



BIOLOGICAL SCIENCES

Gonadal development in pejerrey (*Odontesthes bonariensis*) during spawning season in relation with sex steroids and temperature variation in Gómez lake (Pampas region, Argentina)

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Abstract: Gómez lake (34 ° 39 'S 61 ° 01' W) is a typical shallow lake of Pampas region placed in the upper area of the Salado river. The most abundant fish species in this lake is the pejerrey (*Odontesthes bonariensis*) valued due to the quality of its flesh and its attractiveness as a game fish. The aim of this study was to describe for the first time in this pejerrey wild population the gonadal stages during three consecutive spawning seasons (August to December) in relation with sexual steroids and temperature in this lake. In general, pejerrey gonadal development, the gonadosomatic index and the plasma levels of estradiol and testosterone varied in relation to air temperature. During the sampling period, pejerrey females started to ovulate in early August (winter), with a peak in October and ending in December with some of them with atretic oocytes. For males, it was possible to find spermiating animals during the whole spawning season and some arrested animals only in December. Our results confirm the relationship with pejerrey maturation and temperature and can be useful for decision making in the management of this natural resource.

Key words: Atherinopsidae, environment cues, estradiol, reproduction, shallow lake, testosterone.

INTRODUCTION

Lagoons are distinctive elements of the Pampas landscape and represent a very important resource for the regional economies. In general, they are shallow water bodies with an average depth of about 3 meter and are characterized by a high degree of natural eutrophication, which is often increased by different anthropic activities (Quirós et al. 2002, Diovisalvi et al. 2015). Due to these characteristics, shallow lakes are sensitive to climatic variations, influencing their functioning (Jeppesen et al. 1998, Diovisalvi et al. 2015, Elisio et al. 2018). In this sense, the temperate climate of the Pampas region shows

great seasonal, spatial and interannual variations in both temperatures and precipitation, defining wet and dry periods that condition the structure and functioning of the lakes in the region (Berasain et al. 2015, Colautti et al. 2015, Elisio et al. 2015a). As a consequence, Pampas lakes are very dynamic ecosystems, exposed to changes caused by external and internal factors that force the organisms that inhabit them to make the corresponding adjustments to adapt and avoid extinction (Padisák & Reynolds 2003).

Gómez lake (34 ° 39 'S 61 ° 01' W) is in the upper area of the Salado river basin next to several lakes (Mar Chiquita, Carpincho and Rocha) with

different degrees of connectivity to this river. All these shallow lakes have a great mixture of water by wind forcing and are characterized by being eutrophic or hypertrophic (Quirós et al. 2002). The Salado is a lowland river with 690 km of extension and includes one of the main ecoregions of central Argentina (López & García 2001) comprising an area of 179,000 km² (Rosso & Quirós 2010). The dominant fish fauna of Gómez lake is the pejerrey (*Odontesthes bonariensis*), followed by the porteño (*Parapimelodus valenciennesi*, Rosso & Quirós 2010). For this reason, the attendance of sport fishermen is of great importance in this lake.

The reproductive seasonality in fish is a characteristic that can be interpreted as an evolutionary advantage that ensures the best conditions for the growth and survival of the progeny (Bromage et al. 2001). This seasonality is mainly regulated by the photoperiod and temperature, although other variables such as lunar cycles, social interactions and salinity may also become important (Pankhurst & Porter 2003, Miranda et al. 2013). In temperate fish, the increase of the photoperiod is the environmental signal for the beginning of the reproductive season, while the temperature acts as a secondary modulator regulating the speed of the processes of gametogenesis and the ending of the spawning (Pankhurst & Porter 2003, Migaud et al. 2010). Besides, in fish the timing and regulation of gonadal differentiation, the reproductive cycle and the production of gametes are under precise endocrine control by a set of hormones secreted by the hypothalamic-pituitary-gonadal axis. These processes are modulated by the environment through gonadotrophin releasing hormones (GnRH), gonadotrophins (GtHs) and gonadal sex steroids (Zohar et al. 2010). Within the steroids involved in the stimulation of fish gonadal development we can distinguish androgens,

estrogens and progestogens (Nagahama 1998, Schulz et al. 2010, Lubzens et al. 2010).

The interaction between the different environmental variables that regulate reproduction in fish and the functioning of the reproductive endocrine axis is important both for the understanding and prediction of ecological population phenomena and for their possible use in activities related to the control of reproduction in captivity. Fish, being ectothermic animals, are directly affected by temperature, such that small variations of this variable in water affect all physiological processes (Ficke et al. 2007). It has recently been observed that conditions of highwater temperatures alter the functioning of the reproductive axis of fish and can block spawning, mainly by decreasing the levels of sex steroids in both sexes (Strüssmann et al. 2010, Pankhurst & Munday 2011, Miranda et al. 2013). Since probably as a consequence of global warming it has been reported that the water temperature of the Chascomús lagoon (Pampas region, Buenos Aires, Argentina) increased by an average of 1.4 °C in the last 50 years (Elisio et al. 2015a), being relevant to evaluate reproduction condition of living fish in natural environment.

