

Reproductive biology and development of gill glands in the inseminating characid, *Macropsobrycon uruguayanae* Eigenmann, 1915 (Cheirodontinae: Compsurini)

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The reproductive biology and development of the gill gland are described for *Macropsobrycon uruguayanae*, an inseminating characid species of the tribe Compsurini, subfamily Cheirodontinae. Between April 2001 and March 2002, 117 males and 143 females of this species were collected in the rio Ibicuí, Uruguay basin in the State of Rio Grande do Sul, Brazil. Reproductively active individuals were present during most months sampled, indicating lack of a well-defined seasonal reproductive period. Several maturing females were found to be inseminated before completing full maturation. Histological analyses demonstrated spermatozoa within the ovaries of females in different stages of gonadal maturation collected during most months. No immature females had inseminated ovaries. Standard length at first gonadal maturation was estimated to be 24 mm for both males and females. Mean absolute fecundity was 191.08 (\pm 48.83 SD) oocytes per female, one of the lowest among characids. Relative fecundity was 0.539 (\pm 0.069 SD) oocytes per mg weight of the female, a value similar to that found for the majority of species of Cheirodontinae. The presence of two cohorts of oocytes within ovaries of *M. uruguayanae* indicates synchronous development, with total spawning. The mean diameter of mature oocytes was 0.6711 (\pm 0.1252 SD) mm, smaller than that found for the majority of species of Characidae. Gill glands occurred in all mature males, as well as in males undergoing advanced maturation. In the latter case, fewer gill filaments comprised the glands. Gill glands were not observed in immature males, males undergoing the initial stages of maturation, or in any female. A given gill gland may comprise as many as 24 filaments of the lateral hemibranch of the first gill arch. Secondary lamellae within most of the gill gland are greatly reduced, with columnar cells being present between them. These columnar cells contain abundant vesicles, suggesting secretory activity. The morphology of the gill gland of *M. uruguayanae* resembles that found in the majority of characid species that possess this structure.

São descritos a biologia reprodutiva e o desenvolvimento da glândula branquial de *Macropsobrycon uruguayanae*, uma espécie de caracídeo inseminador da tribo Compsurini, subfamília Cheirodontinae. Foram capturados 117 machos e 143 fêmeas da espécie entre abril de 2001 e março de 2002 no rio Ibicuí, bacia do rio Uruguay no Estado do Rio Grande do Sul, Brasil. Indivíduos em reprodução foram observados na maioria dos meses amostrados, não havendo período reprodutivo sazonalmente definido. Fêmeas em maturação apresentavam-se inseminadas antes de completar a maturação plena. Análises histológicas mostraram espermatozoides nos ovários de fêmeas em diferentes estádios de maturação gonadal coletadas na maioria dos meses. Nenhuma fêmea imatura apresentou ovários inseminados. O tamanho de primeira maturação gonadal foi estimado em 24 mm de comprimento padrão para machos e fêmeas. A fecundidade absoluta média foi de 191,08 (\pm 48,83 SD) ovócitos por fêmea, uma das mais baixas entre caracídeos. A fecundidade relativa foi de 0,539 (\pm 0,069 SD) ovócitos por mg do peso da fêmea, valor semelhante ao encontrado para a maioria das espécies de Cheirodontinae. A espécie mostrou desenvolvimento ovocitário do tipo sincrônico em dois grupos, indicando desova total. O diâmetro médio dos ovócitos maduros foi de 0,6711 (\pm 0,1252 SD) mm, menor do que o encontrado para a maioria das espécies de Characidae. A glândula branquial ocorreu em todos os machos maduros analisados, sendo também observada em machos em maturação avançada, porém envolvendo um número menor de filamentos branquiais. A glândula branquial não foi observada em machos em maturação inicial ou imaturos e em fêmeas em qualquer fase de maturação. Esta glândula pode compreender até 24 filamentos da hemibrânquia externa do primeiro arco. As lamelas secundárias da glândula branquial são reduzidas e há proliferação de células secretoras colunares entre elas. Estas células são preenchidas por inúmeros vacúolos, sugerindo intensa atividade secretora. A morfologia da glândula branquial de *M. uruguayanae* é muito semelhante à da maioria das espécies de Characidae que possuem esta estrutura.

Key words: Reproductive period, Fecundity, Insemination, Sexual dimorphism.

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Introduction

Cheirodontinae is a subfamily of Characidae composed of small Neotropical freshwater fishes commonly found in lentic environments. They are present in the majority of river drainages in South and Central America. Adult individuals of most species reach a maximum standard length of 30-40 mm. Two tribes are recognized in the subfamily (Malabarba, 1998), as well as some genera *incertae sedis*. One of the tribes, Compsurini, comprises five genera, including species that are inseminating, in addition to having modifications of scales, fin-rays and fin hooks (Burns *et al.*, 1997, 1998; Malabarba, 1998; Malabarba & Weitzman, 1999, 2000; Malabarba *et al.*, 2004). Insemination is characterized by the transfer of spermatozoa by males to the ovaries of females. However, the exact moment of fertilization of the oocytes is unknown for any inseminating characid (Burns *et al.*, 1995, 1997, 1998, 2000). Several investigators have hypothesized that insemination within the Cheirodontinae may have evolved independently from that in other groups of characids (Burns *et al.*, 1997; 1998), with Malabarba & Weitzman (2003) suggesting at least three separate origins within Characidae.

