gonads in different development stages helped in this determination [12]. We use an average of ten ovaries of each stage of development compared with the histological preparations of the same, and then the oocytes were counted (decoupled) in the more advanced degree, and 20 of them were measured to determine their diameter and then the diameter classes.

To evaluate the ovaries characteristics, the oocytes were counted and majored. Only the ovarian with the highest gonadosomatic index (GSI) which lose oocytes in the cavity or with hyaline aspect were considered. With further knowledge of the gonad total weight (W), samples were taken, counted (n), and reweighted (w) and have been used to estimate the number of oocytes in the whole ovary (N), $N = n * W/w$ according to Vazzoler [12]. To apply this method, mature ovaries fixed in formaldehyde (10%) were used, where the count of n considered only those oocytes with diameter greater than that determined by comparative analysis of frequency distributions.

From all this the relative fecundity was estimated in relation to the total weight (W_t), using the formula $\mathrm{Fr} = aW_t^b$ and fecundity per reproductive period (FRP) of the fish conformed the results of Vazzoler [12] and Marccuci et al. [14].

The spawning site was obtained based on the temporal variation of the frequency of the stages of gonadal maturity and the gonadosomatic index (GSI), which was later used in the calculation of the reproductive activity index (RAI) [12, 15].

Juveniles and adults were distinguished by macroscopic and microscopy determination of the stage of gonadal maturation [8, 12], which considered the distinctive characteristics for each stage, such as size, transparency, coloration and vascularization of the gonads. Based on these analyses, these fish were classified according to four different

stages: 1 : immature, 2 : beginning the maturation process, 3 : mature, and 4 : spawned.

3. Results

A total of 696 individuals of *M. intermedia* were captured during the study period the total average length was 8,17 cm and average total weight was 10,12 g for females, and average total length of 6,89 cm and average weight of 8,46 g were determined for males, demonstrating a sexual dimorphism in size for the species.

In relation to maturity, 92,4% of these fish were considered adults, and only 7,47% of the individuals captured were identified as juveniles. Figure 2 shows the distribution of juveniles and adults for each stretch sampled, and in Figure 3 are the standard length classes. Cinzas was the site with the highest number of juveniles captured (38), and 14 individuals were caught at Sertanópolis, while in the other stretches (Cruzália and Porecatu), no juvenile specimen was captured.

During the whole sampling period, the species *M. intermedia* was captured in four stretches and in all seasons of the year. Cruzália was the site with the highest abundance of specimens, representing almost 35% of the individuals, and spring was the season of the year in which the most individuals were captured (45,83%) (Table 1).

The sex ratio revealed a predominance of females during the study period. From the total fish collected, 84,48% were females and only 7,90% were males and 7,62%, could not be sexed. Tables 2 and 3 show the distribution of males and females according to stretch and season of the year. The standard length variations among matured individuals were 5,6 to 10,8 cm for females and 5,1 to 9,0 cm for males. The analyses of L_{50} and L_{100} show the respective values 6,1 cm and 8,5 cm for females ($\mathrm{Rf} = 1 - (e^{-2.41*10(-14)} * L_t{}^{14.62})$) and 5,5 cm and 8,5 cm ($\mathrm{Rf} = 1 - (e^{-2.09*10(-12)} * L_t{}^{11.31})$) for males (Figure 3 and Table 4).

Analyses of the RAI show that females capable of reproduction were present in all sampling stretches, but at Cinzas, reproductive intensity was less than at other sampling sites, as can be observed in Figure 4.

Reproductive activity, according to the classification of Vazzoler [12], was very intense in the Cruzália, Sertanópolis and Porecatu stretches, which are sites that have waters with lentic and semilotic characteristics. However, in the Cinzas stretch, which is a region of lotic waters where the conditions are closer to natural, the level of reproductive activity was considered moderate, but it should be noted that the majority of catches occurred in a marginal lake with good characteristics and ample communication with the river all year round.

With regard to type of spawning, it can be stated that *M. intermedia* releases its oocytes in divided manner (synchronous in groups), being evidenced by the distribution of different batches of oocytes according to the diameter and stage of development, indicating that multiple spawning is hard (Figure 5).

