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Reproductive strategy of the Patagonian catfish *Hatcheria macraei*

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This study describes the reproductive strategy of the stream-dwelling catfish *Hatcheria macraei* in the Pichileufu River, Argentina. Gonad maturity phases, classified on the basis of histological analysis, stages of gamete development and the frequency distribution of oocyte size, were correlated with macroscopic features of the gonads. *Hatcheria macraei* has a cystovarian ovary, asynchronous oocyte development and lobular testes. Five oocyte and four spermatogenic stages were identified and related to macroscopic gonad characteristics, making it possible to divide gonad development into five phases for females and males. Mature oocyte diameter ranged from 922 to 1935 µm. Absolute fecundity in mature females varied from 115 to 480 oocytes. *Hatcheria macraei* has multiple spawning during a protracted reproductive season that extends from December to April. This, together with its small size, is characteristic of an opportunistic reproductive strategy, commonly found in species that inhabit adverse and unpredictable environments, such as the low-order rivers of Patagonia.

Key words: fecundity; Fulton's condition index; gonadal development; gonado-somatic index; histology; oocyte diameter.

INTRODUCTION

Reproductive strategies in fishes are extremely variable (Balon, 1990). They differ in traits such as size and number of eggs, number of breeding opportunities, ovarian development organization, type of spawning, parental care and system of sexual differentiation (Murua & Saborido-Rey, 2003). The final outcome of any strategy is to maximize the production of viable offspring for the next generation in a specific environment. For this reason, identifying the reproductive strategy of a given species is crucial, not only for the determination of its reproductive potential, but also for community or ecosystem studies such as the quantification of extinction risk (Sternberg *et al.*, 2014).

The environment has a strong influence on the evolution of fish life history, which depends on its predictability, cycles or stability (Winemiller, 2005). Winemiller & Rose (1992) classified three main life-history strategies based on survival, fecundity and

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the beginning of reproductive life. Opportunistic strategists have early maturation, low juvenile survivorship and live in habitats with high disturbance. Equilibrium strategists present moderate age at maturation, low fecundity per spawning and high juvenile survivorship. These fish species live in more stable environments with low disturbance. Periodic strategists have late maturation, high fecundity and low juvenile survivorship, and usually live in highly periodic or seasonal environments. Fish size is also related to each type of strategy: opportunistic are the smallest, equilibrium have small to medium bodies, and periodic are characterized by large bodies.

The small benthic freshwater Patagonian catfish *Hatcheria macraei* (Girard 1855) belongs to the family Trichomycteridae (pencil or parasitic catfishes) and is endemic to South America (Arratia & Menu-Marque, 1981). It is widely distributed in low-order rivers of Argentina and Chile (Unmack *et al.*, 2009, 2012) and is the southernmost occurring species in the family (Arratia & Menu-Marque, 1981). It is a rheophilic and negatively phototactic fish (Menni, 2004) that inhabits fast flowing, cold and well oxygenated waters (Ringuelet *et al.*, 1967). The diurnal microhabitat use of *H. macraei* is associated with large substrata sizes with conspicuous interstitial spaces (Barriga *et al.*, 2013) and it has a generalist feeding habit based on benthic invertebrates (Di Prinzio & Casaux, 2012). Little is known of its reproductive strategy and the only published study is that by Barriga & Battini (2009) who assessed size at first maturity (standard length, $L_S = 64$ mm for females and 61 mm for males) and observed that spawning occurs from December to February. The aim of the current study was to describe the reproductive biology of *H. macraei* using a combination of macroscopic and microscopic gonadal criteria.

MATERIALS AND METHODS

STUDY AREA AND FISH COLLECTION

Sampling was performed in the Pichileufu River (Río Negro Province, Argentina; Fig. 1), 70 km from San Carlos de Bariloche City. This river begins in the Carrera Mountains and flows north for around 150 km through the Patagonian steppe before draining into Piedra del Águila Reservoir on the Limay River, a major tributary of the Negro River. The Pichileufu River has very variable hydrological characteristics, as it depends mainly on rainfall and snowmelt events (Table I). Capture of the specimens was carried out in the middle section of the river (41° 05′ 24″ S; 70° 49′ 42″ W; 926 m a.s.l.) from October 2008 to March 2010 by electrofishing using a 24 V d.c. backpack electrofishing unit, model 12-B (Smith-Root, Inc.; www.smith-root.com). During each sampling event water, temperature was recorded. Captured fish were euthanized using an overdose of benzocaine >100 mg l^{-1}.

FISH PROCESSING AND HISTOLOGICAL ANALYSIS

In the laboratory, fish were measured to the nearest 0·1 mm L_S from digital photographs of each individual using an Digimizer 4.0 image analyser (MedCalc Inc.; www.medcalc.org). Total body mass (M_T) was measured to 0·0001 g using an AP250D analytical balance (OHAUS; www.ohaus.com). Gonads were extracted by dissection, weighed fresh to 0·0001 g (M_G) and then fixed in 4% formaldehyde. One randomly chosen ovary per female and both testes per male were kept for histological analysis. The histological protocol consisted of dehydration of a gonad (in total 30 testes and 86 ovaries) in a series of alcohol solutions, followed by clearing in xylene, and embedding in paraffin wax. The gonad was then sectioned longitudinally (7 μm) and finally stained with haematoxylin–eosin (Martoja & Martoja-Pierson, 1970).

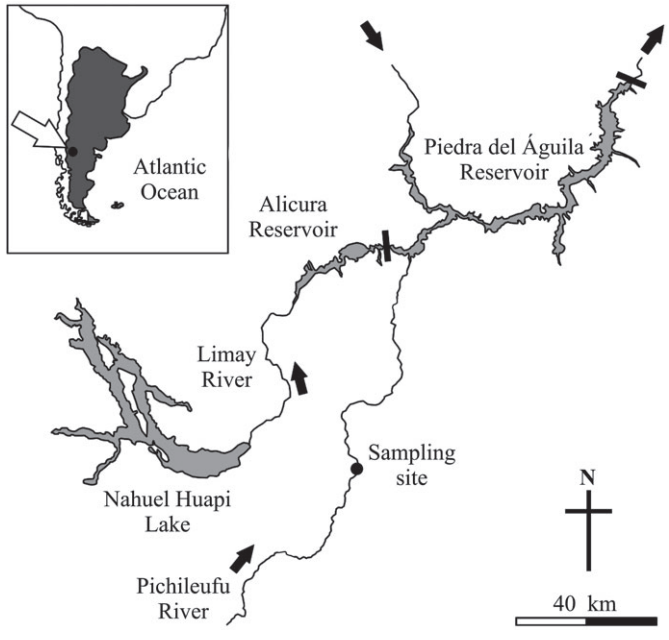

FIG. 1. Study site (●) of *Hatcheria macraei* on Pichileufu River in Patagonia (⇨), Argentina (▮). ⬆, direction of water flow; ▮, dams on Limay River.

