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Reproductive ecology of the catfish, *Hassar affinis* (ACTINOPTERYGII: DORADIDAE), in three lakes of the Pindaré-Mearim Lake System, Maranhão[1]

Ecologia reprodutiva do mandi bico-de-flor *Hassar affinis* (ACTINOPTERYGII: DORADIDAE), em três lagos do Sistema Lacustre Pindaré-Mearim, Maranhão

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ABSTRACT - Reproductive biology affords fundamental features for the establishment of protective measures for fish. The aim of the present work therefore was to determine the reproductive features of the catfish, Hassar affinis, known locally as bico-de-flor, in three lakes of the Pindaré-Mearim Lake System, in the state of Maranhão, with the aim of subsidising the closed season. Monthly collections were carried out between July 2014 and July 2015, when 206 individuals were sampled from Lake Aquiri (LA), 247 from Lake Cajari (LC), and 126 from Lake Viana (LV). In the laboratory, each individual was weighed and measured; a ventral longitudinal incision was then made in order to observe the gonads macroscopically. They were then fixed in Bouin's solution for microscopic analysis and in Gilson's solution for an analysis of fecundity. Positive allometry was recorded at the three study sites. The sex ratio for the total period was 3.29F:1M (LA), 2.43F:1M (LC) and 2.15F:1M (LV). Mean length at first sexual maturity was estimated at 10.60 cm (LA), 10.84 cm (LC) and 11.12 cm (LV). The period from March to May was defined as the breeding season for this species in the three lakes. Mean absolute fecundity was 21,634 oocytes (LA), 16,357 oocytes (LC) and 25,898 oocytes (LV). The information obtained through this study indicates that, in addition to being important spawning areas, the three lakes are interconnected in relation to the migratory dynamics of H. affinis, so it is necessary to arrive at a model that would satisfactorily cover the three lakes, considering that the dam has a negative effect on Lake Viana.

Key words: Catfish. Baixada Maranhense. Conservation. Fecundity. Size at first sexual maturity.

RESUMO - A biologia reprodutiva fornece aspectos fundamentais para o estabelecimento de medidas protetivas aos peixes. Desta forma, o presente trabalho teve por objetivo determinar os aspectos reprodutivos do mandi bico-de-flor (*Hassar affinis)* em três lagos do Sistema Lacustre Pindaré-Mearim, Maranhão, visando subsidiar período de defeso. Foram realizadas coletas mensais entre o período de julho/2014 a julho/2015 onde foram amostrados 206 indivíduos no Lago Aquiri (LA), 247 no Lago Cajari (LC) e 126 no Lago de Viana (LV). Em laboratório, procedeu-se com a pesagem e medidas de cada indivíduo e posteriormente foi feita uma incisão ventro-longitudinal a fim de observar macroscopicamente as gônadas. Em seguida, foram fixadas em solução de Bouin para análise microscópica e em solução de Gilson para análise da fecundidade. A alometria positiva foi registrada nos três locais de estudo. A proporção sexual para o período total foi de 3,29F:1M (LA), 2,43F:1M (LC) e 2,15F:1M (LV). O comprimento médio de primeira maturação sexual foi estimado em 10,60 cm (LA), 10,84 cm (LC) e 11,12 cm (LV). Os meses de março a maio foram definidos como período de reprodução desta espécie para os três lagos. A fecundidade absoluta média foi de 21.634 ovócitos (LA), 16.357 ovócitos (LC) e 25.898 ovócitos (LV). As informações obtidas através deste estudo indicam que, além de serem importantes áreas de desova, os três lagos estão conectados entre si quanto à dinâmica migratória de *H. affinis*, logo, é necessário pensar em um modelo que atenda de forma satisfatória os três lagos, tendo em vista que a barragem tem efeitos negativos sobre o lago de Viana.

Palavras-chave: Bagre. Baixada Maranhense. Conservação. Fecundidade. Tamanho de primeira maturação sexual.

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INTRODUCTION

Over the last few decades, the Baixada Maranhense region of Brazil has been subjected to substantial anthropogenic action, especially through the construction of dams. These dams alter the natural course of rivers, modifying their lotic features with the loss and emergence of new habitats (VONO *et al*., 2002), and in consequence, affecting biological aspects of the aquatic fauna.

