

# Comparative Analysis of Pejerrey Fish (*Odontesthes bonariensis*) Gonadal Development During Two Consecutive Spawning Seasons in Relation to Sex Steroids and Temperature Variation in Cochicó Lake (Pampas Region, Argentina)

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## Abstract

Cochicó belongs to “Encadenadas del Oeste” system of lakes being a typical water body of the Pampas region. The most abundant fish species in this lake is the pejerrey (*Odontesthes bonariensis*) valued due to the quality of its flesh and as a game fish. The aim of this study was to compare the gonadal stages of pejerrey during two consecutive spawning seasons (August to December) in relation to sexual steroids and temperature in this lake. In general, pejerrey gonadal development, the Gonadosomatic index and the plasma levels of estradiol and testosterone fluctuated in relation with temperature. In 2014 samplings, females started to ovulating in early August, with a peak during September-October and ending in December with many of them with atretic oocytes. However, in 2015, a marked delay in maturation was observed with ovulated fish only in October and December. This fact may be because the minors mean temperatures recorded in this year. For males, it was possible to find spermiating animals during the whole spawning season and only arrested animals in December. Unexpectedly, histological gonadal analysis revealed for the first time pejerrey with testis-ova, probably due to the intensive use of agrochemicals in this region.

## Introduction

In the Pampas region of Argentina, the typical water bodies are shallow lakes. These lakes are commonly eutrophic environment with a high limnological variability. Some of them are permanent and others are temporary with periodic flooding and drought conditions (Diovisalvi *et al.*, 2015). Because of these special characteristics, they are highly sensitive to climatic variation (Mooij *et al.*, 2008; Jeppesen *et al.*, 2010). In the last years, it was demonstrated that this region is experiencing a large-scale change in climate such as elevated temperatures, more abundant

precipitations, and increased frequency of floods and droughts events (Berasain *et al.*, 2015; Colautti *et al.*, 2015; Elisio *et al.*, 2015a; Elisio *et al.*, 2018). Moreover, Pampas lakes are the natural habitat of a fish species with high biological and economic value, both for their commercial importance and for their sporting or recreational interest, the pejerrey *Odontesthes bonariensis* (Somoza *et al.*, 2008).

One of the most important systems of the Pampas lakes, is “Encadenas del Oeste” localized in Buenos Aires Province, Argentina. This system marks the beginning of the productive area of the west of this province and is located in a climate transition zone from a sub-humid to

semi-arid climate. The main lakes belonging to this system, presented from west to east are: *Epecuén, del Venado, del Monte, Cochicó and Alsina*. It is important to mention, that the lands surrounding these lakes are subjected to an intensive agricultural activity, highlighting the crops of drought-resistant cereals. In these lakes pejerrey is one of the most abundant fish, being its recreational fishing activity very important especially in Cochicó lake. This water body is an endorheic and eutrophic lake with a surface of approximately 40 km<sup>2</sup>, with diurnal oxygen deficit in depth, without permanent thermal stratification and it has been classified according to the soluble solid residue, as a body of mesohaline water to oligohaline.

It is known, that fish reproduction is regulated by different hormones and enzymes that jointly control the functioning of the brain-pituitary-gonad axis in order to stimulate gametogenesis and spawning (Lubzens *et al.*, 2010). Besides, in temperate regions environmental cues (mainly photoperiod and temperature) modulate fish reproductive axis and thereby promote the spawning seasonality (Pankhurst & Porter, 2003), as it was also demonstrated in pejerrey (Elisio *et al.*, 2014; 2015b). It was reported in the wild that pejerrey is a multiple spawner with a major reproductive period at the end of the winter with a peak during spring and a minor one in autumn, depending of the environmental conditions (Elisio *et al.*, 2014; 2015b). In females pejerrey it was observed that a new oocyte clutch starts to develop when the leading clutch approaches ovulation, being able to spawn frequently in short intervals of 20 to 30 days in captivity (Chalde *et al.*, 2016; Chalde & Miranda, 2020). In this specie, it was also demonstrated that the increase of water temperature and photoperiod modulate gametogenesis in Chascomús lake (Elisio *et al.*, 2014; 2015b). Also, it has been reported that high temperatures above 23° C decreased sex steroids plasma levels, provoking a complete regression and spawning impairment (Soria *et al.*, 2008; Elisio *et al.*, 2012; Miranda *et al.*, 2013).