Histological characterization of gamete development was based on the dye affinities of cytoplasm and nucleus, and by the presence or absence of different cell structures. The diameters of each germ-cell type, different cell structures (*e.g.* nucleus and nucleoli diameter), and radiata zone and follicular layer thickness were measured with the image analyser on digital images. Photographs were taken using a Sony SSC-DC50A digital interface camera (www.pro.sony.com) attached to an Olympus BX40 optical microscope (www.olympus.com). Oocytes and spermatogenic stages were described following the criteria of West (1990) and Grier (1981).

Gonad maturity phases were identified on the basis of macroscopic and microscopic scales of gonad development following Gomes *et al.* (2011) for both sexes. The degree of irrigation, colour and size of the gonads was taken into account for macroscopic characterization. At microscopic level, the germ-cell types present, their abundance and degree of gamete development were recorded. The gonado-somatic index (I_G) was determined as: $I_G = M_G M_T^{-1} \times 10^2$, where M_G and M_T are in g. Once the spawning season had been estimated using the I_G gonad development was analysed monthly from September to May (the austral summer), using the relative frequency of adult gonad phases present to determine the exact period and amplitude of the spawning season. Somatic condition was assessed using Fulton's condition factor, $K = M_T L_S^{-3} \times 10^5$, where M_T is in g and L_S is in mm.

Sex ratio was assessed monthly and contrasted with the hypothetical 1:1 proportion by using χ^2-distribution. The relationship between L_S and M_T was assessed separately for males and females using a linear regression analysis with $\log_{10}$-transformed data. Differences between sexes were then tested by applying one-way ANCOVA, where M_T was the dependent variable, sex was the factor and L_S was the co-variable. Between-sex differences in K were tested using t-tests. To explore the relationship between K and I_G, and also between oocyte diameter and both L_S and M_T, Spearman correlation analyses were performed. The relationship between gonad development (I_G) and environmental variables (*i.e.* water temperature and photoperiod) was also assessed using Spearman correlation analyses.

TABLE I. Hydrological characteristics of Pichileufu River

	Maximum flow ($m\,s^{-1}$)				Total precipitation (mm)			
Date	2008	2009	2010	Mean ± S.D.	2008	2009	2010	Mean ± S.D.
January	2·3	1·3	3·2	2·3 ± 1·0	11	0	1	4 ± 6
February	1·8	0·7	1·6	1·4 ± 0·6	1	1	14	5 ± 8
March	0·8	0·8	1·8	1·1 ± 0·6	4	1	21	9 ± 11
April	1·1	3·4	1·1	1·9 ± 1·3	0	14	1	5 ± 8
May	37·1	177·5	2·2	72·3 ± 92·8	131	105	10	82 ± 64
June	8·2	11·4	16·6	12·1 ± 4·2	25	21	97	48 ± 43
July	102·5	20·2	33·8	52·2 ± 44·1	28	29	34	31 ± 3
August	58·1	129·2	24·4	70·6 ± 53·5	84	35	29	49 ± 30
September	97·2	76·5	30·8	68·2 ± 34·0	14	16	8	13 ± 4
October	32·6	38·6	14·2	28·5 ± 12·7	1	21	11	11 ± 10
November	15·7	29·5	14·4	19·9 ± 8·4	18	25	0	15 ± 13
December	5·2	8·0	5·1	6·1 ± 1·6	12	1	19	11 ± 9
Annual	102·5	177·5	33·8	104·6 ± 71·9	329	269	245	281 ± 43

Data provided by Autoridad Interjurisdiccional de las Cuencas de los ríos Limay, Neuquén y Negro (AIC), Argentina; www.aic.gov.ar.

FECUNDITY

To estimate fecundity only ovaries characterized by the absence of post ovulatory follicles (POF) and before the spawning period were analysed. This was done in order to avoid an underestimation due to the loss of mature oocytes because of partial spawning (Fitzhugh & Hettler, 1995). Oocytes in vitellogenesis were counted under stereo-microscope in one of the two ovaries (*i.e.* the one not used for histology). Absolute fecundity (the total number of vitellogenic oocytes at any time in the ovary; Hunter *et al.*, 1986) was estimated by relating the number of oocytes counted in the ovary to the total mass of both ovaries. The relative fecundity, or the number of oocytes g^{-1}, was calculated from absolute fecundity and M_T. Batch fecundity, defined as the number of oocytes spawned per batch (De Vlaming, 1983), was estimated taking into account only the number of oocytes in the most advanced mode (Walsh *et al.*, 2011). The relationships between absolute, relative and batch fecundity and both L_S and M_T were assessed using linear regressions and tested by ANOVA. In order to meet normality and homoscedasticity assumptions of relative fecundity, the data were previously $\log_{10}$-transformed. The relationships between fecundity (absolute and relative) and oocyte diameter (*i.e.* average for each female) were evaluated with Pearson correlations.

RESULTS

SEX AND SIZE COMPOSITION

Five hundred and eighteen *H. macraei* were captured, which ranged from 31 to 118 mm L_S. In females ($n = 284$) the mean ± S.D. L_S was 64·58 ± 15·96 mm and M_T was 2·26 ± 1·62 g, while male ($n = 234$) L_S was 64·03 ± 16·88 mm and M_T was 2·16 ± 1·66 g. Specimens of indeterminate sex, all juveniles, had a mean ± S.D. of $L_S = 41{\cdot}25 \pm 6{\cdot}88$ mm and $M_T = 0{\cdot}436 \pm 0{\cdot}186$ g.