One such aspect is reproduction, which determines the success of colonisation in the environment. It is expected that species showing high plasticity in their spawning habitats will be among the most successful colonisers (AGOSTINHO *et al*., 1999). Studies of the reproductive biology of fish in these environments can therefore provide data about their condition, aiding strategies of conservation and management (VONO *et al*., 2002).

The reproductive efficiency of different fish species is dependent on several factors that act together, for reproduction to be effective and produce a large number of healthy larvae (ANDRADE *et al*., 2015). Among the main aspects making up the reproductive biology of fish species, size at first sexual maturity, reproductive period and fecundity stand out. Understanding these parameters can be considered as the first step in establishing the principal life history patterns of fish (MAZZONI; SILVA, 2006). In turn, these studies can indirectly warn about the possible effects that current fishing activity can have on fish populations and consequently, on the economy of a region (CAMARGO; LIMA JÚNIOR, 2008).

Among the regional species that inhabit the lakes of the Baixada Maranhense, the catfish, *Hassar affinis* (Steindachner, 1881) plays an important role in catfish production (7,048.1 t year^{-1}) (IBAMA, 2011) and is much appreciated for its flavour, being of great ecological and economic importance for the State of Maranhão (MA). However, studies into its reproduction are rare (CANTANHEDE *et al*., 2016), which hinders the setting up of preservation measures. Accordingly, estimates of the reproductive parameters can be used to evaluate population dynamics, particularly in determining length or age limits for young and adult stocks, as well as of the reproductive period, with a view to preservation (ARAÚJO *et al*., 2000).

In view of the above, this aim of this work was to contribute to the conservation of *H. affinis* in three lakes of the Pindaré-Mearim Lake System through the determination of reproductive aspects.

MATERIAL AND METHODS

Under the regionalisation of the Superintendency of Environmental Management (SUDEMA, 1970), the Baixada Maranhense is described as one of the seven ecological regions of the state. With an area of 1,775,035.6 ha covering 23 municipalities, it makes up a complex ecosystem, with several lakes that are part of the Pindaré-Mearim Lake System, where the specimens used in this study were obtained, more specifically in Lake Aquiri (03º09'34º S, 45º00'13" W), Lake Cajari (03º18'58" S, 45º11'08" W) and Lake Viana (03º14'08" S, 45º05'09" W), located in the municipalities of Matinha, Penalva and Viana respectively (Figure 1).

Collecting the fish included the use of netting with from 4 to 10 cm between opposite knots. The nets remained in the water for a period of from 4 to 8 hours at twilight. To make up the sample of 20 specimens per month, individuals were purchased when necessary. Collections were made monthly, and samples from the campaigns were packed in plastic bags, labelled and kept on ice for transportation. All the samples were analysed in the Laboratory of Fish and Aquatic Ecology of the State University of Maranhão (UEMA), where they had earlier been identified. The total length (precision of 1 cm), total weight, gutted weight, gonad weight in grams (precision of 0.01 g), sex and stage of sexual maturity were all recorded.

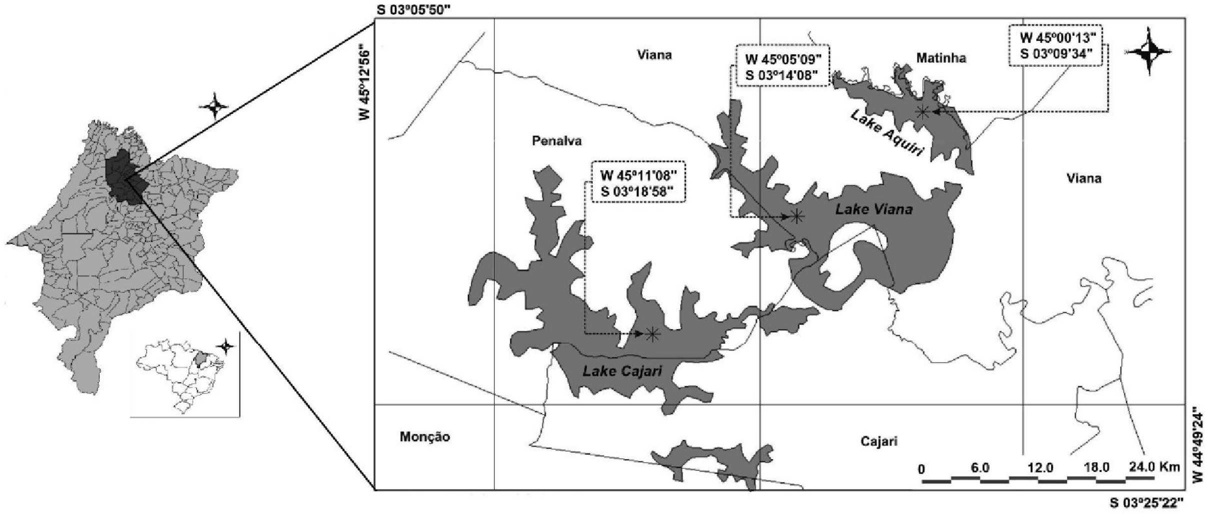
The stages of sexual maturity and the sex of *H. affinis* were determined by macro and microscopic analysis, as per Vazzoler (1996). A ventral longitudinal incision was made in each specimen for extraction of the gonads and macroscopic identification, noting such characteristics as colour, vascularity, volume in relation to the abdominal cavity, blood supply, visibility of oocytes, presence of sperm and consistency. A previously established maturity scale was used for the macroscopic classification of the gonads, including the following categories: A = immature, B = maturing, C = mature and D = empty (VAZZOLER, 1996). The total weight (WT) was then obtained with a 0.01 g precision balance.