Pejerrey (*O. bonariensis*), inhabits the shallow lakes of Pampean region and is considered emblematic of the freshwater ichthyofauna of Argentina (López & García 2001, Somoza et al. 2008, Berasain et al. 2015, Colautti et al. 2015). Pejerrey is a multiple spawner, presenting simultaneously oocytes in all stages of maturation and the same female can spawn several times within a defined reproductive time (Strüssmann 1989). Two periods of reproductive activity have been described in this species, one in spring and one in autumn (Calvo & Morriconi 1972, Strüssmann 1989, Miranda et al. 2006, Chalde et al. 2014, 2016).

Previous analyzes of the gonadosomatic indexes of gonadal stages determined by histology and plasma levels of sex steroids in pejerrey captured in Chascomús lake, demonstrated the strong relationship of the reproductive cycle with the environment. It was observed that vitellogenesis occurs in cold months and short photoperiod (between May and August), final maturation and spawning between September and November (slight increase in temperature and photoperiod) and signs of regression are observed at the end of November with temperatures greater than 21° C and long photoperiods (Elisio et al. 2014, 2015b).

It is important to note, that the studies carried out in Gómez lake are related to the abundance of fish species (Rosso & Quirós 2010), the composition of algal communities (Izaguirre & Vinocur 1994, Izaguirre et al. 2015) and zooplankton communities (Schiaffino et al. 2019). But, in spite of the importance of pejerrey as economic resource in Junín County, there is not information about reproductive aspects of the pejerrey wild population that inhabits in this lake. In this context, the objective of this work was to describe for the first time the gonad stages of pejerrey *O. bonariensis* during the spawning season in three consecutive years in relation to sex steroids plasma levels and the temperature in Gómez lake.

The results obtained can be useful for decision making in the correct management of this value natural resource.

MATERIALS AND METHODS

Study Area

Gómez lake (Fig. 1) has an area of 40 km² and an average depth of 1.2 m. The water is alkaline with pH between 8.3 and 9.9, oligohaline (conductivity ranges from 1.52 to 4.19 mS/Cm),

turbidity of 19 cm (Secchi) and salinity of around 3 g/L (Izaguirre et al. 2015).

Animal sampling

Adult pejerrey of both sexes were sampled in Gómez lake throughout the spawning season (during the first days of August, October and December) in 2013, 2014 and 2015. A net gang composed by eight floating multifilament gill nets with a height of 1.3 m, differing in lengths (4.5; 7.4; 8.6; 13.4; 20.2; 30.2; 45.4; and 70.2 m), and mesh sizes (bar distance: 14, 19, 21, 25, 28, 32, 36, and 40 mm) was used (Berasain et al. 2015). All samplings were carried out at night, and the fish were taken alive to the Hydrobiology Station of Junín in the morning. Immediately, adult pejerrey were anesthetized with a bath of benzocaine (100ppm) and measured (Total Length: TL, Standard Length: SL in cm, and Weight: W in g; Table I).

Only selected fish above the length of the first maturation (>14cm of SL) were used (Calvo & Morriconi 1972, Strüssmann 1989). Immediately after, blood samples were taken from the caudal vein using heparinized syringes, and plasma samples were obtained by centrifugation (3,000 g for 15 min.) at 4° C and stored at -80° C for sex steroids measurements. Gonads were excised and weighted in order to calculate the gonadosomatic index (GSI = 100xGW/W). A section of each gonad was fixed in formalin 10% and processed by routine methods for embedding in Paraplast Plus and posterior histological analysis. All fish were handled and sacrificed in accordance with the UFAW Use and Care Committee Handbook on the Care and Management of Laboratory Animals (<http://www.ufaw.org.uk/pubs.htm#Lab>) and local regulations.