Many inseminating species of Characidae are characterized by modifications of both the testis and spermatozoon. Within Compsurini, the sperm nucleus is moderately elongate in the majority of species, including *Macropsobrycon uruguayanae* (Oliveira *et al.*, 2008), being spherical only in the two species of the genus *Kolpotocheirodon* (Burns *et al.*, 1997; Malabarba *et al.*, 2004). Some studies have also shown unusual characteristics relating to other aspects of reproduction in inseminating species, such as fecundity and duration and seasonality of the reproductive period (Kramer, 1978; Winemiller, 1989; Menni & Almirón, 1994; Azevedo *et al.*, 2000; Silvano *et al.*, 2003; Oliveira *et al.*, 2010). Although some studies on reproduction in species of Cheirodontinae have been carried out (Sendra & Freyre, 1981a, 1981b; Winemiller, 1989; Menni & Almirón, 1994; Gelain *et al.*, 1999; Oliveira *et al.*, 2002; Silvano *et al.*, 2003; Oliveira *et al.*, 2010), the only data available for an inseminating species comes from the work of Oliveira *et al.* (2010) on *Compsura heterura*. The presence of gill glands, characterized by hypertrophied glandular tissue on the first gill arch, has been described in both externally fertilizing and inseminating species of Characidae, including some species of the Stevardiinae and Compsurini (Burns & Weitzman, 1996; Bushman *et al.*, 2002; Burns & Weitzman, 2005; Oliveira *et al.*, 2010).

In the present work, data on the reproductive biology of the inseminating cheirodontine species, *Macropsobrycon uruguayanae*, of the tribe Compsurini, are presented, including analyses of possible seasonal reproduction, period of insemination, fecundity, type of ovarian development, diameter of mature oocytes and size at first gonadal maturation. In addition, the morphology of the gill gland is described, as well as its development associated with testis maturation.

Material and Methods

Specimens of *M. uruguayanae* were collected monthly between April 2001 and March 2002 in the rio Ibicuí (29°50' 14"S 54°47' 53"W), Uruguay River basin, in the divide between the municipalities of Cacequi and São Vicente do Sul, State of Rio Grande do Sul, southern Brazil. Fishes were caught with nets and dipnets (meshes 5 mm and 2.5 mm between knots, respectively). The rio Ibicuí, at the sampling point, is characterized by having transparent to cloudy water, with a sandy bottom and current varying from medium to rapid, with the presence of adjacent pools and flooded areas.

In the field, fish were fixed in 10% formalin. Water temperature was also taken. Rainfall data for the area were obtained from 8th District of Meteorology, and data on day length were obtained with a GPS instrument.

In the laboratory, specimens were transferred to 70% ethanol, and standard lengths (SL) and total weight (WT) determined for each individual. Specimens were dissected to establish sex and stage of gonadal maturation. Both gonads were then removed and weighed (Wg). The gonadosomatic index (GSI) was calculated according to the following formula (Vazzoler, 1996): $GSI = (Wg \times 100) / WT$.

The reproductive period was analysed according to the monthly variation of GSI of males and females, and from the frequencies of males and females with mature gonads and those in advanced stages of maturation. Staging of gonadal maturation followed Vazzoler (1996) and Azevedo *et al.* (2000).

To estimate size at first gonadal maturation for males and females, distributions of the relative frequencies of juveniles (individuals with gonads not developed) and adults (individuals with developed gonads or in development) per class of standard length were determined. The results were adjusted according to the following mathematical expression (Santos, 1978):

$$Fr = 1 - (e^{-aL^b})$$

where: Fr = relative frequency of adult individuals; e = base of natural logarithm; Lt = standard length in mm; a and b = constants. The size at first gonadal maturation was considered that at which 50% of the population are adults (Santos, 1978).

Ovaries of females caught during various months of the year were analyzed histologically to determine if spermatozoa were present (= insemination). Absolute fecundity was estimated based on the total counts of vitellogenic oocytes of thirteen mature females, whereas relative fecundity was determined by the number of vitellogenic oocytes per milligram of body weight for these same females (Adebisi, 1987). Type of ovarian development followed Vazzoler (1996) and was based on analysis of the relative frequencies of diameter classes of oocytes in mature females. The variation and means of the diameters of mature oocytes in these females were also determined. Values for absolute fecundity, relative fecundity, and mature oocyte mean are presented.

Testes, ovaries and first gill arches were processed for microscopic analysis. Right and left first gill arches were removed from selected males undergoing different stages of gonadal maturation collected during different months, as well as from some females, to determine the occurrence of gill glands and to describe its structure using light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). For light microscopy, gonads and gill arches were routinely processed and embedded either in glycol methacrylate resin or paraffin. Sections were stained with toluidine blue, hematoxylin and eosin (H&E), periodic acid-Schiff reagent/hematoxylin (PAS/H) or a modified Masson's trichrome (Schreibman, 1964). For TEM analysis, tissues were dehydrated in acetone, post-fixed in osmium tetroxide, stained with 0.5% uranyl acetate and embedded in araldite resin. Semi-thin sections were obtained using a Leica RM2165 ultramicrotome. Ultra-thin sections obtained using a Leica Ultracut UCT ultramicrotome were stained with a saturated solution of uranyl acetate in 50% ethanol and 0.2% lead citrate solution in 1 N NaOH. Sections were viewed with a Phillips CM 100 transmission electron microscope. For SEM analysis, specimens were dehydrated in an ethanol series, critical point dried, attached to carbon strips on stubs, coated with carbon and gold and viewed with a Phillips XL30 scanning electron microscope.

Results

A total of 260 individuals of *M. uruguayanae* were studied, which included 117 males (21.93-33.08 mm SL) and 143 females (17.18-34.04 mm SL). No specimens were obtained during the months of August, October, December and January. Water temperature at the sampling site varied between 17°C and 27°C over the course of the year, increasing between October and March. Rainfall showed peaks in June, September and March, and day length was longest from September to March (Table 1).

With respect to mean GSI values, no clear seasonal pattern was evident for males (Table 1; Fig. 1A). Specimens with high

GSI values were observed along all sampled months. For females, GSI means were lower in May, June, July and November, and higher in September, February and March (Table 1; Fig. 1B). Mature males and/or males in an advanced stage of maturation were present during all months sampled (Fig. 2A). For females, on the other hand, mature fish and/or those in an advanced stage of maturation were present only during May, June, September, February and March (Table 1; Fig. 2B). No females showing these stages were collected during July or November. Thus, a well-defined reproductive season was not evident for this species.