The overall sex ratio was 1·00:1·21 (234 males and 284 females) which did not deviate from the hypothetical distribution of 1:1 (χ^2, $z = 1{\cdot}88$, $P > 0{\cdot}05$). This proportion

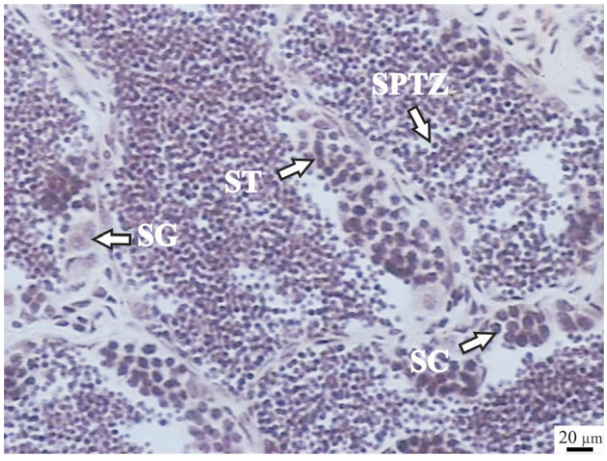

FIG. 2. Photomicrograph of *Hatcheria macraei* mature testis. Stages of sperm maturation: SG, spermatogonium; SC, spermatocyte; ST, spermatid; SPTZ, spermatozoa.

was maintained monthly except for December (1·00:0·47, χ^2, $z = 16{\cdot}90$, $P < 0{\cdot}05$), and April (1·00:0·52, χ^2, $z = 5{\cdot}26$, $P < 0{\cdot}05$).

GONAD DEVELOPMENT

The gonads of males and females of *H. macraei* are paired organs located dorsal to the gastrointestinal tract. As development progresses they occupy almost the entire abdominal cavity, being more pronounced in females.

The testes of *H. macraei* are of the lobular type (Nagahama, 1983) with abundant seminiferous tubules of different diameters. Each tubule is supported by a basement membrane that holds the germ cells. Germ cells are enclosed individually [*i.e.* spermatogonium (SG)], or by groups of cells, in a cystic cell. Each tubule contains multiple cysts and every cyst in turn contains germ cells at the same stage of development (Fig. 2). Four germ cell stages were recognized from the histological analysis. The SG cell had a diameter of $7{\cdot}7 \pm 1{\cdot}4$ µm (mean ± S.D.) and a shape from round to oval. There was one per cyst, with a prominent nucleus and low chromatin condensation. The spermatocyte (SC) cell was rounded and its central nucleus occupied most of the cell because of reduced cytoplasm. Cell diameter was $4{\cdot}9 \pm 0{\cdot}5$ µm. These characteristics were observed in males >61 mm L_S. The spermatid (ST) cell had a diameter of $3{\cdot}0 \pm 0{\cdot}5$ µm, with a basophilic nucleus as a result of the chromatin condensation, whereas the diffuse cytoplasm exhibits poor affinity for the dyes. Finally, spermatozoa (SPTZ) were the smallest ($1{\cdot}4 \pm 0{\cdot}3$ µm) and most dense cells, with a strong affinity for haematoxylin, and were found in the cyst lumen.

Immature testes (phase I) were small, translucent and filiform, difficult to distinguish from immature ovaries. Microscopically, seminiferous tubules were undefined and spermatogonia were agglomerated. Cysts were not evident. During maturing (phase II) the testes increased in size and colouration turned from transparent pinkish to milky white, irrigation becoming evident. Cysts with spermatogonia and some with STs were seen. At the end of this phase a large number of SPTZ were found in

the tubules. Mature phase (III) was the longest lasting stage, when testes reach the largest size. Gonads were white and highly irrigated; lumen was anastomosed and full of SPTZ. Spawning phase (IV) was characterized by milt flowing freely with gentle pressure. During the resting phase (V) the testes became smaller, more flaccid and whitish; irrigation diminished and was superficial. Tubules rapidly reduced their size, with fewer residual SPTZ. Spermatogonia could occasionally be identified (Table II).

The ovaries of *H. macraei* were of the cystovarian type (*sensu* Nagahama, 1983), in which an oocyte matures within a follicle in the ovarian sheets and is released into the ovary lumen at the time of ovulation. Five oocyte stages were recognized from histological analysis. The chromatin nucleolus (CN) stage was characterized by an oocyte of irregular shape with strongly basophilic cytoplasm. The diameter was $74{\cdot}7 \pm 22{\cdot}0$ μm (mean ± S.D.) with a central nucleus (diameter $34{\cdot}9 \pm 12{\cdot}9$ μm) with few small-sized nucleoli (diameter $10{\cdot}9 \pm 4{\cdot}9$ μm). It was surrounded by a single thin layer of loosely attached flat cells (prefollicular cells) with a thickness of $2{\cdot}0 \pm 0{\cdot}7$ μm (Fig. 3). Oocytes in the perinucleolus (PN) stage had a diameter of $179{\cdot}8 \pm 57{\cdot}2$ μm and basophilic cytoplasm. The nucleus was in a central position and had a diameter of $75{\cdot}5 \pm 27{\cdot}3$ μm with numerous nucleoli ($14{\cdot}6 \pm 5{\cdot}7$ μm). The follicular layer had the same characteristics as CN oocytes with a thickness of $2{\cdot}4 \pm 1{\cdot}1$ μm (Fig. 3). The cortical alveoli (CA) stage was characterized by the presence of unstained drops on the periphery of the cytoplasm, the cortical alveoli, which proliferated towards the nucleus. Oocyte diameter was $659{\cdot}2 \pm 90{\cdot}9$ μm with a nucleus of $131{\cdot}5 \pm 34{\cdot}0$ and $26{\cdot}4 \pm 12{\cdot}2$ μm nucleoli. At this stage the radiata zone and two follicular layers (*i.e.* granulose and thecal) were observed. The radiata zone had a thickness of $4{\cdot}0 \pm 1{\cdot}4$ μm while the granulose layer consisted of cubic cells with a thickness of $8{\cdot}2 \pm 6{\cdot}0$ μm and a thecal layer of $2{\cdot}3 \pm 1{\cdot}1$ μm. The smallest female in which these characteristics were observed was 69 mm in L_S (Fig. 3). Oocytes in the early vitellogenesis (EV) stage had a diameter of $842{\cdot}8 \pm 80{\cdot}1$ μm with significant cell growth due to the incorporation of small yolk granules with eosin affinity (acidophilus). The cytoplasm partially retained its basophilic colouration. The nucleus, $154{\cdot}4 \pm 38{\cdot}5$ μm in diameter, was in a central position with a number of $26{\cdot}0 \pm 12{\cdot}0$ nucleoli of peripheral location. The radiata zone had a thickness of $4{\cdot}2 \pm 1{\cdot}7$ μm, while the follicular layer was $24{\cdot}8 \pm 12{\cdot}5$ μm, the granulose cell layer was $22{\cdot}5 \pm 11{\cdot}9$ μm and the thecal $2{\cdot}2 \pm 1{\cdot}4$ μm in thickness. Advanced vitellogenesis (AV) stage oocytes had a spongy, acidophilus cytoplasm due to the inclusion of yolk granules. Oocyte diameter was $1309{\cdot}8 \pm 243{\cdot}5$ μm, nucleus $176{\cdot}5 \pm 15{\cdot}1$ μm and radiata zone thickness was $3{\cdot}1 \pm 1{\cdot}2$ μm. The nucleus was proportionally smaller and difficult to find in histological sections. At this stage the follicular layer was at its thickest, being $92{\cdot}0 \pm 38{\cdot}5$ μm. Granulose and thecal cell layer thickness were $86{\cdot}0 \pm 35{\cdot}7$ and $5{\cdot}5 \pm 4{\cdot}7$ μm respectively (Fig. 3). Histological examination of the gonads revealed two follicular structures: POF and atretic follicles (FA). The former were recognizable because there was only a retracted layer of granulose cells, since the oocyte had been released into the lumen of the ovary (Fig. 4). In FA oocyte cytoplasm was invaded by granulose cells that digested the yolk. During this initial phase, also known as α-atresia, the oocyte is irregular in shape. The minimum and maximum lengths were 136·0 and 248·0 μm, respectively (Fig. 4).

