Reproductive biology and development of sexually dimorphic structures in *Aphyocharax anisitsi* (Ostariophysi: Characidae)

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The reproductive period of *Aphyocharax anisitsi* is described through the analysis of specimens collected monthly from April 2001 to March 2002 in the arroio do Salso (30°22'27"S, 55°02'06"W), Rosário do Sul, Rio Grande do Sul, Brazil. The monthly variation of the Gonadosomatic Index mean (GSI) of both males and females allowed the recognition of a defined seasonal reproductive period occurring between September and February, showing a positive correlation with temperature and daylength. Absolute fecundity was 344.8 oocytes and relative fecundity 0.68 oocytes per mg of body weight. Absolute fecundity was correlated with standard length and total body weight. It was possible to identify two categories of males through the analysis of the number of rays bearing hooks on the anal fin: those with few or no rays bearing hooks identified as young males hatched in the last reproductive season, and those with a higher number of anal-fin rays bearing hooks identified as males that had already reproduced once or that are maturing and participating in their first reproductive period. Once developed, anal-fin rays are maintained permanently. A positive correlation was found between the GSI of maturing and mature males and the number of anal-fin rays bearing hooks from August to January. All specimens bearing gill glands were mature or undergoing maturation, and the frequency of occurrence of the gill glands increased with a rise of GSI.

O período reprodutivo de *Aphyocharax anisitsi* é descrito através da análise de espécimes coletados mensalmente entre abril de 2001 e março de 2002 no arroio do Salso (30°22'27"S, 55°02'06"W) em Rosário do Sul, Rio Grande do Sul, Brasil. A variação mensal das médias do IGS (Índice Gonadossomático) de machos e fêmeas mostrou um período reprodutivo sazonalmente definido, ocorrendo entre os meses de setembro e fevereiro, e apresentou correlação positiva com a variação da temperatura e do fotoperíodo. A fecundidade absoluta média da espécie foi de 344,8 ovócitos. A fecundidade relativa média foi de 0,68 ovócitos por mg de peso total. A fecundidade absoluta apresentou correlação com o comprimento padrão e com o peso total. Através da análise do número de raios com ganchos da nadadeira anal dos machos foi possível identificar duas categorias, aqueles com nenhum ou poucos raios com ganchos que seriam indivíduos jovens nascidos no último período reprodutivo e que ainda não entraram em atividade reprodutiva e aqueles machos com um número alto de raios com ganchos que seriam indivíduos que já se reproduziram e mantiveram os ganchos ou que estão participando pela primeira vez do período reprodutivo. Foi observada uma correlação positiva entre o número de raios com ganchos e o IGS de machos maduros ou em maturação entre os meses de agosto a janeiro. Todos os indivíduos que apresentaram glândula branquial estavam em maturação ou maduros. A freqüência da ocorrência desta estrutura aumentou com a elevação das médias de IGS.

Key words: fin hooks, gill gland, gonadosomatic index, reproduction.

Introduction

Characiformes is a group of Neotropical and African freshwater fishes that exhibits a wide range of behaviors and comprises the most morphologically diverse assemblage among ostariophysan fishes (Vari, 1998). The family Characidae, with over 1352 species (Reis *et al.*, 2003), is the largest within the order and one of the most diverse families of Neotropical fishes. *Aphyocharax* is the single genus currently in the apparently monophyletic characid subfamily Aphyocharacinae,

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which includes a total of 10 species (Lima, 2003).

Aphyocharax anisitsi is found in the rios Paraná, Paraguay and Uruguay and the laguna dos Patos drainages in southern South America. It is a small species that reproduces by means of external fertilization. Mature males have small bony hooks on several anal and pelvic-fin rays, a putative apomorphic character shared by most characids (Malabarba & Weitzman, 2003). Mature males of this species possess gill glands, which have thus far been described in a few characid species (Burns & Weitzman, 1996; Bushmann *et al.*, 2002).