No ovaries of immature females examined histologically were inseminated, *i.e.*, no spermatozoa were present. However, ovaries of some females undergoing the initial or intermediate stages of maturation contained spermatozoa (Fig. 3A). The ovaries of all females in an advanced stage of maturation or fully mature were inseminated (Fig. 3B). Inseminated ovaries were observed in females collected during the months of May, June, September, February and March. No female collected during July or November was inseminated. During the other months of the year, no females were collected. Ovaries lacked any distinct sperm storage regions, with spermatozoa being found throughout the organ. Spermatozoa possessed elongate nuclei as described and figured in Oliveira *et al.* (2008), but no evidence of sperm packaging was seen either in the testis (Fig. 3C-D) or ovary (Fig. 3A-B).

Standard length at first gonadal maturation was estimated to be approximately 24 mm for both males and females. Standard lengths of females used in the fecundity analysis varied between 25.1 mm and 31.4 mm. GSI of these females varied from 7.0 to 9.8. The number of mature oocytes in the ovaries of these females varied between 98 and 260. Mean absolute fecundity of the species was estimated to be 191.08 (± 48.83 SD) oocytes and relative fecundity 0.539 (± 0.069 SD) oocytes per mg body weight (Fig. 4).

Analysis of the frequencies of diameter classes of oocytes of mature females of *M. uruguayanae* (Fig. 5) showed two peaks of oocyte size. The first peak comprises oocytes of

Table 1. Monthly variation of day length (min) and rainfall (mm) for the region of Cacequi, RS, water temperature of the rio Ibicuí, and monthly means of gonadosomatic index (GSI) of males and females of *Macropsobrycon uruguayanae*. n = number of individuals captured. IGS means were calculated based only in adult specimens. There were no males collected in August, October, December, and January. A single adult male was captured in November.

Month	Photoperiod (min)	Rainfall (mm)	Water temperature (°C)	GSI males	n	GSI females	n
May	641	196.8	17.0	1.898	13	1.342	13
June	614	319.2	-	1.109	39	0.914	44
July	617	162.8	17.7	1.294	10	0.678	3
August	648	116.9	20.1	-	-	-	-
September	722	430.4	17.8	1.921	9	7.208	22
October	778	181.6	22.5	-	-	-	-
November	836	174.5	22.9	-	4	0.836	4
December	846	063.6	27.0	-	-	-	-
January	817	084.8	26.0	-	-	-	-
February	808	023.4	24.0	2.096	27	2.902	51
March	753	342.6	22.5	1.944	15	4.259	6
Total					117		143

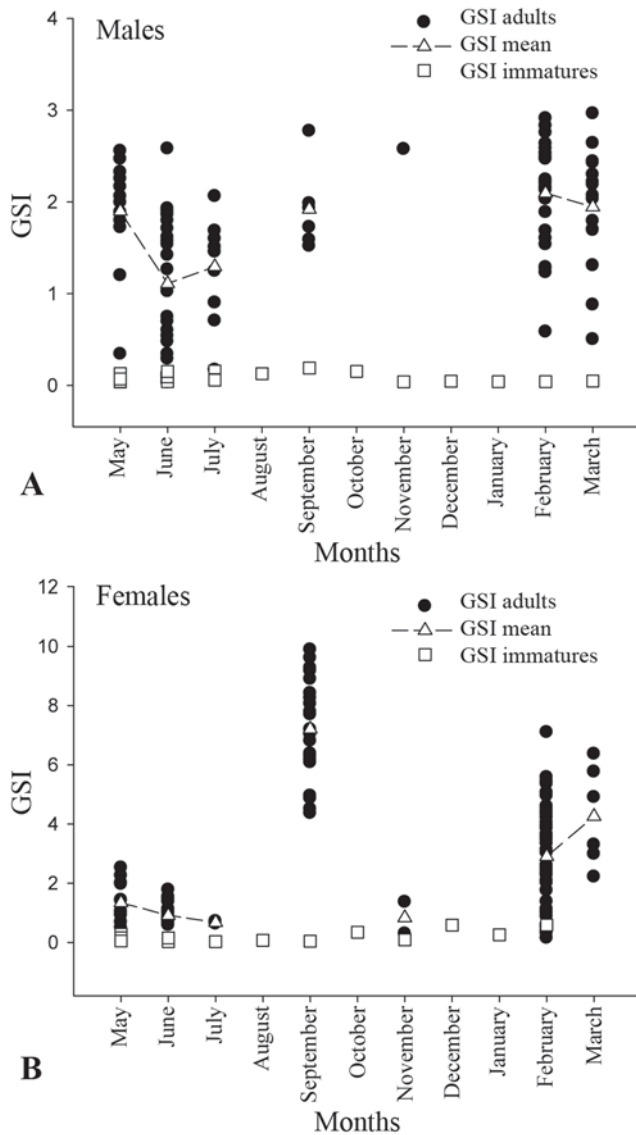


Fig. 1. Monthly distribution of the gonadosomatic index (GSI) values of males (A) and females (B) of *Macropsobrycon uruguayanae*. IGS means (triangles) were calculated based only in adult specimens (circles), excluding immature specimens (squares). There were no males collected in August, October, December, and January. A single adult male was captured in November.

small diameter, representing oocytes in reserve, whereas the second peak consists of larger oocytes, representing mature yolky oocytes. Thus, two distinct cohorts of oocytes are present in mature ovaries, indicating synchronous ovarian development (Vazzoler, 1996). The diameter of mature oocytes varied between 0.4056 mm and 1.0140 mm, with a mean of 0.6711 (± 0.1252 SD) mm.