When determination of the stage of sexual maturity at the macroscopic level was not feasible, the gonads underwent microscopic analysis, using several histological techniques, where they were fixed in Bouin solution, embedded in paraffin wax, and stained with hematoxylin-eosin.

The sex ratio of the individuals sampled was obtained for the total period. To verify the existence of significant differences in sex ratio, the chi-square test (χ^2) with Yates correction was used at a significance level of 5%.

The relationship between total length and total weight was established by nonlinear regression; this can be positive allometric (b> 3), negative allometric (b <3) or isometric (b = 3). The fit of the curve represented by the mathematical expression $WT = a \times LT^b$, was obtained

Figure 1 - Location of the Baixada Maranhense, highlighting the study areas (*): Lake Aquiri, Lake Cajari and Lake Viana



by the method of least squares, suggested by Zar (1996), where WT is the total weight of the fish, a is the linear regression coefficient, LT is the total length of the fish, and b is the angular regression coefficient.

The reproductive period was defined based on the monthly frequency of the maturity stages, variation of the mean values for the gonadosomatic index (ΔGSI), and the condition factor (ΔK).

The gonadosomatic index (ΔGSI) is the difference between GSI_1 and GSI_2, given by the equations:

$$GSI_1 = \left(\frac{Wg}{WT}\right)\times 100$$

$$IGS_2 = \left(\frac{Wg}{WC}\right)\times 100$$

where: Wg = gonad weight; WT = total weight of the individual; WC = WT-Wg.

The condition factor (ΔK) is the difference between the two models K_1 and K_2 (allometric condition factor indices), given by the equations:

$$K_1 = \frac{WT}{LT_b}$$

$$K_2 \frac{WC}{LT_b}$$

where: K_1 = total condition factor; WT = total weight of the individual; LT = total length of the individual; b = angular coefficient of the weight to length ratio; K_2 = somatic condition factor; WC = WT-Wg, where: Wg = gonad weight.

To analyse size at first sexual maturity ($L_{50\%}$), the maturity stages were grouped into immature (stage A) and mature (stages B + C + D), as proposed by Vazzoler (1996) and Ortiz-Ordónez *et al*. (2007). The percentage of mature individuals per class length was calculated and considered as the dependent variable (Y), with the total length considered the independent variable (X). These values were later fit to a logistic curve, using the Statistica 7.0 software, under licence from the Laboratory of Fish and Aquatic Ecology, UEMA, according to the equation:

$$P = \frac{1}{\left(1+\exp\left[-r\left(LT-L_{50\%}\right)\right]\right)}$$

where: P = proportion of mature individuals; r = slope of the curve; LT = total length; $L_{50\%}$ = average length at sexual maturity.

Fecundity (F) was estimated by the volumetric method, as per Vazzoler (1996). Ten gonads from each site were selected and placed in modified Gilson’s solution to dissociate the oocytes. The total oocyte volume was then recorded and three subsamples extracted. Relative fecundity was established through the relationship between total length (LT), total weight (WT) and fecundity (F), expressed by the equations: FR = a x LT^b and FR = a x WT^b, where: FR is the relative fecundity; a is the linear regression coefficient; LT is the total length; b is the angular regression coefficient, and WT is the total weight. Analysis of the correlation of fertility with both weight and length was carried out using the Pearson correlation coefficient ($p<0.05$).