In spite of reproduction being one of the most studied aspects of fishes, the available information is still very limited given the large number of fish species. Vazzoler & Menezes (1992) estimated that information on reproductive patterns is available for only about 10% of Neotropical characiforms.

In the current report, the reproductive cycle of *Aphyocharax anisitsi* is described from the analysis of the monthly variations of the gonadosomatic index (GSI) and stages of gonadal maturation in both sexes. We also determine female absolute and relative fecundity, as well as the influence of water temperature, day-length, rainfall, and stomach repletion index (SRI) on the reproductive cycle. Male secondary sex characters (gill glands and anal-fin hooks), which develop during sexual maturation, are also described.

Material and Methods

Specimens of both sexes were collected monthly from April 2001 to March 2002 in the arroio do Salso (30°22'27" S, 55°02'06" W), a stream of the rio Uruguay drainage, Rosário do Sul, Rio Grande do Sul, Brazil. This stream has a sandy bottom and slow current flow. Marginal vegetation is sparse and the abundance of floating macrophytes moderate.

Specimens were obtained with a seine net (5 mm mesh), and preserved in 10% formalin just after capture. Water temperature, oxygen saturation, pH and conductivity were measured in the field during each collection. Specimens were left in fixative a minimum of 10 days, and later transferred to 70% ethanol. Standard length (SL) and total body weight (Wt) were measured for each specimen, as well as gonad weight (Wg) and stomach weight (Ws) after dissection. All weights were calculated to the nearest 0.0001 g. Gonadosomatic index (GSI) and stomach repletion index (SRI) were calculated according to Vazzoler (1996): GSI = (Wg x 100)/ Wt and SRI = (Ws x 100)/Wt.

Total fecundity was estimated by counting the number of yolky oocytes in both ovaries of 10 mature females, and relative fecundity by dividing the total number of oocytes by total body weight (Adebisi, 1987). Stages of gonadal maturation were determined through macroscopic analysis following Azevedo *et al*. (2000) and confirmed through histological analysis, when necessary. For histology, formalin fixed gonads were dehydrated in an ethanol series, embedded in paraffin, sectioned at 0.5 μm and stained with hematoxylin-eosin. The fusion of gill filaments during gill gland formation was

determined using scanning electron microscopy (SEM). For SEM analysis, the first gill arch of formalin fixed and alcohol preserved specimens was extracted, dehydrated with critical point dryer and covered with coat and gold. A stereomicroscope was used to analyze the presence and number of rays bearing hooks on the anal and pelvic fins.

Correlations were calculated between GSI and abiotic factors (water temperature, day-length and rainfall) and one biotic factor (SRI) using the non-parametric test of Spearman. Correlations were also calculated between absolute fecundity and the biometric factors (SL and Wt) using the correlation test of Pearson. Voucher specimens are deposited at Departamento de Zoologia of the Universidade Federal do Rio Grande do Sul fish collection, and numbered as UFRGS 6485.

Results

A total of 285 specimens were analyzed, comprising 130 males (19.59-31.62 mm SL) and 155 females (20.80-32.31 mm SL). Male GSI means ranged from 0.31 (March) to 1.09 (December) and female GSI means ranged from 0.45 (April) to 6.14 (January). The monthly variation of GSI of both sexes (Fig. 1) indicated a well defined reproductive period extending from September through February, which corresponds to spring and summer in the southern hemisphere. The monthly variation of mean GSI of both sexes was significantly correlated with the monthly variation of water temperature (r = 0.6200, p = 0.0347 for males and r = 0.5884, p = 0.0489 for females) and day-length (r = 0.7692, p = 0.0034 for males and r = 0.7622, p = 0.0055 for females) (Fig. 1). A significant negative correlation with rainfall was found for females only (r= -0.7343, p= 0.0065). No significant correlation was found between GSI and SRI for either sex (Table 1). Absolute fecundity ranged from 203 to 491 oocytes (mean = 344.8 ± 116.4), and the mean relative fecundity was 0.68 ± 0.14 oocytes/mg Wt. Absolute fecundity was significantly correlated with SL (r= 0.7693, p= 0.0093) and Wt (r = 0.8337, p = 0.0027).