Tall columnar cells within the gill lamellae of the first gill arch, as well as fusion of adjacent gill filaments, occurred in all mature males analyzed. These “gill glands” were also

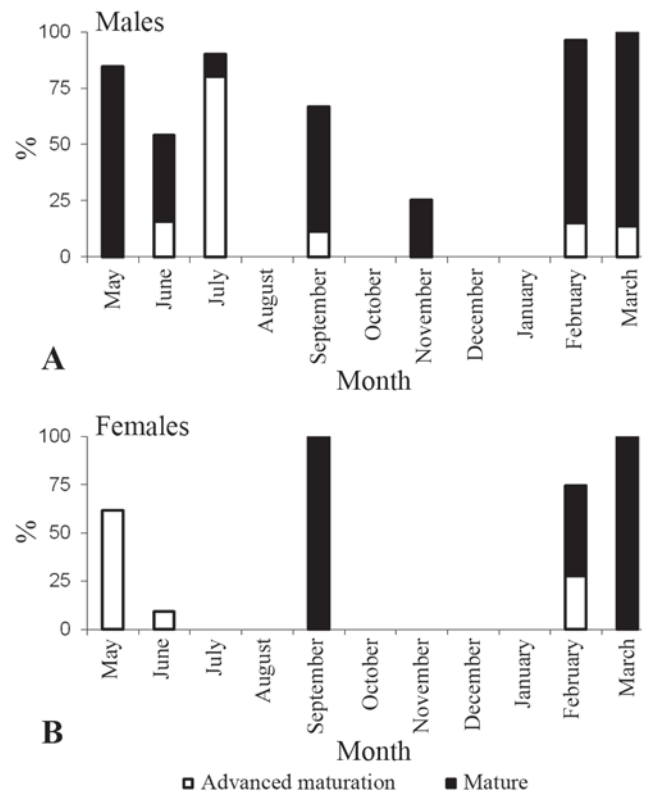


Fig. 2. Relative frequency of males (A) and females (B) of *Macropsobrycon uruguayanae* with mature gonads (dark bars) and gonads in advanced stages of maturation (white bars).

observed in males in an advanced stage of maturation where they were comprised of fewer gill filaments. In females, immature males or males undergoing the initial stages of maturation, modifications of the gill tissues were not observed. The gill glands of *M. uruguayanae* can comprise upwards of 24 gill filaments of the lateral hemibranchs of the first gill arches, thus occupying a substantial portion of the first gill arches (Figs. 6A, 7A-B). During gill gland development, fusion of adjacent gill filaments begins on the most anterior filaments, starting at the base of each filament by expansion of the epithelium that covers it (Fig. 6B). This epithelium eventually covers nearly the entire length of the filaments, with only the most distal portions remaining free. These unfused distal regions constitute the opening of the chambers that are formed internally (Fig. 6C-D). Light microscopy showed that the secondary lamellae of the filaments involved in the formation of the gill gland are greatly reduced (Fig. 6D). Between the secondary lamellae, there is a proliferation of tall columnar cells whose nuclei are localized near the base (Fig. 7C-D). These cells form a single layer lining the inside of the individual gill gland chambers (Fig. 7A-D). With TEM, the columnar cells contain abundant electron-lucent vesicles, some of which contain more electron-dense material (Fig. 8A-B).

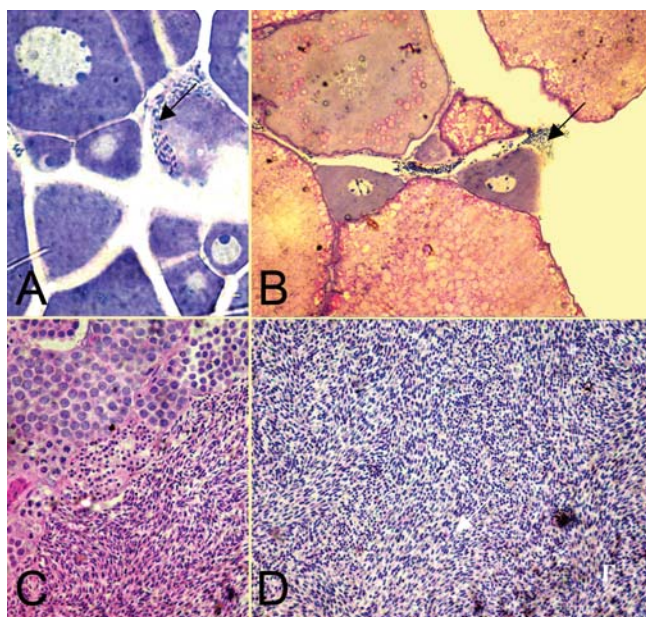


Fig. 3. Ovaries and testis. Sagittal sections (3mm), stained with hematoxylin and eosin (H&E). A, ovary of a maturing female with previtellogenic oocytes containing spermatozoa (arrows) in the lumen (specimen collected in July 2001; 27.8 mm SL; IGS=0.89). Magnification: 10x10. B, ovary of a mature female containing spermatozoa in the lumen (arrows) (specimen collected in September 2001; 28.9 mm SL; IGS=4.87). C, testis showing initial phases of spermiogenesis and spermatozoa. D, testis containing spermatozoa. B, C, D magnification: 40x10.

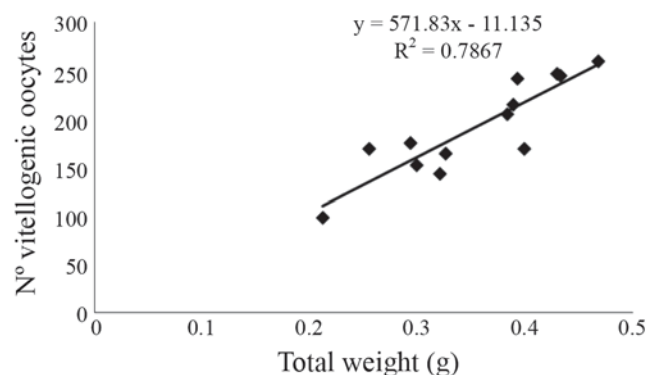


Fig. 4. Total counts of vitellogenic oocytes versus body weight (g) of thirteen mature females of *Macropsobrycon uruguayanae*.