To determine whether the period of greatest rainfall in the region coincides with the reproductive period of the species, rainfall data were obtained from the Northeastern Region Real-time Climate Monitoring Program (PROCLIMA), with the relationship between the variables (GSI and rainfall indices) tested using the Pearson correlation coefficient ($p<0.05$).

RESULTS AND DISCUSSION

During the study period, 579 specimens were analysed. The largest number of individuals were caught in Lake Cajari, with 247 specimens: 109 females and 17 males during the dry season, and 66 females and 55 males during the rainy season, giving a total of 175 females and 72 males analysed. This was followed by Lake Aquiri, with 206 specimens: 104 females and 8 males during the dry season, and 54 females and 40 males during the rainy season, giving a total of 158 females and 48 males analysed; then Lake Viana with 126 specimens, where the total sample was 86 females and 40 males, caught during the rainy season only (January to June) (Table 1).

In Lake Viana, the same phenomenon was seen as found by Cantanhêde *et al*. (2016), in which the species was absent from the lake throughout the dry season (July to December); this was probably related to a migratory event connected to feeding, since the species returned to the lake during the rainy season (January to June) capable of breeding, suggesting an allocation of energy during the previous period. This is due to the dams in the neighbouring lakes (Cajari and Aquiri), which generate critical conditions of food and water in Lake Viana.

The sex ratio in Lake Aquiri for the complete period was 3.29 females for each male (χ^2 = 58.73, $p<0.05$). In Lake Cajari, this ratio was 2.43 females for each male (χ^2 = 42.95, $p<0.05$) and in Lake Viana, it was 2.15 females for each male (χ^2 = 16.79, $p<0.05$). Throughout the life cycle of the fish, the sex ratio can vary according to several factors that act differently on the individuals of each sex (SOUZA; CHELLAPPA; GURGEL, 2007). The result presented by the three lakes is an indication of possible sexual segregation in the species, perhaps caused by the females reaching a larger size and becoming more attractive for sale, since when necessary, the samples were complemented with commercial specimens. Another possible explanation is that the males may have different habits than the females, or a preference for sites far from the points sampled. In Lake Viana, the absence of the species throughout the dry season may have directly interfered in the sex ratio found there. Similar results for sex ratio in *Hassar affinis* were found by Cantanhêde *et al*. (2016) in Lake Viana (3.4F:1.0M), with a significant difference favouring the females.

Positive allometry was recorded for males and females in Lakes Aquiri and Cajari. In Lake Viana, positive allometry was recorded for males and for both sexes simultaneously. In the females, allometry was negative, however Student's t-test (t = 0.002, $p> 0.05$) did not show a significant difference for the total weight to total length ratio between the two sexes, thereby defining the allometry of *H. affinis* in Lake Viana as positive (Table 2).

Analysing this result it can be concluded that development conditions were similar for the three sites under study, favouring an increase in weight. This may have been caused by the genetics of the species itself, or by the high availability of food. In the study by Cantanhêde *et al*. (2016) of the species in Lake Viana in 2012, negative allometry was recorded, however Gurgel and Mendonça (2001) reported that growth type may be conditioned by an adaptive response to the environment, therefore, as two of the lakes under study are favoured by the presence of the dam increasing the availability of food and water, positive allometry was to be expected. Conditions in Lake

Table 1 - Population structure for *H. affinis* in Lakes Aquiri, Cajari and Viana, MA

Class	n	LT (Min - Max) (cm)	Mean ± SD (cm)	WT (Mín-Max) (g)	Mean ± SD (g)
			Lake Cajari		
Females	175	10.3 – 17.5	14.0 ± 1.2	16.2 – 70.1	36.2 ± 12.1
Males	72	11.3 – 16.2	13.5 ± 1.0	21.5 – 58.3	31.2 ± 9.7
Grouped	247	10.3 – 17.5	13.9 ± 1.2	16.2 – 70.1	34.7 ± 11.7
			Lake Aquiri		
Females	158	10.8 – 20.0	14.8 ± 1.6	20.3 – 129.2	48.8 ± 17.2
Males	48	10.5 – 16.5	12.9 ± 1.1	14.7 – 79.5	30.3 ± 10.4
Grouped	206	10.5 – 20.0	14.4 ± 1.7	14.7 – 129.2	44.5 ± 17.6
			Lake Viana		
Females	86	13.5 – 21.0	15.6 ± 1.2	28.5 – 134.4	55.2 ± 16.0
Males	40	12.0 – 16.5	15.0 ± 0.8	23.7 – 76.4	46.8 ± 9.8
Grouped	126	12.0 – 21.0	15.4 ± 1.2	23.7 – 134.4	52.5 ± 14.8