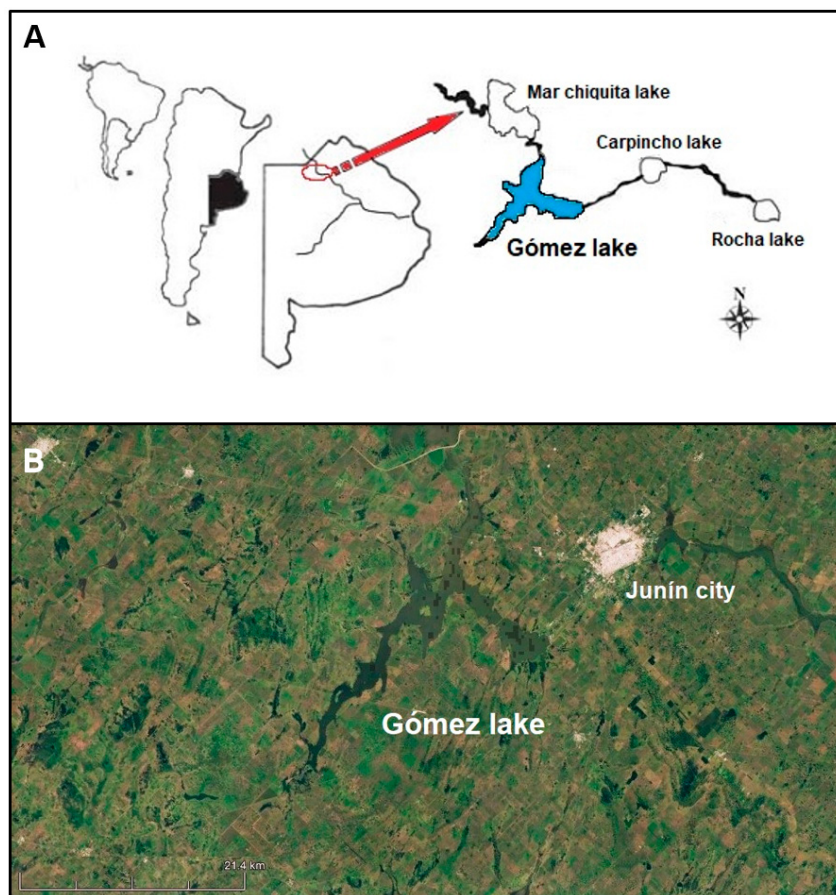


Figure 1. (a) Schematic and satellite diagram (b) of the location of Gómez lake, Junín, Buenos Aires, Argentina.

Table I. Morphometric data of pejerrey captured in Gomez lake.

Year	Date	Females				Males			
		SL (cm)	TL (cm)	W (g)	N	SL (cm)	TL (cm)	W (g)	N
2013	August 5 th	21.4 ± 6.8	25.3 ± 7.9	164.3 ± 189.1	13	20.0 ± 2.7	23.6 ± 3.3	98.9 ± 45.2	4
	October 7 th	20.8 ± 2.7	24.7 ± 3.4	111.2 ± 49.8	9	20.9 ± 2.9	26.4 ± 4.5	110.0 ± 45.2	6
	December 15 th	16.5 ± 0.8	19.3 ± 1.1	54.93 ± 11.9	12	16.1 ± 1.7	19.0 ± 2.2	51.1 ± 17.6	6
2014	August 6 th	20.9 ± 2.9	23.1 ± 3.6	85.9 ± 23.9	13	19.7 ± 1.7	21.5 ± 0.9	74.4 ± 7.6	5
	October 1 st	20.3 ± 2.4	23.4 ± 1.7	98.9 ± 22.5	10	19.2 ± 1.6	22.55±1.17	90.5 ± 17.0	11
	December 9 th	18.7 ± 1.7	36.5 ± 33.9	107.4 ± 31.7	7	18.1 ± 0.8	22.61±1.92	89.8 ± 28.8	9
2015	August 11 th	18.7 ± 2.6	21.5 ± 2.7	69.9 ± 44.0	10	20.5 ± 2.3	22.4 ± 2.8	73.8 ± 35.4	10
	October 13 th	21.2 ± 4.5	25.4 ± 5.9	134.6 ± 77.0	10	15.3 ± 1.6	18.5 ± 1.9	44.1 ± 15.3	10
	December 15 th	24.6 ± 2.8	29.1 ± 3.3	201.9 ± 64.1	12	23.3 ± 1.9	27.6 ± 2.4	176.2 ± 50.5	8

Total length (TL). Standard length (SL) and weight (W). Values are mean ± Standard Deviation.

Histological analysis

Gonad sections of 6µm thick were stained with hematoxylin and eosin for observation of histological characteristics in order to determine different reproductive stages in each animal. Ovarian stages were defined following the guidelines proposed by (Elisio et al. 2014): Primary growth (**PG**), cortical alveoli (**CA**), initial vitellogenesis (**VtgA**), advanced vitellogenesis (**VtgB**), final maturation (**FM**), atretic (**AT**) and ovulated (**OV**).

Testicular stages were redefined following the guidelines proposed by Elisio et al (2015b): Arrested (**A**), spermatogonial stage (**SG**), spermatocytary stage (**SC**) and spermiogenic stage (**SP**). This last stage was reclassified as: Initial spermiogenic stage (**ISP**): 4 – 6 layers of spermatogonia (Spg); spermatogenic lobules well developed; testicular lumen full of spermatozoa (Spz); GSI ~ 1.8 %. Spermiogenic stage (**SP**): Number of Spg (2 – 3 layers) and spermatocytes (Spc) relatively scarce; most of spermatogenic lobules possess Spz; testicular lumen full of Spz; GSI: ~ 2 %. Final spermiogenic stage (**FSP**): 2 – 3 layers of Spg, spermatogenic lobules less developed than in ISP and SP and full of spermatids (Spd) and Spz; testicular lumen has scarce Spz; GSI: ~ 1%.

The identification of the stages of both sexes was performed on micrographs taken with a light microscope Nikon Eclipse E600, equipped with a digital photomicrographic system (Nikon Digital Sight DS-U2). The percentages of each gonadal stages were calculated per each date of sampling.