Table 1. Monthly mean values of GSI (± SD) and RSI (± SD) for males and females of *A. anisitsi* collected from April 2001 to March 2002 in the arroio do Salso, Rosário do Sul, RS, Brazil. GSI = Gonadosomatic Index; RSI = Stomach Repletion Index; SD = Standard Deviation.

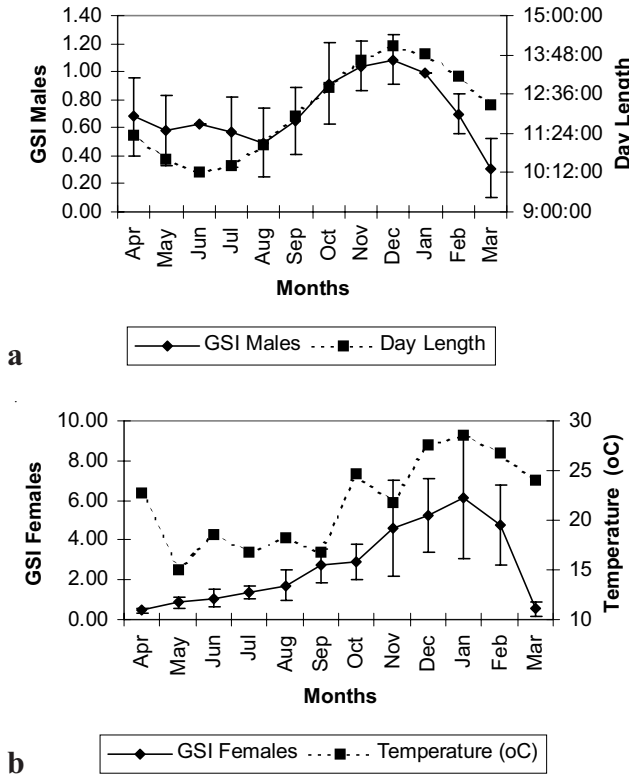|	Males		Females	
Month	GSI (±SD)	RSI (±SD)	GSI (±SD)	RSI (±SD)
April	0.68 (±0.28)	1.13 (±0.22)	0.45 (±0.11)	1.08 (±0.35)
May	0.58 (±0.25)	1.43 (±0.18)	0.85 (±0.27)	1.72 (±0.52)
June	0.63 (±0.0)	1.34 (±0.0)	1.07 (±0.44)	1.81 (±0.69)
July	0.57 (±0.25)	1.82 (±0.25)	1.37 (±0.31)	2.49 (±0.86)
August	0.49 (±0.24)	1.54 (±0.57)	1.73 (±0.78)	2.26 (±1.19)
September	0.65 (±0.24)	1.52 (±0.39)	2.71 (±0.85)	1.36 (±0.39)
October	0.91 (±0.29)	1.47 (±0.46)	2.91 (±0.90)	1.32 (±0.39)
November	1.04 (±0.18)	2.20 (±0.59)	4.62 (±2.40)	1.73 (±0.53)
December	1.09 (±0.18)	1.68 (±0.94)	5.21 (±1.86)	2.04 (±0.74)
January	0.99 (±0.0)	1.02 (±0.0)	6.14 (±3.08)	1.64 (±0.06)
February	0.70 (±0.14)	1.52 (±0.99)	4.73 (±2.03)	1.44 (±0.48)
March	0.31 (±0.21)	1.07 (±0.25)	0.54 (±0.36)	1.17 (±0.42)

Fig. 1. Monthly variation of the mean values of GSI (± SD) in males (a) and females (b) of *Aphyocharax anisitsi* collected from April 2001 to March 2002 in the arroio do Salso, Rosário do Sul, RS, Brazil, and monthly variation of the day-length (a) and water temperature (b) mean values. (GSI = Gonadosomatic Index; SD = Standard Deviation represented by vertical bars).