Discussion

Most species of Characiformes studied to date show some type of seasonal reproductive pattern, with peak reproduction normally occurring during the spring and summer months (Vazzoler & Menezes, 1992). Within temperate regions, annual fluctuations in temperature and day length appear to be the

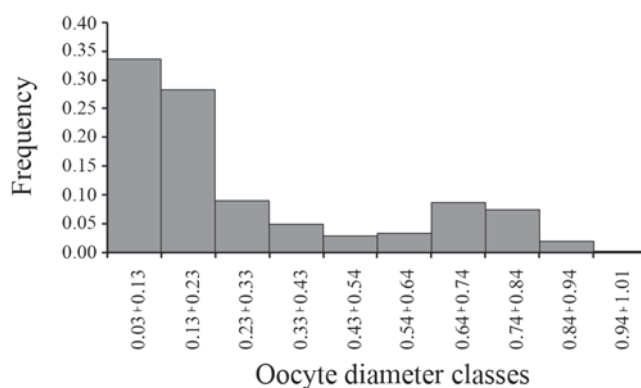


Fig. 5. Relative frequency of the ranges of oocyte diameter of mature females of *Macropsobrycon uruguayanae*.

most important factors influencing seasonal reproduction (de Vlaming, 1974; Burns, 1976). In tropical areas, on the other hand, the seasonal increase in rainfall, often associated with a concomitant increase in the availability of food, is considered to be the most important parameter affecting reproductive seasonality (Vazzoler & Menezes, 1992). In *M. uruguayanae*, the presence of mature males, as well as males in advanced stages of maturation, during all months sampled, along with finding inseminated females during most of the sampling period, suggest that this species lacks a well-defined seasonal reproductive period, with reproduction possibly occurring during most months or even throughout the year.

Regarding other species of Cheirodontinae whose annual reproductive patterns have been studied, some show a single annual reproductive period, as described by Vazzoler & Menezes (1992) for most Characiformes, whereas others display two peaks of reproductive activity annually. Populations of the externally fertilizing *Odontostilbe pulcher* and *Cheirodontops geayi* in Venezuelan streams showed single seasonal reproductive periods lasting approximately five and two months, respectively (Winemiller, 1989). A population of the externally fertilizing *Cheirodon interruptus* in the Chascomus Lagoon, Argentina, showed two periods of maturation, one between February and May and the other between June and September (Sendra & Freyre, 1981a). Another population of this same species was studied by Menni & Almirón (1994) in man-made lakes close to La Plata, Argentina, with the results showing high frequencies of mature individuals between August and November (end of winter and spring) and January and April (summer). Oliveira *et al.* (2002) studied a population of the externally fertilizing *Cheirodon ibicuihensis* in a stream in the south of Brazil and found a long seasonal reproductive period beginning in September and lasting through February (months corresponding to spring and summer). Gelain *et al.* (1999), studying a population of the externally fertilizing *Serrapinnus calliurus* in the same stream, also observed extended seasonal reproduction during the spring and summer months. Silvano *et al.* (2003) studied a population of the externally fertilizing