Sex steroid measurements

The plasma levels of estradiol (E_2) in females and testosterone (T) in males were measured by an enzyme-linked immunosorbent assay (ELISA), using commercial kits and following the manufacturer protocols (DRG International Inc.,

Mountainside, NJ, USA; E2: EIA-2693 and T: EIA-1559) previously validated by Chalde et al (2016). Serum samples were extracted with diethyl-ether and suspended in their initial volume of PBS buffer. A standard curve was run for each ELISA plate. The lower limits of detection were 9.7 pg/mL for E_2 and 83 pg/mL for T. The optical density was read at 450 nm. The intra-assay coefficients of variance were < 10 %.

Environmental variables

The air temperature and precipitation data were provided by the National Meteorological Service (Junín station, Buenos Aires Province, Argentina: 34° 39' S 61° 01' 0). Maximum, mean and minimum monthly temperatures were calculated from July to December. Mean annual precipitation (mm) was calculated from daily data. Water salinity was measured in each sampling date using an optical refractometer (Atago Co, Tokyo, Japan) to the nearest of 0.1 g/L.

Statistical analysis

Gonadosomatic indexes and hormonal data are presented as the mean ± standard error of the mean (SEM). Compliance of data with the normal distribution was analyzed by the Shapiro–Wilk test, and the Levene test was used to check the homogeneity of variance. Statistical differences between months or stages were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The results were considered statistically significant at $p < 0.05$. When the data lacked the assumptions of the ANOVA, logarithmic transformations were used. Statistical analyses were performed using GraphPad Prism 5.0 Software and InfoStat Software.

RESULTS

The morphometric data and the number of animals captured during the study periods are shown in Table I. Mature specimens of both sexes were captured on all sampling dates with sizes between 16.1 ± 1.7 to 24.6 ± 2.8 cm of SL and weights from 51.1 ± 17.6 to 164.3 ± 189.1 g.

In August 2013, the majority of the females were in CA (80%) and the rest of them were in

vitellogenic stages. In October, the first females showed signs of ovulation (22%), the majority were vitellogenic (VtgA: 22% and VtgB: 34%) and the rest in FM and CA stages. In December, most of the females captured were in PG (75%), 15% in CA, one in FM and another atretic (Fig. 2a). In October, there was a peak in the values of the GSI and the plasma values of E_2 ($3.6 \pm 0.9\%$ and 530.6 ± 84.5 pg / ml, respectively) with minimum values in December ($0.7 \pm 0.3\%$ and 255.1 ± 26.7 pg