According to our analyses the period of spawning was related to the warmest periods of the year with highest rates in the spring and summer (most intense reproductive activity) declining to moderate levels in the autumn (Figure 6).

Females spawn oocytes with diameter of 0,71 mm in average and present a mean relative fecundity of 1065 oocytes/grams (Table 5), and the absolute fecundity overage was 9611 oocytes per gonad. The coefficients a and b used in the formula $\mathrm{Fr} = aW_t^b$ to calculate the relative fecundity were equivalent to 14,62 and 2,41, respectively.

The reproductive activity declines more at the approach of winter (incipient), a period in which practically no reproduction occurs for this species. In Figure 6, can be observed and compared the reproductive intensity of this species among the seasons.

4. Discussion

Vazzoler [12] considered that the proportion between males and females is important information for the characterization of the structure of a species or population, besides helping in the study of other aspects such as the evaluation of the reproductive potential and estimation of stock size. According to Vazzoler [12], this parameter in fish varies over the life cycle with successive events that affect male and female individuals distinctly, resulting in an efficacious reproductive strategy.

Various authors [12, 16, 17] believe that mortality is one of the factors that can act differentially on males and females. This mortality can be associated with the high rate of predation in one of the sexes or also with intensive predatory fishing (considered little for this species). Raposo and Gurgel [18] also argue that the sex ratio can be unbalanced due to an elevated birthrate of individuals of a determined sex.

It is believed that the differential behavior between males and females of *M. intermedia* in Capivara Reservoir is the principal cause of the large sex disproportion existing in this species. An example of this was observed during the samplings, when males and females of this species were captured in separate shoals, utilizing the same fishing gear. Reynolds [19] explains that in some fish species there is a segregation of the sexes when matured where they may differ in preference for a particular habitat, possibly making one of the sexes more vulnerable to capture and predation.

It is also known that this difference between males and females could be related to sampling methodology, but it is believed that it is not valid for this study because of the applied mechanisms, but Orsi et al. [11] after carrying out sampling Sertanópolis between 1990 and 1995 found proportion (56,14% for females and 43,86% for males) different than that in this study, and it is also true that in this related period the presence of *Cichla cf. kelberi* (an important nonnative predator) was not observed by Orsi and Agostinho [20].

One way to establish how species explore the environment and carry out their life cycle is by determining the population first gonad maturation standard length (L_{50}) [12]. This maturation is variable, according to Godinho [21], depending mainly on the species and the conditions of its environment.

Table 1: Total number of individuals captured in the four stretches and per season of the year. The values between parentheses are the percentages of fish collected for each stretch and each season.

Season	Stretches				Total (%)
	Cinzas	Cruzália	Sertanópolis	Porecatu	
Autumn	24	61	55	24	164 (23,56)
Winter	56	3	9	9	77 (11,06)
Spring	22	166	116	15	319 (45,83)
Summer	36	13	33	54	136 (19,54)
Total (%)	138 (19,83)	243 (34,91)	213 (30,60)	102 (14,66)	696 (100,00)

Table 2: Absolute frequency of males and females collected in each season of the year, in Capivara Reservoir.

Seasons	Sex		Total (%)
	Males	Females	
Autumn	11	148	159 (24,73)
Winter	03	55	58 (9,02)
Spring	39	269	308 (47,90)
Summer	02	116	118 (18,35)
Total (%)	55	588	643 (100,00)

Table 3: Absolute frequency of males and females captured in each stretch sampled, in Capivara Reservoir.

Stretches	Sex		Total (%)
	Males	Females	
Cinzas (I)	07	93	100 (15,55)
Cruzália (II)	31	211	242 (37,64)
Sertanópolis (III)	13	186	199 (30,95)
Porecatu (IV)	04	98	102 (15,86)
Total (%)	55	588	643 (100,00)

Vazzoler and Menezes [22], in a work on the Characiformes of South America, observed that *M. intermedia* showed a mean standard length of first maturation of 3,6 cm, while in another study, in the upper Rio Paraná, Vazzoler [12] found a mean total length at first maturation of 5,5 cm. However, Hojo et al. [23] found that *M. intermedia* had a mean standard length at first gonadal maturation estimated at 6,6 cm, which was greater than that stated the other reports, mainly considering that the analysis was of standard length. Orsi et al. [11] also studied this parameter for *M. intermedia* and found values close to those obtained in the present work.