It is important to mention that this system of lakes locates in the south west of Pampas region, was poorly studied, and also it has been recently observed a decrease in pejerrey population specially in Cochicó lake. In spite of the importance of this species as economic resource this is the first study that described the gonadal stages of pejerrey during two consecutive spawning seasons in relation with sex steroids and air temperature in Cochicó lake. The findings obtained can help government authorities to make decisions in order to manage this value natural resource.

## Materials and Methods

### Study Area

Cochicó lake belongs to a System of Pampas lakes that extends between the parallels 36° 30" and 37° 30" S and meridians 61° 00' and 63°30' W (Figure 1) in Guaminí county, in the south-west of Buenos Aires Province, Argentina. This system is well distinguished by a defined east – west gradient salinity from fresh to saline water.

### Animal Sampling

Adult pejerrey of both sexes were sampled in Cochicó lake throughout the spawning season (August, October and December) in 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 Cochicó in the morning. Immediately, adult pejerrey were anesthetized with a bath of benzocaine (100ppm) and measured (Total Length; TL, Standard Length: SL in cm; Weight: W in g; Table 1). Only selected fish above the length of the first maturation (>14cm of

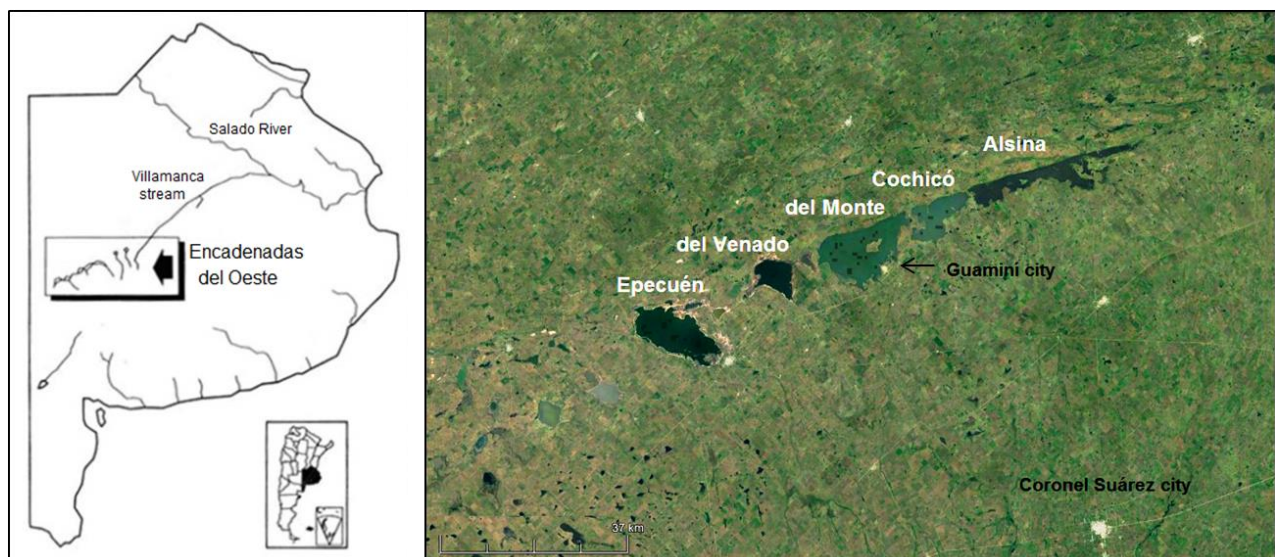


Figure 1: "Encadenadas del Oeste" System lakes, Buenos Aires, Argentina.