n = number of individuals; LT = length; WT = total weight; SD = standard deviation

Table 2 - Parameters of the weight to length ratio for males and females of *H. affinis*, in Lakes Aquiri, Cajari and Viana, MA

Class	a	b	r²	Allometry
		Lake Cajari		
Females	0.0054	3.31	0.91	Positive
Males	0.0039	3.43	0.86	Positive
Grouped	0.0047	3.37	0.91	Positive
		Lake Aquiri		
Females	0.0149	3.00	0.85	Positive
Males	0.0113	3.06	0.85	Positive
Grouped	0.0115	3.09	0.86	Positive
		Lake Viana		
Females	0.0167	2.93	0.80	Negative
Males	0.0084	3.17	0.73	Positive
Grouped	0.0128	3.03	0.80	Positive

a = linear regression coefficient; b = angular regression coefficient; r² = coefficient of determination

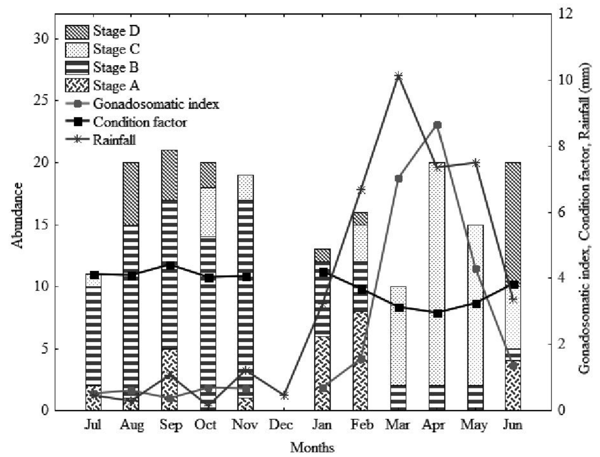
Viana may have changed in relation to the period of study of Cantanhêde *et al*. (2016), generating these differences in allometry.

If the frequency of gonadal development stages and the gonadosomatic index are taken into account, it is possible to verify that in Lake Aquiri, the reproductive period of the species occurred between March and May, when the greatest number of individuals were at stage C (mature) and the highest values for the gonadosomatic index were seen. The period in which the species presented the highest values for condition factor, was between July and January, this was then followed by a decrease in value, which represented the spawning season, when the species expended the allocated energy (Figure 2).

This increase in condition factor occurs because reproduction is considered a highly energy-intensive process, and takes place only when the animals are in their comfort zone (RIBEIRO; MOREIRA, 2012). The GSI expresses the percentage represented by the gonads of the total weight of an individual, and varies mainly for species, type of spawning, time of year and environmental conditions (SOLIS-MURGAS *et al*., 2011), as the period when the highest value for the gonadosomatic index was seen coincided with one of the highest values for rainfall. The greatest occurrence of individuals at stage D (spawned) was in July 2014 and June 2015, confirming the end of the spawning period.

In Lake Cajari, the same pattern was seen in relation to the gonadosomatic index and condition factor for the same period seen in Lake Aquiri. During April and May, the highest occurrence of individuals at stage C (mature)