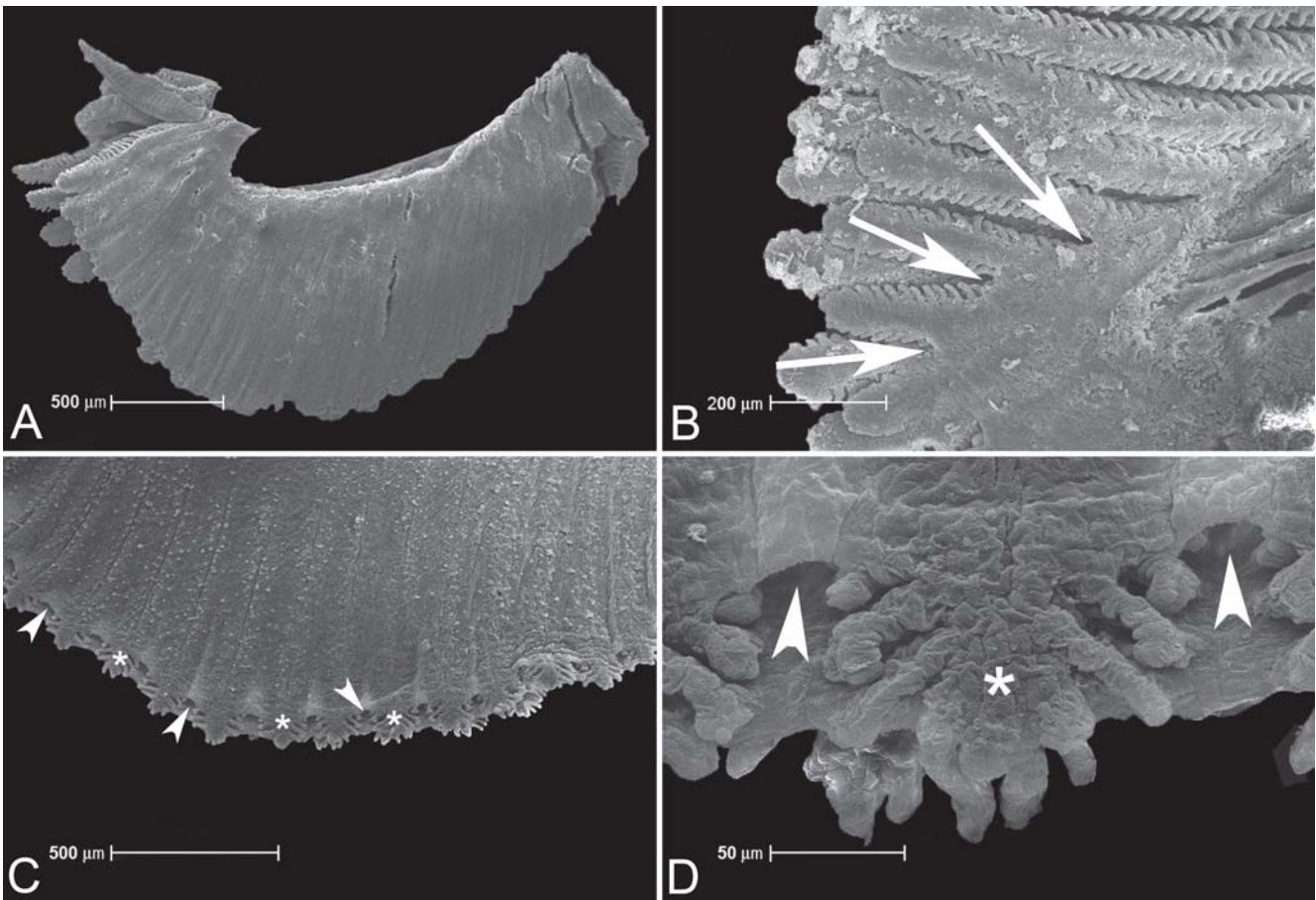


Fig. 6. Scanning electron microscope images of a gill gland on the first right gill arch of males of *Macropsobrycon uruguayanae*; anterior is to the right; A, general appearance of the gill arch of a mature male bearing a gill gland, showing 24 modified gill filaments that are fused and covered by epithelial tissue; B, gill arch of a maturing male, showing the development of the epithelial tissue (arrows) starting to fuse the gill filaments at their bases to form the gill gland; C, D, ventral edge of the gill gland of a mature male showing the free ends of the filaments (asterisks) and the opening of the gill gland chambers (arrowheads). (A, C, D - specimen collected in February 2002; 32.0 mm SL; IGS = 2.48; B - specimen collected in June 2001; 26.9 mm SL; IGS = 0.60).

Serrapinnus piaba in a river in northeast Brazil and found a well-defined reproductive period (summer and beginning of fall) only for the females of the species, with males having active gonads throughout the year. The mean GSIs of the collections for the females of this species showed a positive correlation with rainfall and water temperature.

Oliveira *et al.* (2010) studied populations of the externally fertilizing *Odontostilbe* sp. (= *O. pequirá*, LRM pers. obs.) and inseminating *Compsura heterura*, two species of Cheirodontinae from the south and northeast of Brazil, respectively. *Odontostilbe* sp. showed two reproductive peaks, between September and October and between January and February, being significantly correlated with day length. For the compsurin *C. heterura*, a seasonal reproductive period occurred between January and April. Monthly GSIs showed correlations with water temperature in both sexes, but only female GSIs were correlated with rainfall. No other cheirodontine studied appears to spawn throughout the year as observed for the inseminating *M. uruguayanae*. Such an

extended period of reproduction may represent a novel reproductive strategy among cheirodontines, allowing the insemination to occur all along the year and females to spawn always when favorable conditions are available.

Regarding other inseminating species of Characidae, data on reproduction are available only for members of the Glandulocaudinae and Stevardiinae. Some species of Stevardiinae have a single reproductive period during spring and summer (Menni & Almirón, 1994; Azevedo *et al.*, 2000), others are characterized by more than one reproductive period during the year (Menni & Almirón, 1994) and some show mature individuals during most months or even the entire year (Kramer, 1978; Winemiller, 1989). Regarding the Glandulocaudinae, *Mimagoniates microlepis* reproduces during the winter, whereas *M. rheocharis* shows sexually active specimens throughout most of the year (Azevedo, 2000).

Males and females of *M. uruguayanae* reach their first gonadal maturation at approximately the same length (24 mm). Thus, there does not appear to be differences in the rates of