SL) were used (Elisio *et al.*, 2014). After this, blood samples were taken from the caudal vessels using heparinized syringes, and plasma samples were obtained by centrifugation at 4°C and stored at -80°C for sex steroids measurements. Then, gonads were excised and weighted in order to calculate the gonadosomatic index (GSI = 100GW/W). After, 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.

### Histological Analysis

Ovarian and testicles sections of 6 µm thick were stained with hematoxylin and eosin for observation of histological characteristics and estimation of the reproductive status of each animal. Female gonad 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).

Male gonadal stages were defined 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 in: Initial spermiogenic stage (ISP): 4-6 layers of spermatogonia (Spg); spermatogenic lobules well developed; testicular lumen is full of spermatozoa (Spz); GSI ~ 1.8 %. Spermiogenic stage (SP): The number of Spg (2-3 layers) and spermatocytes (Spc) is relatively scarce. Most of spermatogenic lobules possess Spz. The testicular lumen is full of Spz; GSI: ~ 2 %. Final spermiogenic stage (FSP): 2-3 layers of Spg, spermatogenic lobules are 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-Fi1).

Then, the percentages of gonadal stages of both sexes were calculated per each date of sampling.

### Sex Steroid Measurements

The plasma levels of estradiol (E<sub>2</sub>) 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; E<sub>2</sub>: 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<sub>2</sub> 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 from the National Meteorological Service (Coronel Suarez, Buenos Aires Province, Argentina 37° 28' S, 61° 56' O) 50 miles far from Cochicó lake. We decided to analyze these data instead of water temperature because this lake is 600 Km far from INTECH and it was impossible to set up and equipment to record water temperature continuously. In Pampas shallow lakes it was demonstrated that water showed a tight coupling with air temperature, therefore suggesting that their thermal conditions depend largely on the local climate (Elisio *et al.*, 2015a). Maximum, mean and minimum temperatures were calculated monthly 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 profiles data are presented as the mean ± standard error of the mean (SEM). Normal distribution for data was analyzed by the Shapiro–Wilk test, and the Levene test was used to check the homogeneity of variance. The differences

**Table 1:** Morphometric data of pejerrey captured in Cochicó Lake.

Year	Date	Females				Males			
		SL (cm)	TL (cm)	W (g)	N	SL (cm)	TL (cm)	W (g)	N
2014	August 3 <sup>rd</sup>	35.2±3.7	41.3±3.9	741.9±234.9	12	41.5	35.3	641.0	1
	October 1 <sup>st</sup>	14.8±0.8	17.2±0.9	30.3±3.6	10	14.4±0.5	16.7±0.6	28.4±2.4	10
	December 9 <sup>th</sup>	14.9±0.6	17.5±0.6	32.2±2.1	9	14.7±0.3	17.3±0.4	31.0±2.3	6
2015	August 5 <sup>th</sup>	18.9±1.4	22.3±1.9	80.3±23.7	4	20.1±1.4	23.6±1.6	100.9±21.2	10
	October 7 <sup>th</sup>	17.6±7.7	20.3±9.1	101.4±191.5	8	14.9±1.4	17.4±1.6	36.6±9.2	10
	December 15 <sup>th</sup>	15.6±0.7	18.0±0.6	39.4±3.9	7	15.7±0.8	18.1±0.8	40.2±5.3	10

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

for these parameters 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 statistical test, logarithmic transformations were used. Statistical analyses were performed using GraphPad Prism 5.0 Software and InfoStat Software.