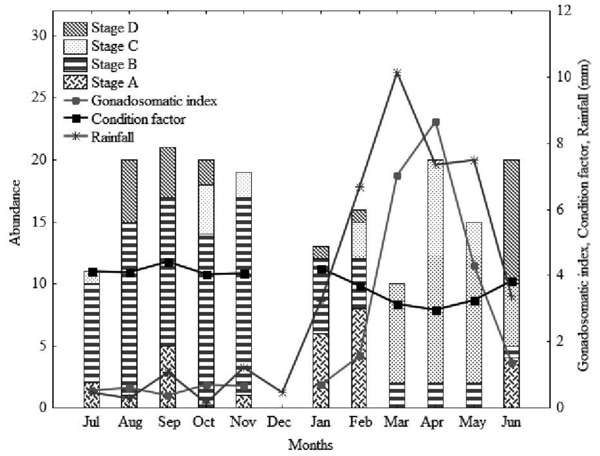
Figure 2 - Frequency of maturity stages, gonadosomatic index (ΔGSI), condition factor (ΔK) and rainfall (mm) indicating the spawning season of *H. affinis*, in Lake Aquiri, MA



was recorded, coinciding with the highest values found for the gonadosomatic index and the highest values for rainfall. The highest values for condition factor were from July to December (Figure 3).

In Lake Viana, as previously mentioned, the species was absent from the lake for almost the entire dry season, from July 2014 to November 2014, only being caught in December 2014. The same phenomenon was seen by Cantanhêde *et al*. (2016) for the same species in Lake Viana, where it did not occur from June to July or from August to September, but reoccurred from October to November.

Figure 3 - Frequency of maturity stages, gonadosomatic index (ΔGSI), condition factor (ΔK) and rainfall (mm) indicating the spawning season of *H. affinis*, in Lake Cajari, MA



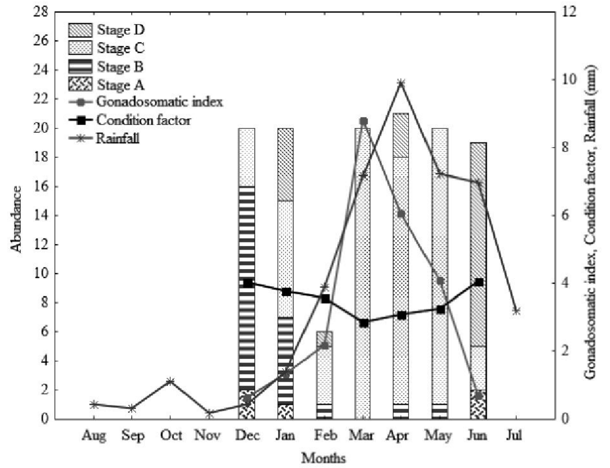
Accordingly, Cantanhêde *et al*. (2016) suggest that the absence of the species at the site during this period was due to a migratory event to the neighbouring lakes, Lake Aquiri and Lake Cajari, which was connected to feeding and maintaining energy reserves to be used in the reproductive event. However, the two lakes have dams that would have prevented the return of *H. affinis* to Lake Viana during the dry season (July, August, September and October), this being possible only during the rainy season (November to May) due to the dams overflowing.

Thus, analysing the results found for Lake Aquiri and Lake Cajari, it is possible to verify what was proposed by Cantanhêde *et al*. (2016), since during the time the species was absent from Lake Viana, the populations of Lake Aquiri and Lake Cajari displayed large values for condition factor, indicating feeding and the allocation of energy. When the population of *H. affinis* returned to Lake Viana in December, it was able to reproduce, expending the allocated energy, since according to Lima-Junior and Goitein (2006), the condition factor provides important information about the physiological state of the animals, based on the assumption that individuals with greater body mass are in better physical condition.

It is therefore assumed that the population of *H. affinis* in Lake Viana migrated to Lakes Aquiri and Cajari during the dry season and returned during the rainy season to spawn, as can be seen by the frequency of maturity stages, where from March to May most of the individuals were at stage C (mature) and capable of spawning, coinciding with the greatest values seen for the gonadosomatic index. The high occurrence of individuals at stage D (spawned) during June confirms the end of the spawning season (Figure 4), just as happened in Lake Aquiri.

Ribeiro (2002) reports that for *Conorhynchus conirostris*, a siluriform of the family Pimelodidae, in the River São Francisco, in the Pirapora Region of Minas Gerais, there were no samples taken between June and September, as the species was not caught. The author relates this absence to the individuals migrating to feeding areas, as happened with *H. affinis* in Lake Viana, where the possible feeding areas would be Lake Aquiri and Lake Cajari.