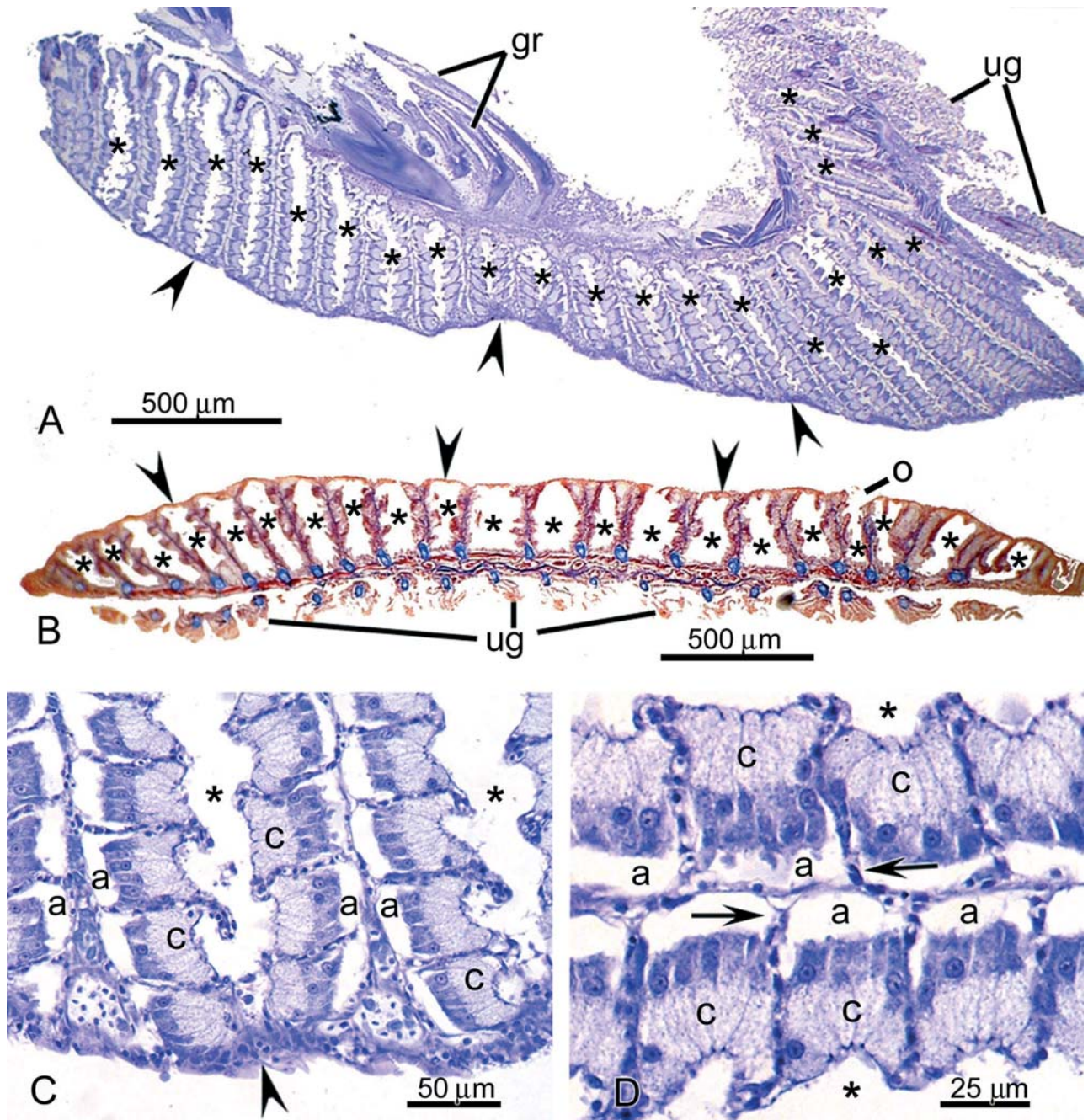


Fig. 7. Light micrographs of sections through gill glands of two mature male *Macropsobrycon uruguayanae*; A, C, D, MCP 11939, 28.2 mm SL, glycol methacrylate, toluidine blue; B, MCP 18588, 39.0 mm SL, paraffin, modified Masson's trichrome. A, sagittal section showing gill gland of first gill arch formed from at least 23 modified gill filaments that are fused and covered distally by epithelial tissue (arrowheads) thus forming enclosed chambers (lumen, asterisks); anterior is to the left; unmodified gill filaments (ug) are seen at the posterior region of the gill arch; gill rakers (gr). B, frontal section through ventral region of first gill arch showing a gill gland made up of at least 24 modified gill filaments; each chamber (lumen, asterisk) that is covered distally by epithelial tissue (arrowheads) over most of the surface of the gland eventually opens (o) into the gill chamber ventrally. Note that the gill gland is only formed on the distal side of the first gill arch, unmodified gill filaments (ug) are seen medially. C, higher magnification of the gill gland in "A" showing columnar cells (c) between reduced secondary lamellae; epithelial cover (arrowhead) of gill chambers (lumen, asterisk); artifact space (a). D, higher magnification of "C" showing columnar cells (c) with basal nuclei and granular apical cytoplasm in between greatly shortened secondary lamellae (arrow); artifact spaces (a) due to shrinkage during tissue preparation actually help demonstrate the integrity of the reduced secondary lamellae; gill gland chamber lumen (asterisk).

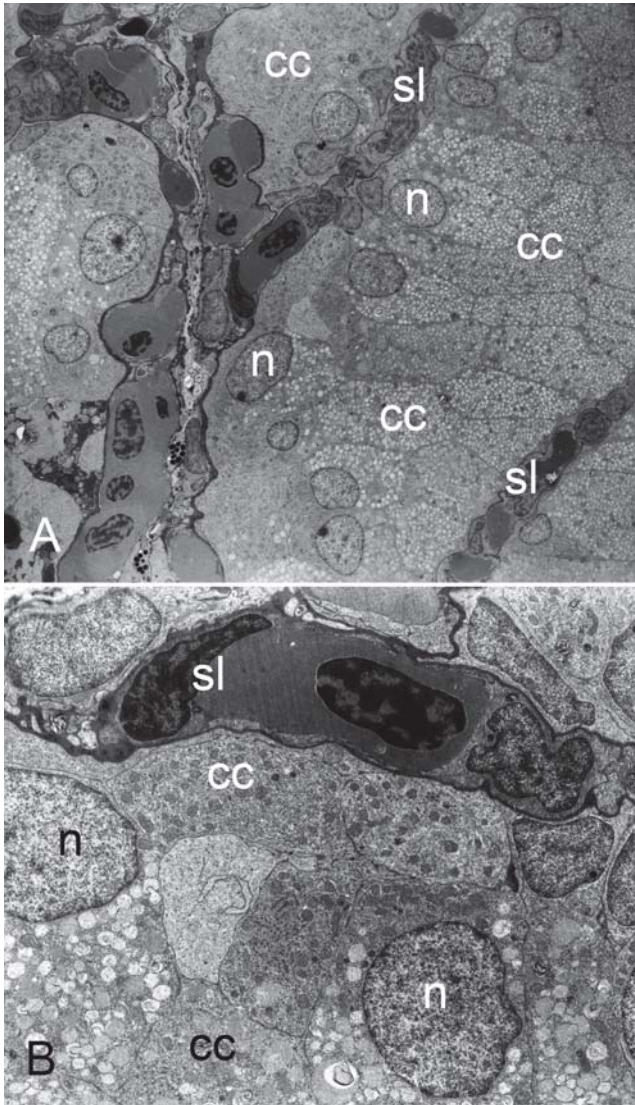


Fig. 8. Oblique section through the gill gland on the first right gill arch of a mature male *Macropsobrycon uruguayanae* examined by transmission electron microscopy, showing columnar cells (cc) with nucleus (n) near the base of the cell and cytoplasm filled with electron-lucent vesicles between the reduced gill secondary lamellae (sl). In B, note that some vesicles contain more electron-dense material. A, magnification: 1290x. B, magnification: 4135x.

birth, mortality, growth and maturation between the sexes. During some months, the majority of the population has developed gonads, indicating that most of the males and females are sexually active. These data suggest that there are no dominant individuals in the population that inhibit the development of others, as shown for the stevardiine, *Corynopoma riisei* (Bushmann & Burns, 1994).