The number of anal-fin rays in males ranged from iv (unbranched) + 15 to 20 (branched). Maximal development of bony hooks on this fin was represented by the presence of hooks in the last unbranched ray and along all branched rays. All mature males examined from October through February, when the highest GSI means were seen, had a high number (11-20) of anal-fin rays with bony hooks (Fig. 2). From April through September 2001 and March 2002, however, it was possible to discern two categories of males: those with few to no rays (0-8) bearing hooks and those with a high number (11-20) of rays with hooks (Fig. 2). The range of body size of males with no hooks or fewer rays (0-8) bearing hooks is similar to and largely overlaps the range of body size of males with a greater number of anal-fin rays (11-20) bearing hooks (19.6-28.2 mm SL, mean 24.7 mm, *vs.* 21.5-31.6 mm SL, mean 27.8 mm, respectively). Body size of males with few or no hooks, however, was significantly smaller than those with a greater number of rays bearing hooks when submitted to a Mann-Whitney U test (U = 456, p = 0.001). A significant correlation between GSI and number of anal-fin rays with hooks (r = 0.7409, p < 0.001) was found for males from August 2001 through January 2002, when we observed a progressive increase in the number of anal-fin rays bearing hooks.
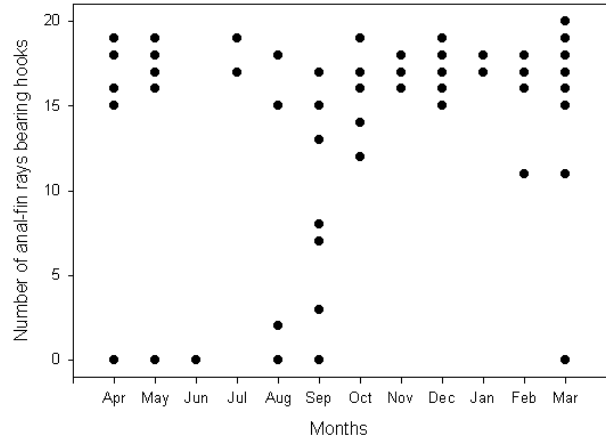

Fig. 2. Monthly variation of the number of anal fin rays bearing hooks in males of *Aphyocharax anisitsi* between April 2001 and March 2002 in the arroio do Salso, Rosário do Sul, RS, Brazil (each dot may represent more than one specimen). At the bottom, males with few or no hooks identified as young males that were probably hatched during the previous reproductive season. At the top, males that matured during previous reproductive season or are maturing for the first time.

Males with few to no hooks on the anal-fin rays from April through September 2001 and March 2002 were identified as young males that were probably hatched during the previous reproductive season. The presence of hooks on at least some fin rays suggest that some males had begun to mature, but once environment conditions (day-length, temperature) become unfavorable, further maturation was inhibited. Males with a high number of anal-fin rays with hooks captured outside of the reproductive period, *i.e.*, from April to August 2001 and March 2002, have apparently already reproduced during the previous season. It appears that, once developed, the hooks remain on the anal-fin rays permanently.

Gill glands were either observed or not in males of *A. anisitsi*. All specimens that possessed distinct gill glands were males that were already mature or undergoing sexual maturation. When present, gill glands were formed from the fusion of two to five gill filaments on the first gill arch on either side of the body (Fig. 3). Higher mean GSIs were associated with a greater number of males possessing gill glands (Fig. 4). However, immature, maturing and even some mature males lacked discernible gill glands.