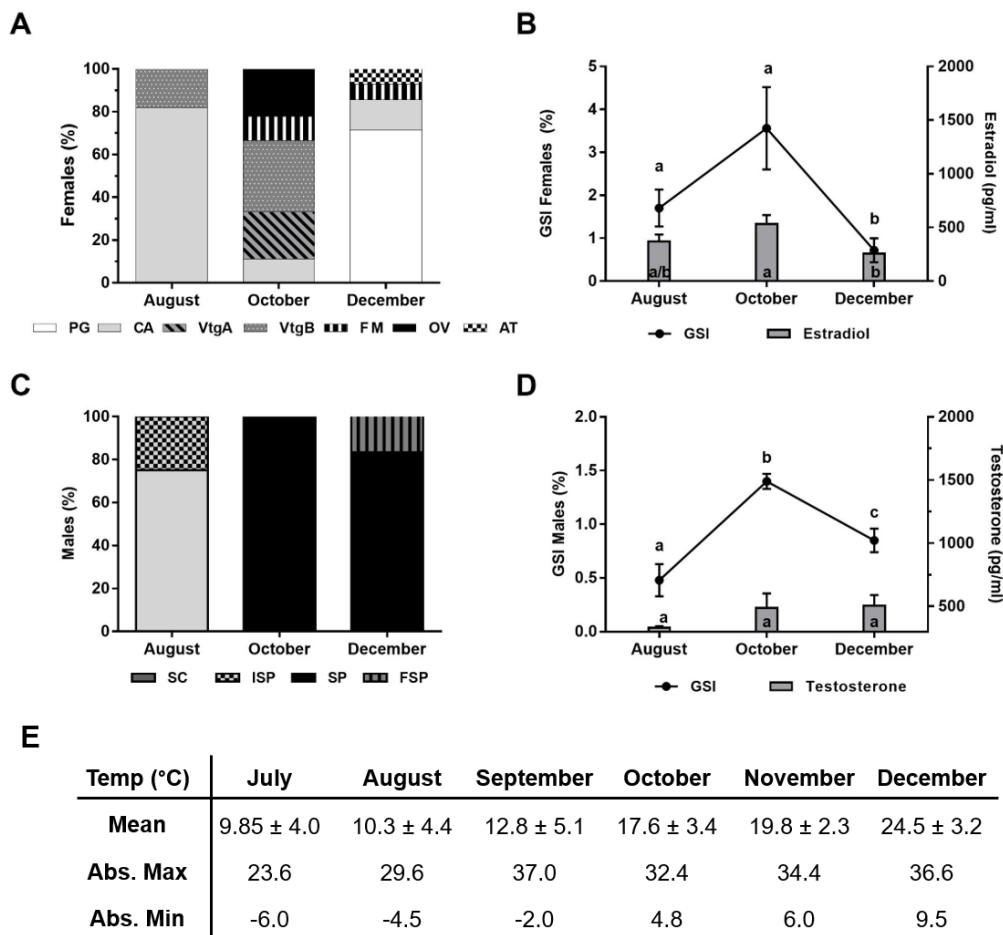


Figure 2. Pejerrey (*O. bonariensis*) sampling in Gómez lake (2013). a) Percentage of females at different gonadal stages. CA: cortical alveoli stage; VtgA: initial vitellogenesis stage; VtgB: advanced vitellogenesis stage; FM: final maturation stage; OV: ovulated females; AT: atretic stage. b) Female gonadosomatic indexes (GSI: dots) and E_2 plasma levels (bars). Values are mean \pm SEM. Different letters represent significant differences between sampling dates (Tukey’s multiple comparison test. $p < 0.05$). c) Percentage of males at different gonadal stages. ISP: Initial spermiogenic; SP: spermiogenic; FSP: final spermiogenic; A: arrested. d) Male gonadosomatic indexes (GSI: dots) and T plasma levels (bars). Values are mean \pm SEM. Different letters represent significant differences between sampling dates (Tukey’s multiple comparison test. $p < 0.05$). e) Air temperature ($^{\circ}C$) during the spawning season. Mean: Monthly mean temperature; Abs. Min.: Absolute Minimum; Abs. Max.: Absolute Maximum.

/ ml, respectively, Fig. 2b). In the case of males in August, few were releasing sperm (ISP: 25%) and the highest proportion were in spermatogenesis (SG: 75%). In October and December all the males were releasing sperm. In October, 100% was in SP whereas in December 15% was found ending the spermiation (Fig. 2c). A GSI peak was registered in October ($1.4 \pm 0.1\%$) with minimum values in December ($0.9 \pm 0.1\%$). Higher values of T were found in October and December (487.2 ± 115.9 pg / ml and 504.6 ± 85.5 pg / ml, respectively, Fig. 2d). The air temperature during the 2013 sampling period are shown in Fig. 2e. The mean air temperature of the spawning period was 15.8 ± 5.8 °C and varied between 9.9 ± 4.0 °C in July to 24.5 ± 3.2 °C in December. Besides, the absolute maximum temperatures ranged between 23.6 °C to 37.0 °C and the absolute minima from -2.0 °C to 9.5 °C.

Regarding the females captured in 2014, it is important to note that OV females were found in the three sampling dates. In August, 15% were OV, the same proportion were FM, 50% vitellogenic (VtgA: 20% and VtgB: 30%) and 15% in CA. In October, most of the females were OV and only one was in FM, while in December the proportion of OV decreased to 28%. The same percentage was found in FM and VtgB and one female in PG (Fig. 3a). The highest values of the GSI and E_2 were registered in October ($5.4 \pm 2.9\%$ and 2093.9 ± 1745.9 pg / ml, respectively) with