Absolute fecundity of *Macropsobrycon uruguayanae* was shown to be low (191 oocytes) in comparison with externally fertilizing species of Cheirodontinae such as *Odontostilbe pequirá* (722 oocytes; Oliveira *et al.*, 2010),

Cheirodon ibicuhiensis (513 oocytes; Oliveira *et al.*, 2002), *Cheirodon interruptus* (400 oocytes; Sendra & Freyre, 1981b; Vazzoler & Menezes, 1992) and *Serrapinnus calliurus* (406 oocytes; Gelain *et al.*, 1999), and the inseminating *Compsura heterura* (434 oocytes; Oliveira *et al.*, 2010). Absolute fecundity of *M. uruguayanae* is very low among all other Characidae that have this information available (Azevedo, 2004). Relative fecundity of *M. uruguayanae* (0.539 oocytes per mg of female weight), on the other hand, was closer to that found for the above-mentioned species (*O. pequirá*, 0.71; *C. ibicuhiensis*, 0.5; *S. calliurus*, 0.631; and *C. heterura*, 0.55), as well as to other Characidae that have this information available (Azevedo, 2004).

Information on the diameter of mature yolky oocytes is lacking for the majority of characid species. These data can be useful for interpreting reproductive strategies used by a given species. Some species produce larger oocytes, a strategy that presumably increases the probability of survival of the eggs and larvae, whereas others invest energy in the production of a greater number of smaller oocytes. Kramer (1978) presented ranges of mature oocyte diameters for six species of Characidae from Panama (*Bryconamericus emperador*, 1.2-1.5 mm; *Brycon petrosus*, 1.6-1.9 mm; *Piabucina panamensis*, 1.5-1.7 mm; *Hyphessobrycon panamensis*, 0.7-0.8 mm; *Gephyrocharax atricaudata*, 0.7-0.8 mm; *Roeboides guatemalensis*, 0.85-1.0 mm). For the inseminating stevardiine, *D. terofali*, the diameter of mature oocytes varied from 0.54 to 1.21 mm, with a mean of 0.83 mm (Azevedo, 2004). The diameters of mature oocytes of *M. uruguayanae* ranged from 0.40 to 1.01 mm (mean 0.67 mm), being smaller than those of the majority of species reported above.

Macropsobrycon uruguayanae shows small body size, small fecundity and small size of the oocytes, with relative fecundity similar to that observed in other characid species. Thus, there appears to be little difference in the production of oocytes by weight among characids, suggesting a similar allocation of energy in species of the Cheirodontinae or Characidae, whether or not they are inseminating.

The presence of two cohorts of oocytes within ovaries of *M. uruguayanae* indicates synchronous development. One batch of oocytes (previtellogenic) presumably functions as a reserve stock, while the other (yolky oocytes) will be eliminated during the spawning period (Wallace & Selman, 1981). This type of ovarian development often results in "total" spawning. According to Vazzoler (1996), total spawning occurs in species that spawn periodically throughout their lives by releasing only one lot of oocytes during each reproductive period, and is common in species that carry out long reproductive migrations. It is not known if spawning *M. uruguayanae* females release their entire batch of yolky oocytes all at once, or if small batches of oocytes, recruited from the yolky cohort, are laid over the course of a given spawning period. Given that *M. uruguayanae* may reproduce throughout the year, multiple spawnings are also a possibility, with new batches of mature oocytes developing from the reserve

cohort. Other species of the Cheirodontinae, such as *S. calliurus* (Gelain *et al.*, 1999) and *C. ibicuihensis* (Oliveira *et al.*, 2002), show asynchronous spawning, that is, more than one lot of oocytes is released during a given reproductive period.

Regarding life history traits, Winemiller (1989) reported various species of fishes of Venezuela as having an “opportunistic strategy,” characterized by short generation time, low fecundity and minimal investment in progeny. This strategy was associated with species of small size that remained reproductively active despite the apparent high rate of mortality during the unfavorable environmental conditions of the dry season. The growth of populations of “opportunist” species was attributed to a combination of multiple spawnings by older surviving adults, with rapid recruitment of new adults due to rapid rates of maturation. According to Pianka (1970), these traits are often found in “r-strategist” species. *Macropsobrycon uruguayanae* is a species of small size, producing reduced absolute numbers of oocytes of small diameter, characterized by continuous reproduction, with females being inseminated before full maturation takes place. In general, these reproductive traits are similar to those displayed by the opportunist species proposed by Winemiller (1989).

The alteration of gill tissues to form the gill gland of *M. uruguayanae* is correlated with sexual maturation in males. Thus, the gill gland may be considered to be a male secondary sex character. This same correlation was also found for other species of Cheirodontinae, such as *Cheirodon ibicuihensis* (Oliveira *et al.*, 2002), and *Odontostilbe pequirá* and *Compsura heterura* (Oliveira *et al.*, 2010). Gill glands are also found in sexually mature male *Aphyocharax anisitsi*, an externally fertilizing species of Characidae (Gonçalves *et al.*, 2005), and many inseminating species of Stevardiinae (Bushmann *et al.*, 2002). Although considerable variation in gill gland morphology has been demonstrated (Bushmann *et al.*, 2002), in all species that possess them, gill glands appear to be formed by the fusion of gill filaments on the anterior portion of the first gill arch on each side of the body, leading to the formation of a series of chambers that open ventrally into the gill cavity. In addition, tall columnar secretory cells are found between adjacent secondary lamellae that are often greatly reduced in length or even absent in some species. The gill gland of *M. uruguayanae* also shows the same general structure seen in these other species, suggesting that gill glands represent homologous structures in the species of Characidae that possess them. It has been suggested that secretions released by gill glands may be involved with some aspect of reproduction, such as serving as chemical signals (Bushmann *et al.*, 2002).

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