Discussion

According to Vazzoler and Menezes (1992), most characiforms, including several characid species, spawn during spring and summer. The peak reproductive period of *Aphyocharax anisitsi* also occurred during spring and summer when temperatures and day-lengths were both maximal. Indeed, in both sexes, mean monthly GSIs were significantly correlated with both water temperature and day-length, which shows an annual variation of approximately four hours. An-
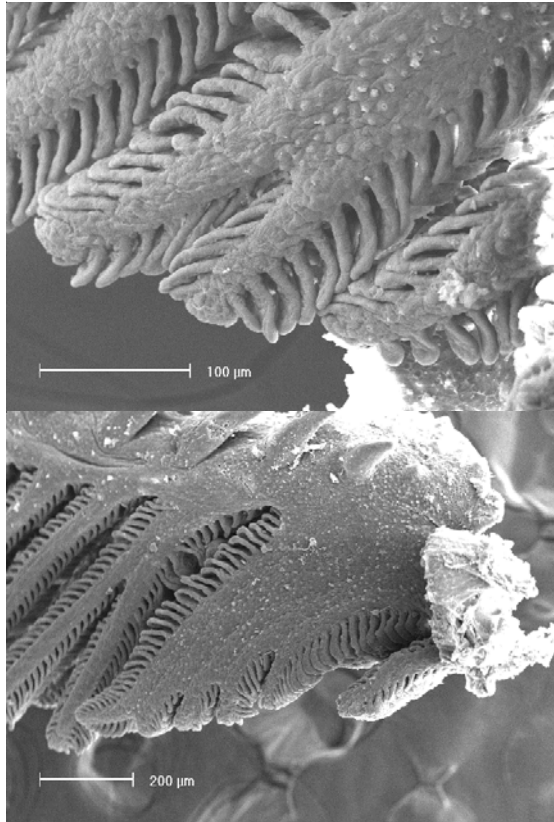
Fig. 3. Lateral view of the ventralmost portion of the first gill arch of two males of *Aphyocharax anisitsi* collected in February 2001 in the arroio do Salso, Rosário do Sul, RS. (A) specimen with the initial development of the gill gland seen through the fusion of two gill filaments. (B) specimen with a fully developed gill gland seen through the fusion of five gill filaments (SEM).

nual day-length variations as small as 1.6 hours have been shown to significantly influence reproduction in low-latitude fishes (Burns, 1985; Burns & Flores, 1981). In temperate southern Brazil, the site of our collections, environmental conditions during spring and summer appear to be most favorable for the survival of the fry, when food availability is greatest.

In tropical regions, where the annual variations of water temperature and day-length may be minimal, rainfall appears to be a major environmental factor influencing reproductive cycling (Vazzoler & Menezes, 1992). In these areas, the increase in water levels provides better shelter for the fry, as well as a more abundant food supply. On the other hand, in southern Brazil, where no definitive pattern in monthly precipitation is evident, fish reproductive cycles are generally not correlated with rainfall (Vazzoler & Menezes, 1992). This is the case with several characid species from southern Brazil (Azevedo, 2000; Azevedo *et al.*, 2000; Oliveira *et al.*, 2002). However, in the present study, the mean monthly GSIs of female *Aphyocharax anisitsi* did show a significant negative correlation with monthly rainfall levels, although male GSIs showed no such pattern.

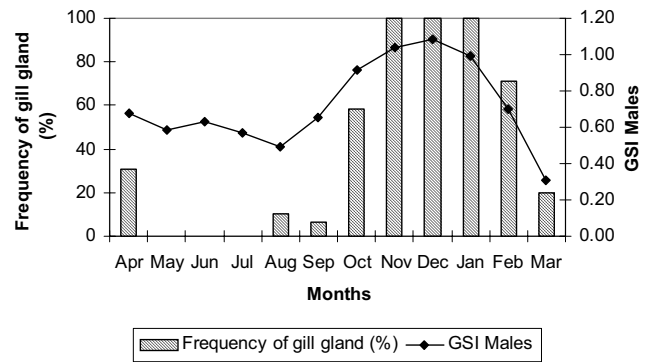The positive correlations between absolute fecundity and

Fig. 4. Monthly variation of the GSI mean values and frequency of occurrence (%) of males of *Aphyocharax anisitsi* bearing gill glands, collected from April 2001 to March 2002 in the arroio do Salso, Rosário do Sul, RS, Brazil.