During the immature phase (I) the ovary was small, cylindrical and hyaline; oocytes were not visible to the naked eye and irrigation was scarcely evident. At microscopic level, laminar organization was clearly observed in this phase, where only CN and PN were found. In this phase oocytes in CN predominated. During maturation

TABLE II. Macroscopic and microscopic features of gonad phases and their seasonal occurrence in *Hatcheria macraei*

	Testes			Ovaries		
Gonad phase	Macroscopic description	Microscopic description	Season	Macroscopic description	Microscopic description	Season
I Immature	Small and translucent without apparent irrigation	Seminiferous tubules undefined, SG agglomerated	All	Small, cylindrical and translucent without apparent irrigation. Oocytes invisible to the naked eye	Laminar organization. Oocytes mostly in CN stage but some in PN stage	All
II Maturing	Middle-sized and whitish with evident irrigation	Cells organized in cysts, seminiferous tubules defined	Autumn	Middle-sized, cylindrical, of whitish-yellowish colour with evident irrigation. Oocytes may be visible	Laminar organization is not evident. Oocytes in CN, PN and CA stages. Follicular wall begins to differentiate during CA	Autumn to winter
III Mature	Cylindrical and whitish with very evident irrigation. The largest size is reached in this phase	Tubules increased in size. Lumen is anastomosed and full of SPTZ	Spring	Large, yellow with very evident irrigation. Oocytes very visible	Oocytes in CN, PN, CA, EV and AV. Follicular wall is well developed during EV and AV stages	Spring to summer
IV Spawning	Middle-sized and opaque-whitish with very evident irrigation	Tubules reduced in size. Lumen with SPTZ	Spring to late autumn	Flaccid, opaque-yellowish with very evident irrigation. Yellow and hyaline oocytes	All oocyte stages mentioned in the previous phase and also POF. No laminar organization is seen	Summer to early autumn
V Resting	Small to middle sized, flaccid and opaque-whitish with superficial irrigation	Smaller tubules in which the amount of SPTZ is reduced. SG occasionally identified	Late summer to autumn	Middle-sized, cylindrical of opaque whitish colour with evident irrigation	Oocyte mainly in PN and CN stages. CA in low number. Some atretic oocytes	Late summer to early autumn

AV, advanced vitellogenesis; CA, cortical alveoli; CN, chromatin nucleolus; EV, early vitellogenesis; FA, follicular atretic; PN, perinucleolus; POF, post ovulatory follicles; SG, spermatogonium, SPTZ, spermatozoa.

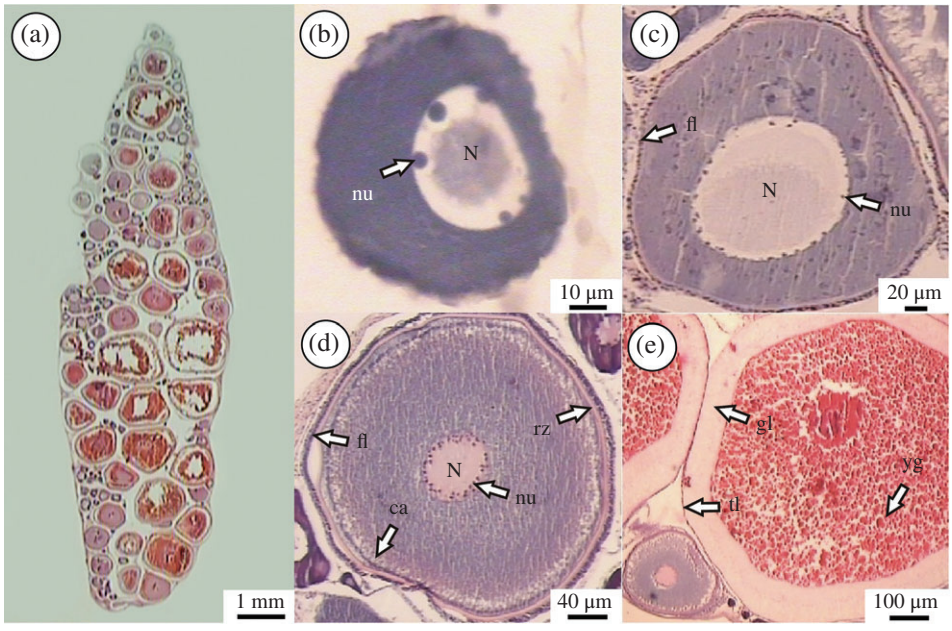

FIG. 3. Photomicrograph of *Hatcheria macraei* mature ovary. (a) Various stages of development; (b) chromatin nucleolus; (c) perinucleolus; (d) cortical alveoli; (e) advanced vitellogenesis. N, nucleus; nu, nucleoli; fl, follicular layer; rz, radiata zone; ca, cortical alveoli; gl, granulose layer; tl, thecal layer; yg, yolk granules.