minimum values in December ($3.7 \pm 1.3\%$ and 1377.0 ± 1437.5 pg / ml, respectively, Fig. 3b). In the case of the males captured in this year, all were releasing sperm apart from some of them (25%) who were arrested in December. In August and October, similar proportions were found in SP (80% and 75%, respectively) and ISP (20% and 25%, respectively) and also in December, 30% were found in FSP and 45% in SP (Fig. 3c). In the case of the GSI, the highest values were registered in October and December ($1.9 \pm 0.3\%$

and $1.6 \pm 0.6\%$, respectively) and for the case of plasma T values in August (1749.4 ± 877.3 pg / ml) with a minimum in December (755.4 ± 549.7 pg / ml, Fig. 3d). The mean air temperature of the spawning period was 16.5 ± 4.5 °C, ranging from 10.6 ± 2.9 °C in July to 22.0 ± 3.2 °C in December with absolute maximum temperatures between 22.5 °C to 36.0 °C and absolute minimum temperatures between -5 °C to 8 °C (Fig. 3e).

As in 2014, for the specimens captured in 2015, it was possible to find OV females in the three sampling dates. In August the first female OV was found, and the rest were in several gonadal stages: 50% were in CA, 20% in VtgB and also one female in FM and VtgA. In October the proportion of OV increased to 40%, the same amount was found in FM and 20% in VtgB, while in December only 15% of OV was found and 50% of the captured females were atretic (Fig. 4a). In October, a peak of the GSI values and the plasma E_2 values ($6.3 \pm 2.5\%$ and 1058.5 ± 1430.0 pg / ml) were recorded with minimum values in December ($3.2 \pm 2.6\%$ and 308.4 ± 121.2 pg / ml, Fig. 4b). In the case of males, in August, 80% did not release sperm and the rest were beginning spermiation. In October, all were releasing sperm while in December 50% of the males were arrested and most of them were ending their spermiation (FSP: 35%, Fig. 4c). The highest values of the GSI were registered in October ($1.8 \pm 0.5\%$) and the highest values of T in August (3021.7 ± 651.2 pg / ml) with minimum values of the two parameters in December ($0.4 \pm 0.2\%$ and 367.9 ± 30.7 pg / ml, respectively, Fig. 4d). The air temperature during 2015 sampling period were shown in Fig. 4e. The mean air temperature of the spawning period was 15.5 ± 4.8 °C, ranging from 10.4 ± 3.5 °C in July to 23.2 ± 3.2 °C. Additionally, the absolute maximum air temperature varied from 22.7 °C to 35.4 °C and the absolute minimum air temperature from -5.0 °C to 8.8 °C. The salinity of the water was greater