both SL and Wt in *A. anisitsi* demonstrate that larger specimens are able to produce greater numbers of eggs. Vazzoler (1996) also found that absolute fecundity increases with female development, being more related to body size than age. Differences in mean body size may explain the disparity between the values for absolute fecundity in *A. anisitsi* (345 oocytes, mean SL 28 mm) in the present study and that of *Aphyocharax alburnus* (617 oocytes, mean SL 37 mm) in the study of Winemiller (1989). There is little information on relative fecundity in characids. Relative fecundity allows the comparison of the energetic effort spent in egg production in species of different sizes. Table 2 presents both absolute and relative fecundity for 11 small characid species from southern Brazil. Mean relative fecundity in *A. anisitsi* was 0.68 oocytes/mg

Table 2. Absolute and relative mean fecundity values (± Standard Deviation) of small size characids. Relative fecundity values = Number of oocytes / mg of total body weight.

Species	Absolute fecundity	Relative fecundity
Aphyocharax anisitsi This study	344.8 (± 116.4)	0.68 (± 0.14)
Bryconamericus iheringii (Lampert, 2003)	933.71 (±303.1)	0.36 (±0.08)
Bryconamericus stramineus (Lampert, 2003)	371.3 (±244.6)	0.35 (±0.08)
Cheirodon ibicuhiensis (Oliveira *et al.*, 2002)	513 (±230.06)	0.5 (±0.11)
Compsura heterura (Oliveira, 2003)	434 (±112)	0.55
Diapoma speculiferum (Azevedo *et al.*, 2000)	491.1 (±159.5)	0.4101 (±0.120)
Diapoma terofali (Azevedo, pers. comm.)	322.63 (±149.35)	0.565 (±0.098)
Mimagoniates microlepis (Azevedo, 2000)	109.33 (±56.51)	0.27 (±0.08)
Mimagoniates rheocharis (Azevedo, 2000)	421.05 (±193.5)	0.356 (±0.098)
Odontostilbe pequira (Oliveira, 2003)	722 (±1.79)	0.71
Pseudocorynopoma doriae (Azevedo *et al.*, 2000)	980 (±254.26)	0.3412 (±0.1342)
Serrapinnus calliurus (Azevedo *et al.*, 2000)	406	0.631

of total body weight, demonstrating that this species expends a large amount of energy in egg production compared to other characid species of similar size.

The presence of fin hooks in males has been occasionally suggested as seasonal for some species of characid fishes (von Ihering & Azevedo, 1936; Collette, 1977; Garutti, 1990), based on the large occurrence of fin rays with hooks in males during the reproductive season and its absence or small occurrence in the remaining periods. In other papers, these structures are considered permanent once developed (Andrade *et al.*, 1984; Silva *et al.*, 1996; Azevedo, 2000; Lampert, 2003). In *A. anisitsi*, all males captured during the reproductive season had well developed anal-fin hooks, whereas during the other months some males had anal-fin hooks and others did not. We hypothesize that these hooks, once developed, are retained by the males, and that males showing no hooks are young males that have not yet begin to mature. The statistically significant smaller size of the males without hooks than that observed in males with hooks supports this hypothesis.

Male secondary sex characters, such as fin hooks and gill glands, appear to depend on gonadal hormones, particularly androgens, for their development. Hence, their appearance and increase in size should parallel gonadal development. Supporting this is the positive correlation between mean GSI and the number of anal-fin rays bearing hooks in *A. anisitsi*. In addition, a greater frequency of males with developed gill glands was found during months with higher mean GSIs. In fact, during the three months of peak mean GSIs (November-January), all males collected had gill glands. Gill glands may be involved with the production of a chemical signal related in some way with reproduction, possibly courtship and/or intermale aggression (Bushmann *et al.*, 2002). Among characids, gill glands are found in a number of genera of the subfamily Glandulocaudinae, perhaps all genera of the subfamily Cheirodontinae, and species in the genera *Bryconamericus*, *Knodus* and *Hemibrycon* (Bushmann *et al.*, 2002), *Attonitus* and *Bryconadenos* (Weitzman *et al.*, 2005).

Acknowledgements

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