Differences in the values of first maturation for the same species or genus are found in the report by Vazzoler [12] who states that gonadal maturation is a very effective reproductive strategy intimately related to genotype-environment interaction and, consequently, to growth, showing intraspecific, spatial, and temporal variations associated with the abiotic and biotic environmental conditions prevailing in the region in which the population was exposed. Therefore, it can be stated that the individuals cited in different studies probably underwent environmental pressures of different forms. Thus, distinct reproductive tactics were chosen, such as a more accelerated growth to maintain equilibrium in the environment in which they live. This means a high ecological value, as these strategies provide a certain adjustment of existing environmental conditions.

A population of fish can mature early or increase its size at first gonadal maturation as needed [24]. For example, Parker and Johnson [25] observed that in situations of high mortality rates, early maturation is advantageous, since it increases opportunities for reproduction over time. However, low mortality rates cause delay in first maturation, consequently increasing survival. Therefore, the influence of environmental variables of Capivara Reservoir can be related to this process of relatively early maturation. Thus, the species tends toward a pattern in this strategy for the hydrographic basin.

Unfortunately, we are not aware of studies on the reproduction of fish before the construction of the dam, hindering the comparison of this biological parameter in this locality.

In studies conducted by Lizama and Ambrósio [6, 26], Braga and Gennari [27] and Vazzoler [12], the maximum total length was about 10,0 cm, where the findings of this work are in line with the patterns found by these authors. Meanwhile, Hojo et al. [23] found females in advanced stages of gonadal maturation with a total length of 11,0 cm and males of 10,2 cm. These values are within the calculations of L_∞ presented by Campos et al. [28], which demonstrates a difference in size between females and males (12,0 cm and 10,0 cm, resp.).

Thus, the mean length at first maturation and the period and area of reproduction are parameters essential for the understanding of the behavior of a species within its habitat [29], and based on this information, it is possible to take preventive and management measures for this species.

The Cinzas stretch showed a RAI well below that of the other stretches, and this can be associated with, besides other factors, water flow rate, since this stretch is the only one with characteristics of lotic waters. A study carried out by Luz et al. [30] showed that this species has a preference for more lentic environments, where till then it was considered an environmental indicator for species with a preference for disconnected lakes. This same author even demonstrated that this preference is a characteristic of various species of the family Characidae.

Also regarding the Cinzas stretch, Figure 2 shows that this was the site where most juveniles were captured. Despite the

Table 4: The mean length of first maturation (L_{50}%) of *M. intermedia* captured in the Capivara reservoir. Smallest adult captured (< adult) and length 100% in reproduction (L_{100}%).

Species	Females			Males		
	< adult (cm)	L_{50}% (cm)	L_{100}% (cm)	< adult (cm)	L_{50}% (cm)	L_{100}% (cm)
Moenkhausia intermedia	5,60	6,10	8,50	5,10	5,50	9,00

Table 5: Mean diameter, number of oocyte (matured), fecundity per reproductive period ((Frp), related to batches spawned), and mean relative fecundity (RF) of *M. intermedia* captured in the Capivara reservoir (*N*: number of females, *n*: number of measured oocyte, *s*: standard mean deviation).

	Oocytes mean diameter (mm)				Total number of oocytes					Frp	RF (oocytes/grams) $Fr = {}_{14,62}Wt^{2,41}$
	N	*n*	mean	*s*	*N*	Mean	Lower	Higher	*s*		
Moenkhausia intermedia	21	201	0,71	0,12	21	9611	6447	10965	4457	4265	1065

number being less than that of adults collected, we believe that a marginal lake had served as a preferential area for reproduction, development, and growth of the juveniles of this species, where this is considered an important shelter area for this and other species existing in the locality. The greater presence of small individuals was interpreted as a differential in the success of effective recruiting of the species, where in this manner there is less need for energy expenditure during reproduction, explaining moderate RAI levels.