in the first year of sampling (2013: 4.2 ± 0.3 g / L) and was decreasing in the following years: in 2014 it was 2.1 ± 0.1 g / L and in the 2015 was 1.2 ± 0.5 g / L. These findings were in agreements

with the increase of the precipitations observed in 2013 (625 mm), 2014 (1357 mm) and 2015 (1540 mm).

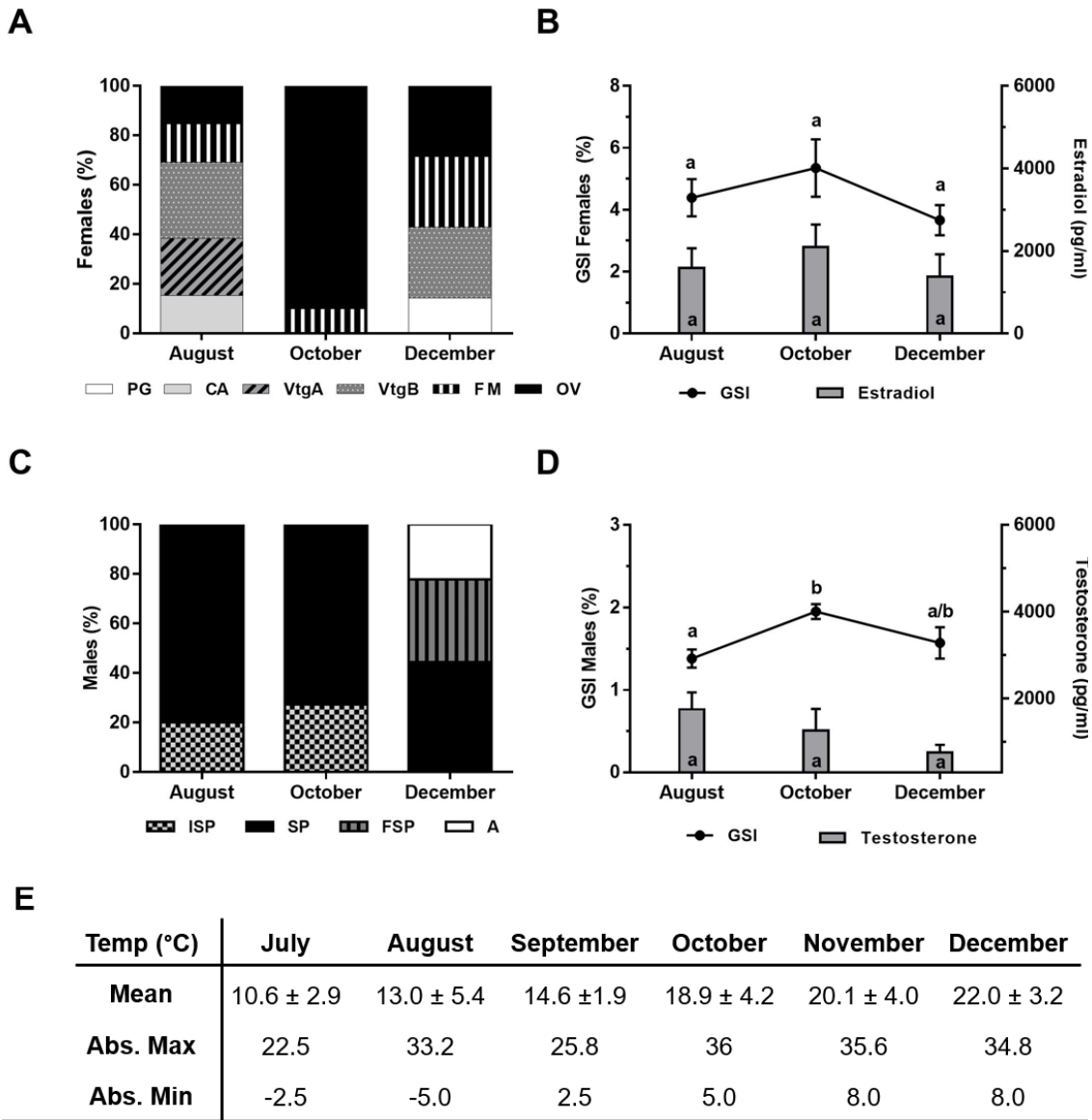
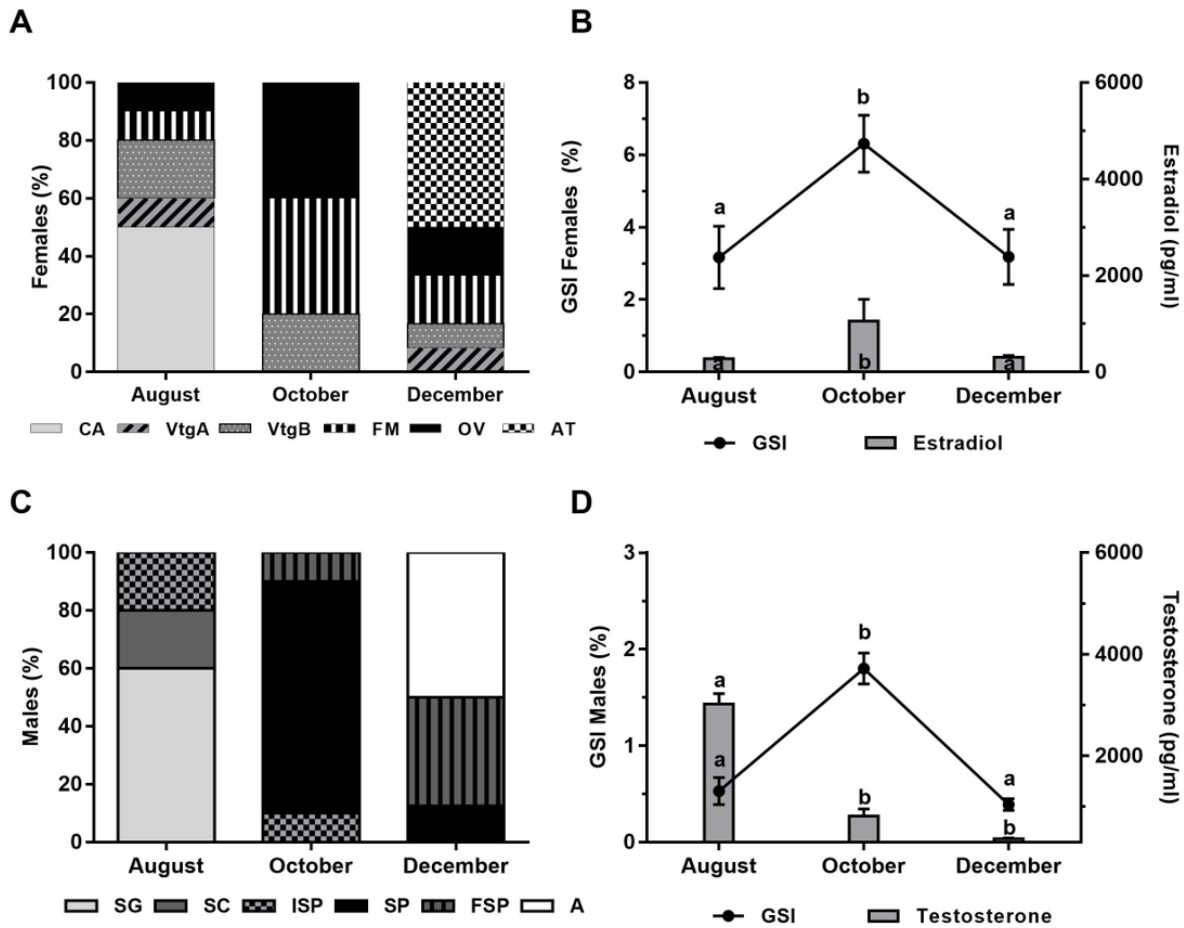