(phase II) ovaries were cylindrical and yellow. Oocytes could be seen macroscopically. The main characteristic was the presence of CA oocytes and also of CN and PN. Laminar organization was not seen. The number of CN oocytes was high even though the maturation process had already begun. In the next stage, mature (phase III), ovaries were irrigated and almost filled the abdominal cavity. Oocytes were yellow and visible. Laminar organization was not clear and oocytes in CN, PN, CA, EV and AV were observed. During spawning (phase IV) ovaries were flaccid and opaque; a decrease in volume was evident. In addition to all the stages of oocytes seen in the previous phase, POF were observed, which were present only in this phase. The resting phase (V) was macroscopically similar to the immature one; the main difference, however, was the higher level of ovarian irrigation. In the ovarian laminas, mainly oocytes in PN and a low number of FA can be distinguished. Some CA and CN were also found (Fig. 5 and Table II).

SPAWNING SEASON

There was a sustained increase in I_G in early October, reaching the highest values for females in December (Fig. 6). For males there was a slow increase in I_G in November to December, peaking in January to February. In females I_G began to decline in February, while in males this decrease was in March.

In both sexes the pattern of variation of K was similar. The highest values were recorded during November to December and the lowest during July. The K values for adult females and adult males, however, were significantly different (t-test, $P < 0{\cdot}05$).

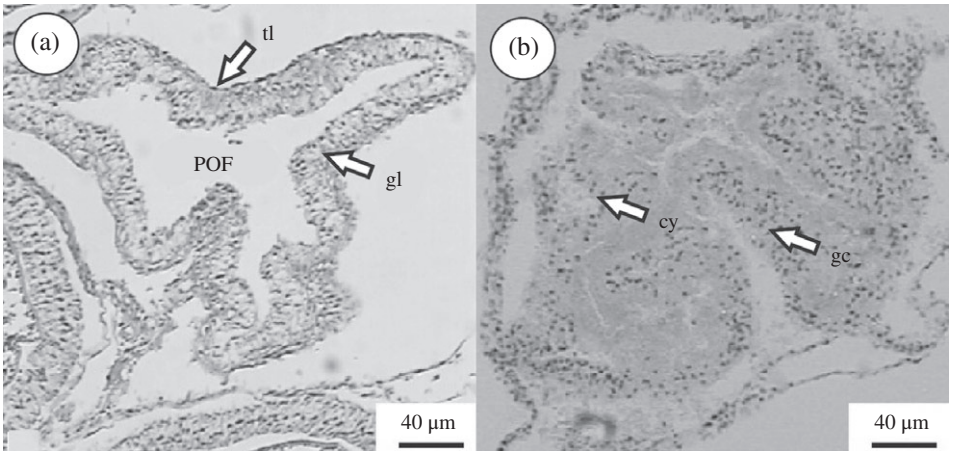

FIG. 4. Follicle types in *Hatcheria macraei*. (a) Post ovulatory follicle (POF) and (b) atretic follicle. tl, thecal layer; gl, granulose layer; gc, granulose cell; cy, cytoplasm.

In females K was directly related to I_G (Spearman, $\rho = 0{\cdot}421$, $P < 0{\cdot}05$, Fig. 6). In males, however, this relationship was not significant (Spearman, $\rho = 0{\cdot}116$, $P > 0{\cdot}05$).

On comparing environmental variables with I_G of females, direct relationships were found with photoperiod (Spearman, $\rho = 0{\cdot}376$, $P < 0{\cdot}05$) and with water temperature (Spearman, $\rho = 0{\cdot}242$, $P < 0{\cdot}05$), while in males a direct relationship was observed only with the photoperiod (Spearman, $\rho = 0{\cdot}191$, $P < 0{\cdot}05$, Fig. 6).

Ovaries of all adult females were in the mature phase from September to November. Earlier-spawned individuals were recorded during December. The number of individuals with ovaries in spawning phase was highest in January (60%). In February the first resting ovary was recorded and after this month females in spawning phase decreased. Concurrently, fish with resting ovaries increased from February to May, when all females had ovaries in this phase (Fig. 7).

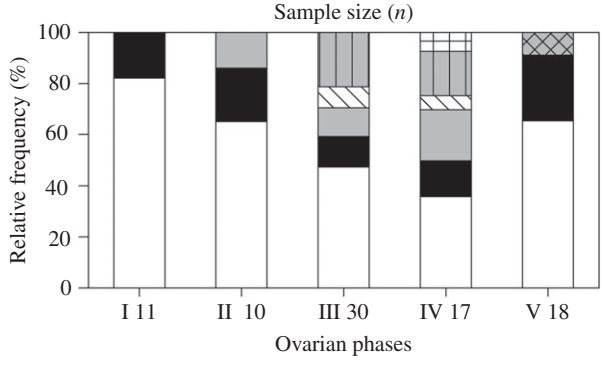

FIG. 5. Relative frequency of oocyte stages according to different ovarian phases of *Hatcheria macraei*: (I) immature, (II) maturing, (III) mature, (IV) spawning and (V) resting. Oocyte stages: □, chromatin nucleolus; ■, perinucleolus; ▨, cortical alveoli; ▧, early vitellogenesis; ▥, advanced vitellogenesis; ⊞, post ovulatory follicles; ⊠, atretic follicle.

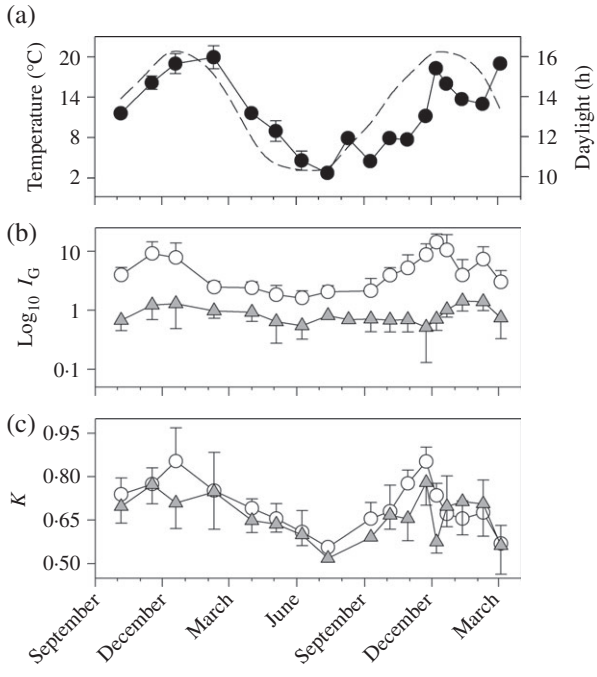

FIG. 6. Mean ± S.D. 2008–2010 (a) water temperature (–●–) and duration of daylight (– – –) at the Pichileufu River sampling site, (b) monthly variation of *Hatcheria macraei* gonado-somatic index (I_G) and (c) Fulton's condition factor (K). ○, adult females; △, adult males.