According to Braga [31], the locations where the number of adults is higher than that of juveniles is considered as an area of reproduction, and when the opposite occurs in which more juveniles are captured than adults this refers to an area for feeding and growth. The fact that mainly adult individuals were captured in the Cinzas stretch indicates that this is an area for reproduction, but for a nonmigratory species and with a ample analysis, it can be an alarming demonstration of a deficiency in effective recruitment detected for this species in this area, since Bennemann et al. [9], who carried out studies in the region of Sertanópolis in the 1990s, noted that *M. intermedia* was the most numerous species during samplings. This deficit could have been caused by increased pressure from predation due to the introduction of allochthonous species of the genus *Cichla* as demonstrated by Orsi and Sodré [8].

Figures 3 and 4 show a highly increased reproductive activity index (RAI). This elevated reproductive pattern for this species was also found by Orsi et al. [11], in which, in comparison with 17 other species, *M. intermedia* showed the highest reproductive activity index.

The results found for the reproductive period in this work were similar to those obtained by Vazzoler and Menezes [22] who noted that the reproductive period of *M. intermedia* begins in November and continues until the month of June, where this same pattern was again later reported by Vazzoler [12]. According to Veregue and Orsi [32], this long spawning period suggests that this is an efficacious strategy in the production of future progeny, because it increases the chances of perpetuation of the species.

Mean absolute fecundity shown in this study (Table 5) is slightly different than that observed by Rodrigues et al. [33] for the Ibitinga Dam, where they obtained a mean value of 6092 oocytes for *M. intermedia* against 9611 oocytes obtained in this study. However, these values indicate that this fish is in a category of species of intermediate fecundity as suggested by Vazzoler and Menezes [22] and Hartz et al. [34]. These authors consider species that undergo extensive reproductive migrations and show total spawning as having high fecundity, while those nonmigratory and without parental care, such as *M. intermedia,* showing an intermediate fecundity, and those with parental care reduced fecundity.

Besides the characteristics regarding oocyte production, various authors [35–38] believe that fecundity is more or less directly related to the availability of food in the environment. Gennari and Braga [37] add that the weight of the individual depends greatly on feeding and that the more food is offered the greater its weight will be, accumulating sufficient energy for increasing ovary size and consequently the level of fecundity. It is believed that the size and number of oocytes produced are related not only to feeding but also to the characteristics of the environment in which the oocytes will develop.

The analysis on fecundity was performed to characterize the species in Capivara Reservoir, with respect to the production of a few or many oocytes and if these were large or small. Araujo and Garutti [39] demonstrated that the evaluation of these reproductive attributes is important, because they represent aids in the estimation of population sizes and can also provide indications of the life strategy of the species. It should be emphasized that it is important to carry out a larger sampling and in shorter periods, because according to Barbieri et al. [24] the size and number of oocytes can vary within a population in different years.

5. Final Considerations

It is known that the fish species *M. intermedia* is an important component of the food chain [6, 9], serving as food for various other animals of greater size and representative of

economic importance. An imbalance of this population, such as in reduced numbers or extinction, can result in other types of alterations in the environment in which they are found, as a cascading effect. According to Lizama and Ambrósio [6] and Duke Energy [40], this species has low relevance for human consumption in this basin.

Evidence of reproductive activity in the system evaluated, despite the reduction in absolute numbers of the individuals, calls attention to this finding. Although this species makes efforts to maintain its presence in the environment, it appears that these efforts are not adequate from the point of view of efficacy and maintenance of recruitment. Therefore, it is necessary to learn about the characteristics of this species, such as reproductive strategies and tactics, because from this information it is possible to implement preservationist and management measures for keeping it in ecological equilibrium. Besides, this information will serve as a basis for comparison with future studies on the species in the hydrographic basin of the Paranapanema River and to evaluate its capacity for permanence and occupation of this environment.

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