Figure 4 - Frequency of maturity stages, gonadosomatic index (ΔGSI), condition factor (ΔK) and rainfall (mm) indicating the spawning season of *H. affinis*, in Lake Cajari, MA



A strong relationship between rainfall and reproductive period ($R = 0.70$, $p<0.001$) was found in the present work, just as seen by Rondineli and Braga (2009) for *Corydoras flaveolus* in the River Passa Cinco, where the greatest values for the gonadosomatic index coincided with the greatest values for temperature and rainfall.

Determining size at first gonadal maturity ($L_{50\%}$, the size at which at least 50% of the population is capable of reproducing) is of paramount importance to the management of commercially exploited populations, as it is the fundamental basis for determining the minimum size allowed for capture (SEVERINO-RODRIGUES *et al*., 2012). Size at first sexual maturity in *H. affinis* displayed similar values for the three lakes (Table 3).

For females, it was not possible to determine average size at first sexual maturity, since all the smaller individuals caught were capable of spawning; it was

Table 3 - Size at first sexual maturity in *H. affinis* for females, males and grouped gender in Lakes Aquiri, Cajari and Viana, MA

Site	Females	Males	Grouped gender
Lake Aquiri	8.9 cm	11.3 cm	10.6 cm
Lake Cajari	10.7 cm	11.7 cm	10.8 cm
Lake Viana	-	11.9 cm	11.1 cm

therefore not possible to determine the size at which the individuals were still juveniles but were able to spawn. In addition, the size at first sexual maturity in the species at the three sites was small when compared to the maximum size reached by the species during the study period. Differences in size at sexual maturity may be related to genotypic variation and to the action of different stressors on the population (e.g. pressure of capture, predation, availability of food and population density), as well as to the results of variations or inaccuracies in the methods of capture used (HINES, 1989; JONES; SIMONS, 1983; ORENSANZ; ERNST; ARMSTRONG, 2007).

As a result, and in view of the reduction in longevity due to the greater probability of capture, and the need of the species to keep genes active in the population, selective pressure could be taking place in the region, favouring those individuals that reach maturity earlier (CARVALHO; CARVALHO; COUTO, 2011).

Cantanhêde *et al.* (2016) report a size at first sexual maturity for females of *H. affinis* in Lake Viana of 11.56 cm, for males of 11.46 cm, and for grouped gender of 11.52 cm, close to the results found in this work.

The results for mean absolute fecundity and relative fecundity by length and weight can be seen in Table 4.

The values for these parameters were found to increase proportionately with body weight and total length. The values for r^2 indicated a correlation of fecundity with body weight and total length in Lake Aquiri, being greater for body weight; and a correlation with total length in Lake Cajari. Sampaio and Sato (2006) found that the values for these parameters also increased proportionately with body weight and total length in *Pseudopimelodus charus* in the São Francisco River Basin, but values for r^2 were better when these parameters were related to body weight. Santos (2002) found a better relationship between total length and fecundity than between body weight and fecundity in *Rhamdia quelen*. Lake Viana displayed a weak correlation for the two variables, since the smallest individual analysed showed an absolute fecundity greater than the largest individuals analysed, demonstrating that for Lake Viana, the size or weight of the individuals had no effect on total fecundity. According to Araujo (2009), high absolute fecundity is a characteristic among fish that display pelagic spawning and among those that do display parental care.

Table 4 - Mean absolute fecundity and relative fecundity by length and weight in females of *H. affinis* in Lakes Aquiri, Cajari and Viana, MA

Site	MAF	RF/L	RF/W	r	
				RF x L	RF x W
Lake Aquiri	21,634 ± 20,532	1,377	353	0.92 (p = 0.025*)	0.98 (p = 0.002*)
Lake Cajari	16,357 ± 9,821	1,055	323	0.98 (p = 0.027*)	0.89 (p = 0.10)
Lake Viana	25,898 ± 9,933	1,957	508	-0.04 (p = 0.930)	-0.04 (p = 0.931)

MAF = Mean absolute fecundity; RF/L = relative fecundity by length (in centimetres); RF/W = relative fecundity by weight (in grams); r = Pearson correlation coefficient; * = Significant values ($p<0.05$)

CONCLUSION

The three lakes are important spawning areas for the species. Size at first sexual maturity was small when compared to the maximum size reached by the species. There was a relationship between rainfall and the reproductive period, with the rainy season favourable to spawning. It was possible to verify that lakes Viana, Aquiri and Cajari are connected, and it is therefore necessary to think of a model that would satisfy all three lakes, considering that the dam has a negative effect on Lake Viana.

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