Most adult male testes were mature from September to October (90%) and all from November to January. In February, the first resting testes were observed, and from March this number began to increase (Fig. 7).

OOCYTE DIAMETER DISTRIBUTION

During vitellogenesis and before spawning, all intervals of oocyte diameters (the entire range of diameters) were observed. After spawning, in contrast, oocytes in the range 300–700 µm diameter (stage CA) were not observed (Fig. 8). Furthermore, no relationship was found between oocyte diameter and L_S (Spearman, $\rho = 0{\cdot}141$, $P > 0{\cdot}05$) or oocyte diameter and M_T (Spearman, $\rho = 0{\cdot}191$, $P > 0{\cdot}05$).

SIZE, MASS AND FECUNDITY

The estimated length–mass relationship (between $\log_{10} M_T$ and $\log_{10} L_S$) proved to be isometric for females, with a coefficient b value of 2·99 (C.I. = 2·92–3·05). Males had a negative allometric growth pattern (coefficient $b = 2{\cdot}89$, C.I. = 2·83–2·96). Comparing coefficients of both sexes, however, there were no significant differences between them (ANCOVA, $P > 0{\cdot}05$).

Absolute fecundity ranged from 115 to 480 oocytes per female, with a mean ± S.D. = 277 ± 84. Relative fecundity ranged between 75 and 149, with a mean ± S.D. = 109 ± 53 oocytes g^{-1} M_T. Absolute fecundity increased significantly with M_T and L_S (ANOVA,

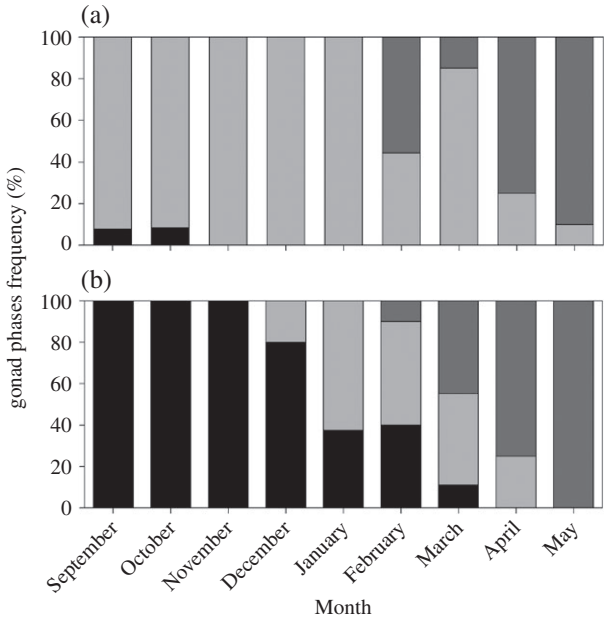

FIG. 7. Monthly relative frequency of gonad phases of adult (a) male and (b) female *Hatcheria macraei*. ■, mature; ■, spawning; ■, resting.

$F_{1,18} = 37{\cdot}942$, $P < 0{\cdot}001$ and ANOVA, $F_{1,18} = 29{\cdot}150$, $P < 0{\cdot}001$, for M_T and L_S, respectively), while relative fecundity decreased significantly with M_T and L_S (ANOVA, $F_{1,18} = 25{\cdot}316$, $P < 0{\cdot}001$ and ANOVA, $F_{1,18} = 6{\cdot}722$, $P < 0{\cdot}01$, for M_T and L_S, respectively; Fig. 9). No significant relationships were found between M_T or L_S and batch fecundity (ANOVA, $F_{1,13} = 0{\cdot}809$, $P > 0{\cdot}05$ and ANOVA, $F_{1,13} = 3{\cdot}086$, $P > 0{\cdot}05$, for M_T and L_S, respectively). Furthermore, no relationship was found between oocyte diameter and absolute fecundity (Pearson, $\rho = 0{\cdot}381$, $P > 0{\cdot}05$), or between oocyte diameter and relative fecundity (Pearson, $\rho = 0{\cdot}335$, $P > 0{\cdot}05$).

DISCUSSION

As far as is known, this is the first detailed study of the reproductive biology of *H. macraei*. In this work macroscopic gonad characterization is paired with development at the microscopic level. The combined use of histological analysis, oocyte size-frequency distribution, macroscopic features of the gonads and I_G analysis proved to be an efficient way to assess gonad status and to ascertain the reproductive strategy of this species.

Testes of *H. macraei* are elongated, without projections, like other siluriform species such as *Hoplosternum littorale* (Hancock 1828) (Loir *et al.*, 1989) and *Loricaria lentiginosa* Isbrücker 1979 (Guimaraes-Cruz *et al.*, 2005). Histological studies showed that *H. macraei* has testes of the lobular type, where germ cells are scattered around the periphery of a lobular structure with a central lumen, into which SPTZ are released after completion of spermatogenesis (Billard *et al.*, 1982). Four spermatogenic stages

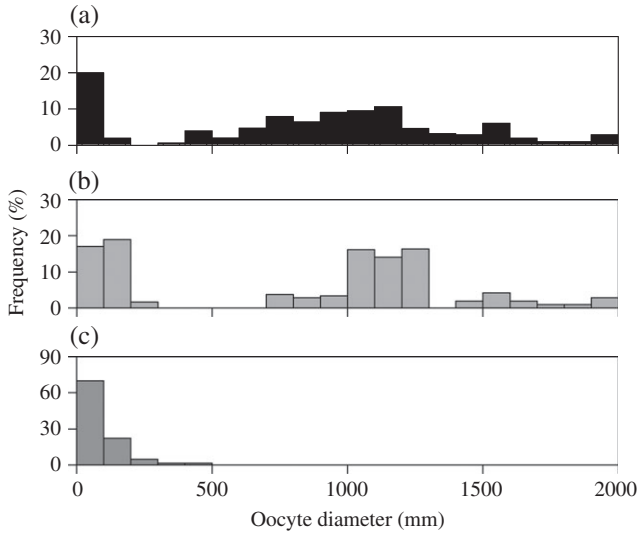

FIG. 8. Relative frequency distribution of oocyte diameters for adult *Hatcheria macraei* (a) during mature ($n = 30$), (b) spawning ($n = 17$) and (c) resting phases ($n = 18$).

were identified. The SG was the largest primary cell observed, mainly found on the periphery and associated with one or more cystic cells. As spermatogenesis progressed, germ cells reduced their cytoplasm content and nuclear chromatin was condensed, a process that occurred within the cysts. By linking these characteristics with the four macroscopic phases, the level of maturity could be established.