Figure 3. Pejerrey (*O. bonariensis*) sampling in Gómez lake (2014). a) Percentage of females at different gonadal stages. CA: cortical alveoli stage; VtgA: initial vitellogenesis stage; VtgB: advanced vitellogenesis stage; FM: final maturation stage; OV: ovulated females; AT: atretic stage. b) Female gonadosomatic indexes (GSI: dots) and E₂ plasma levels (bars). Values are mean ± SEM. Different letters represent significant differences between sampling dates (Tukey's multiple comparison test. p < 0.05). c) Percentage of males at different gonadal stages. ISP: Initial spermiogenic; SP: spermiogenic; FSP: final spermiogenic; A: arrested. d) Male gonadosomatic indexes (GSI: dots) and T plasma levels (bars). Values are mean ± SEM. Different letters represent significant differences between sampling dates (Tukey's multiple comparison test. p < 0.05). e) Air temperature (°C) during the spawning season. Mean: Monthly mean temperature; Abs. Min.: Absolute Minimum; Abs. Max.: Absolute Maximum.



E

Temp (°C)	July	August	September	October	November	December
Mean	10.4 ± 3.5	12.8 ± 2.9	12.8 ± 2.4	14.6 ± 3.4	19.1 ± 2.4	23.2 ± 3.0
Abs. Max	22.7	26.3	25.3	30.0	30.3	35.4
Abs. Min	-5.0	2.0	-0.5	2.6	8.0	8.8

Figure 4. Pejerrey (*O. bonariensis*) sampling in Gómez lake (2015). a) Percentage of females at different gonadal stages. CA: cortical alveoli stage; VtgA: initial vitellogenesis stage; VtgB: advanced vitellogenesis stage; FM: final maturation stage; OV: ovulated females; AT: atretic stage. b) Female gonadosomatic indexes (GSI: dots) and E₂ plasma levels (bars). Values are mean ± SEM. Different letters represent significant differences between sampling dates (Tukey's multiple comparison test. p < 0.05). c) Percentage of males at different gonadal stages. ISP: Initial spermiogenic; SP: spermiogenic; FSP: final spermiogenic; A: arrested. d) Male gonadosomatic indexes (GSI: dots) and T plasma levels (bars). Values are mean ± SEM. Different letters represent significant differences between sampling dates (Tukey's multiple comparison test. p < 0.05). e) Air temperature (°C) during the spawning season. Mean: Monthly mean temperature; Absolute Minimum; Abs. Max.: Absolute Maximum.

DISCUSSION

This study describes for the first time the reproductive status of pejerrey population of Gómez lake using gonadal histology, gonadosomatic indexes and T and E₂ plasma levels during three consecutive spawning seasons. It was observed that the reproductive activity of pejerrey developed according to that reported previously in the same species for Chascomús lake (Elisio et al. 2014, 2015b), showing an increase in gonadal maturation as the air temperature increases from winter to spring, associated with high values of GSI and sex steroids plasma levels. At the beginning of summer with high temperatures, a decrease of all these parameters is observed with arrested and atretic specimens.

Our results showed that the spawning started in August of 2014 and 2015 (ovaries with post-ovulatory follicles) with mean air temperatures in July of 10.6 ± 2.9 °C and 10.4 ± 3.5 °C, respectively. In addition, in 2014 most of the captured males released sperm fact that did not happened in 2015. Although there were ovulated females and males initiating spermiation in 2015 a delay in maturation is observed because a large proportion of females were found in AC stages and males in spermatogenesis. Similarly, for August of the first year of sampling (2013) there was a delay in maturation in both females and males since no OV females have been found. This retardment in maturation and spawning in these two years of sampling may be due to the fact that the mean, and the minimum and maximum absolute air temperatures of July 2013 and 2015 were lower than in 2014.

In October of the three years of sampling, the highest proportion of ovulated females and males in spermiation were captured with mean air temperatures (September) of 12.8 ± 5.1 °C, 14.6 ± 1.9 °C and 12.8 ± 2.4 °C, respectively. These

temperatures were like to those reported for the ovulation and spermiation of pejerrey in Chascomús lake (Elisio et al. 2014, 2015b).

In December of 2014 and 2015 ovulated females and males releasing sperm were found however, for 2013 and 2015 also atretic females, and for 2014 and 2015 also arrested males were found. This fact indicated that spawning season would be finishing associated with an increase of the temperature during the end of spring. Similar observation has already reported by Elisio et al (2014) for Chascomús lake finding females and males in the same gonadal stages with maximum water temperatures above 21 °C. In the case of Gómez lake, the maximum absolute air temperatures for November were greater than 21 °C, which can explain the appearance of males and females finishing the spawning season for 2014 and 2015. In December 2013, also some females were found in PG stage, like to that reported in December for pejerrey of Chascomús lake. These findings showed that pejerrey females after finishing the spawning remain in resting stage to the next reproductive season when the environmental conditions will be favorable (Elisio et al. 2014). The fact that it was not possible to find arrested males in December 2013, could be explained due to that spermatogenesis occurs in a wider range of temperatures than the oogenesis as it was demonstrated in captivity and in the wild for the same species (Chalde et al. 2014, 2016, Elisio et al. 2015b).

It is known in teleost fish, that the GSI of both sexes increased proportionally with the gonadal development associated with sex steroids plasma levels (E₂, T and 11-ketotestosterone (Kadmon et al. 1985, Kumar et al. 2000, Miura et al. 1991, Weltzien et al. 2002, Miranda et al. 2007, Muncaster et al. 2010, Schulz et al. 2010, Zohar et al. 2010, Pham et al. 2011, Adebisi et al. 2013). Our results showed that in

three years of sampling the GSI increased from August to October and decreased in December in both sexes evidencing the peak of spawning / spermiation in October coinciding with the highest proportion of ovulated females and males in full spermiation. This increase in the GSI of females is accompanied by the higher values of E_2 in October and decreasing in December when some females were in PG and AT stages. In the case of T levels, is not observed a clear pattern with animals presenting high values during spermatogenesis (sampling of August 2015), and low values in December in accordance with the presence of arrested males. This finding was coincident with that previously published about the role of T during spermatogenesis and spermiation. It should be noted that also in December 2013 the T values were high since all the males were releasing semen.

In summary, the results obtained in this study, reinforced the concept that pejerrey *O. bonariensis* spawning is strongly influenced by environment, mainly to temperature variation. Due to the sensitivity of this species not only to natural stressors as well also human pollution (Gárriz et al. 2015, 2017, 2019, Menéndez-Hellman et al. 2015) future investigations are needed to clarify these issues. Also, these findings can help local authorities to make decisions in order to protect this emblematic fish and to develop an integrated management of Gómez lake.

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