The dynamics of oocyte maturation in *H. macraei* was of the asynchronous type, where ovaries of mature females had oocytes at all stages of development. This asynchronic pattern is generally observed in species that spawn several times during the course of a prolonged breeding season (Wallace & Selman, 1981; Jobling, 1996). In this type of ovary, yolk accumulation and hence oocyte development depends mostly on food availability in the environment at that moment (Hunter & Leong, 1981). The same spawning pattern has been described for related species such as *Trichomycterus areolatus* Valencienes 1846 in Chile (Chiang *et al.*, 2011), two *Trichomycterus* spp. in Brazil (Casatti, 2003; Rondineli *et al.*, 2009), and *Trichomycterus spegazzinii* (Berg 1897) (Romero & Vera-Mesone, 2010) and *Trichomycterus corduvensis* Weyenbergh 1877 (Marraro *et al.*, 2005) from the north and centre of Argentina, respectively. It has also been described in other members of the Trichomycteridae family: *Copionodon pecten* de Pinna 1992 in Brazil (Zanata & Primitivo, 2013) and *Eremophilus mutisii* Humboldt 1805 in Colombia (Florez & Sarmiento, 1989). This reproductive strategy is considered to be selectively advantageous in unstable and unfavourable environments (Durham & Wilde, 2008). Multiple spawning allows temporary separation of the offspring, which diminishes the probability of an unpredictable catastrophic event destroying all the progeny of the year (Matthews, 1998) and also reduces intraspecific competition at the beginning of exogenous feeding.

The condition factor is mainly related to feeding status and gonad phase (Wootton, 1998). Sex-related differences were not found in *H. macraei K* index. Both sexes had the greatest scores from *c.* October to February, while the lowest values were recorded

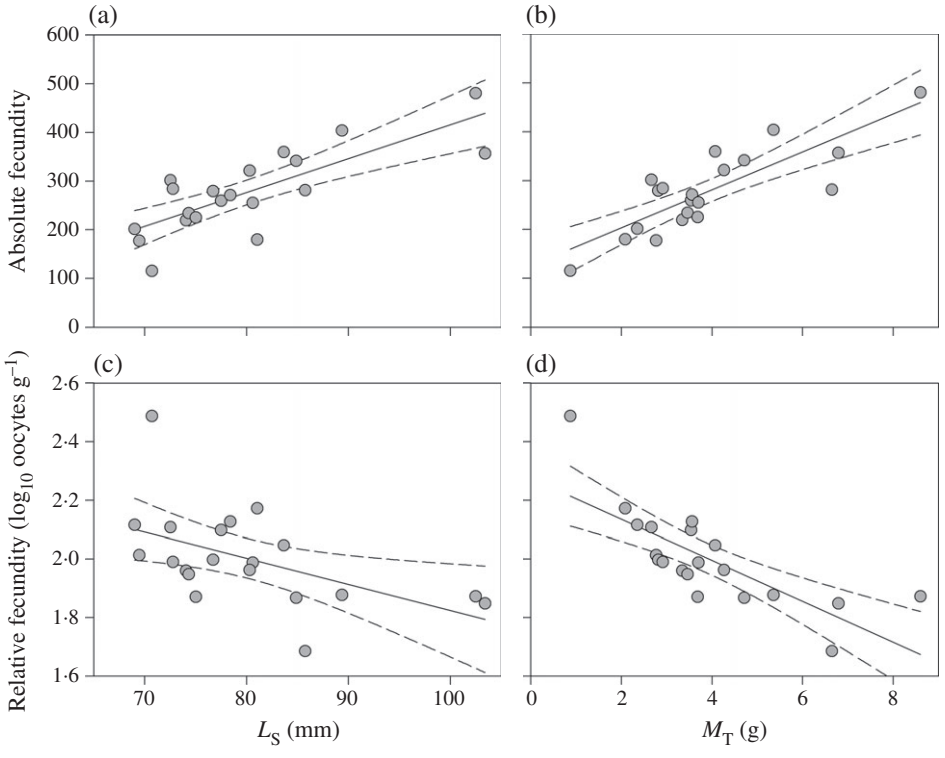

FIG. 9. Linear regressions (±95% C.I.) of (a), (b) absolute and (c), (d) relative fecundity in relation to standard length (L_S) and total mass (M_T) of *Hatcheria macraei*. (a) $y = 6{\cdot}96x - 280{\cdot}12$, $r^2 = 0{\cdot}62$, $P < 0{\cdot}001$; (b) $y = 38{\cdot}84x + 125{\cdot}64$, $r^2 = 0{\cdot}68$, $P < 0{\cdot}001$; (c) $y = -0{\cdot}01x + 2{\cdot}72$, $r^2 = 0{\cdot}27$, $P < 0{\cdot}01$; (d) $y = -0{\cdot}07x + 2{\cdot}28$, $r^2 = 0{\cdot}58$, $P < 0{\cdot}001$.

in July. This is consistent with the reproductive period. One reproductive period per year was evident from I_G values, from September to March (*i.e.* austral spring and summer), suggesting that gonad growth in females began between September and October and continued until February or March, when females had low I_G values. The spawning period of *H. macraei*, based on the proportion of ovarian phases, occurred from December to April, and in May all females were in the post-spawning stage. I_G values correlated with histological findings which makes I_G a good indicator of the duration of the reproductive season. Without additional information, however, such as histological descriptions or oocyte size data, its use is limited only to detection of the reproductive season, and does not give exact information on individual reproductive status. For example, it does not provide reliable measures to distinguish, during the non-spawning season, reproductively inactive, but adult females from immature ones (Alejo-Plata *et al.*, 2011). In this context histological analysis is needed for an accurate assessment of gonad maturity status, this being the main drawback of the method, and the most time-consuming.

The reproductive period reported in this study agrees in part with that recorded by Barriga & Battini (2009) for another population of *H. macraei* from the same basin. They used only I_G and reported that the spawning season took place from December

to February in the Caleufu River population. There is no general pattern regarding spawning season when comparing Trichomycteridae species. High-latitude species, however, seem to spawn during the austral spring and summer [*e.g. T. areolatus* (Manriquez *et al.*, 1988; Habit *et al.*, 2005), *T. corduvensis* (Marraro *et al.*, 2005) and *T. spegazzinii* (Romero & Vera-Mesone, 2010)]. The spawning season of *H. macraei* was associated with periods of high water temperature and long photoperiod. Spawning synchronization with these periods often coincides with peaks of prey availability and optimal developmental temperature levels that maximize offspring survivorship (Wootton, 1998). In contrast, low-latitude species reproduce mostly during the dry season in autumn and winter [*e.g. Trichomycterus caliensis* (Eigenmann 1912) (Roman-Valencia, 2001), *Trichomycterus itacarambiensis* Trajano & de Pinna 1996 (Trajano, 1997)], probably when food availability is still optimum, but the chances of being washed away by floods are low (Trajano, 1997).

Hatcheria macraei fecundity is determinate because new oocytes were not recruited during the spawning season. Moreover, there was a low number of FA and a decrease in the number of vitellogenic oocytes during this season. Absolute fecundity increased with increasing length and mass of the fish, which is an expected pattern in fishes (Winemiller, 1989; Winemiller & Rose, 1992; Jobling, 1996). In *T. corduvensis* and *T. areolatus* in particular, a positive relationship between mass and fecundity has been described (Manriquez *et al.*, 1988; Marraro *et al.*, 2005). Fecundity is a specific characteristic and it is adapted to life-cycle conditions of the species. It varies with growth, population density, food availability and mortality rate (McBride *et al.*, 2013). *Hatcheria macraei* has low potential fecundity and size of mature oocytes, similar to other members of the Trichomycteridae family (Table III). The relationship between oocyte size and fish size has been recorded in many fish species (Kamler, 2005), however, this pattern was not found in *H. macraei*. Relative fecundity, on the other hand, related to mass (g) of fish, does decrease with size or mass. The reduction in fecundity is often compensated for by large oocytes which give rise to individuals with a greater capacity to explore the environment than those which originated from small oocytes (Riesch *et al.*, 2012). *Trichomycterus corduvensis* showed the highest fecundity and the smallest diameter of mature oocytes within the Trichomycteridae (Table III). In contrast, the lowest fecundity and largest oocytes have been observed in *C. pecten* (Zanata & Primitivo, 2013), excluding the miniature trichomycterids such as *Pygidianops amphioxus* de Pinna & Kirovskyi 2011 (Carvalho *et al.*, 2014) and *Stauroglanis gouldingi* de Pinna 1989 (Zuanon & Sazima, 2004), whose extreme specializations constrain both size and number of oocytes. In turn, Pouilly & Miranda (2003), comparing *Trichomycterus* populations from Bolivia, pointed out that low fecundity and also larger eggs were found in the hypogean *Trichomycterus barbouri* (Eigenmann 1911) compared with epigean *Trichomycterus chaberti* Durand 1968 (Table III).

Within the Trichomycteridae there is no clear interspecific pattern of egg size variation according to latitude (Table III). Comparatively, large eggs have larger amounts of yolk that provide more energy for free embryo growth (Balon, 1990) and embryo mortality decreases (Einum *et al.*, 2002) before the start of exogenous feeding, resulting in a larger individual. It has been suggested that egg diameter is usually influenced by temperature, with a larger size in populations living in colder waters where bioenergetic requirements are greater, and individuals need to reach a minimum size to survive

TABLE III. Comparison of reproductive traits of species belonging to the family Trichomycteridae

Species	Latitude	Altitude (m a.s.l.)	Country	Absolute fecundity	Mature oocyte diameter (μm)	Reference
Trichomycterus caliensis	4° 36′ N	1500–1800	Colombia	191	1500	Roman-Valencia (2001)
Copionodon pecten	12° 53′ S	732	Brazil	*c.* 26–150	1500–3000	Zanata & Primitivo (2013)
Trichomycterus chaberti	18° 03′ S	1980	Bolivia	230	850	Pouilly & Miranda (2003)
T. chaberti	18° 05′ S	2020	Bolivia	200	800	Pouilly & Miranda (2003)
T. chaberti	18° 06′ S	2200	Bolivia	150	1250	Pouilly & Miranda (2003)
T. chaberti	18° 07′ S	2840	Bolivia	110	1500	Pouilly & Miranda (2003)
Trichomycterus barbouri	18° 07′ S	2700	Bolivia	40	1520	Pouilly & Miranda (2003)
T. chaberti	18° 08′ S	2740	Bolivia	80	870	Pouilly & Miranda (2003)
Trichomycterus sp.	22° 22′ S	1000	Brazil	74 ± 26	1836	Rondineli *et al.* (2009),
Trichomycterus spegazzinii	24° 48′ S	1198	Argentina	772	1029 ± 260	Romero & Vera-Mesone (2010)
Trichomycterus corduvensis	31° 43′ S	500	Argentina	2947	457–494	Marraro *et al.* (2005),
Trichomycterus areolatus	33° 55′ S	410	Chile	506 ± 301	1510–2100	Manriquez *et al.* (1988).
Hatcheria macraei	41° 05′ S	926	Argentina	277 ± 84	1310 ± 243	This study

the first winter (Shuter & Post, 1990). From this perspective, *H. macraei*, the southernmost Trichomycteridae species, would be expected to have the largest egg diameter, but this is not the case. In this context, local conditions such as food availability or microhabitat conditions seem to have more influence on oocyte diameter than broad geographic effects like temperature.

In summary, the small size of *H. macraei* and the presence of a protracted breeding season with multiple spawnings suggest that this species has an opportunistic strategy (*sensu* Winemiller & Rose, 1992). This strategy is common in unfavourable and unpredictable environments such as low-order rivers, because they are more affected by fluctuations in weather conditions. These conditions in the Patagonian steppe vary greatly seasonally and also within each day; prolonged rainfall events can be experienced as well as periods of drought that would affect water flow. In other species

of Trichomycteridae some reproductive features differ slightly, but the strategy is the same. Knowledge of the abundance of a species and its reproductive traits, such as proportion of spawners and fecundity, allows estimation of the reproductive potential of a given population. The baseline information provided in this study could be used to establish conservation measures in different populations of this species.

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