## Results

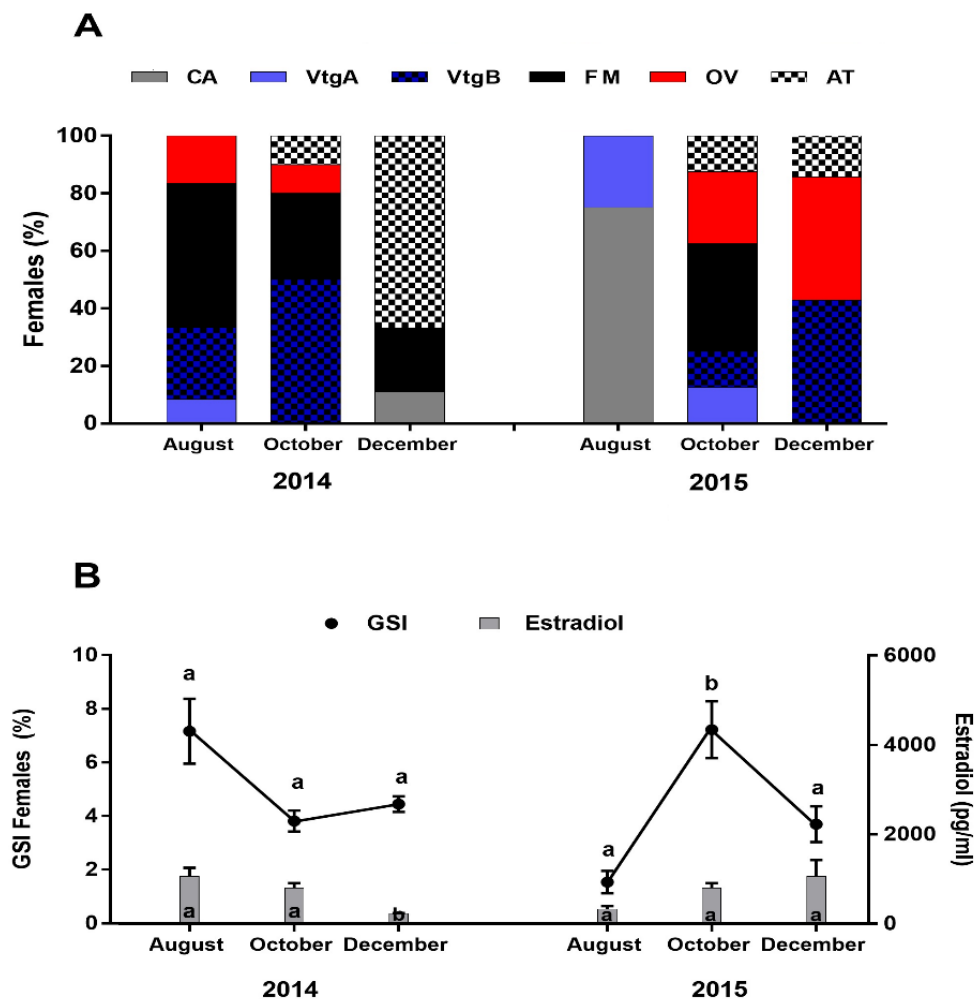
### Animal Sample Analysis

The morphometric data and number of pejerrey captured in 2014 and 2015 of both sexes are shown in Table 1. The differences in fish sizes between samples dates may be due to the different areas where the nets were placed in the lake and to the segregation by sex at the peak of spawning (Elisio *et al.*, 2014).

In August of 2014, some females were ovulated and the rest were at FM or at vitellogenic stages indicating the beginning of spawning. In October similar proportion of the same stages were observed, however,

one atretic female was found. In December, most of females were atretic (70%), the rest at FM stage and only one was at CA stage, indicating the end of reproductive period. In August of 2015, most females were at CA stage and one was vitellogenic. In October and December, similar proportion of OV (30% - 45%) and AT females (10%) females were found. A difference observed between these months was that in October the rest of them were at FM stage and in December the rest were at VtgB stage (Figure 2A).

The analysis of GSI and  $E_2$  in 2014 showed the highest GSI values in August ( $7.2 \pm 1.2\%$ ), and a slight decreased in October ( $3.8 \pm 0.4\%$ ) and December ( $4.5 \pm 0.3\%$ ). The  $E_2$  plasma values were also high in August ( $1051.0 \pm 200.9$  pg/ml) and in October ( $801.2 \pm 105.1$  pg/ml), whereas a strong and statistically significant (ANOVA,  $P < 0.05$ ) decline was observed in December ( $229.5 \pm 11.8$  pg/ml). In 2015, the GSI showed a clear peak in October with a maximum value of  $7.2 \pm 1.1\%$  being statistically significant different when compared with the other months. For  $E_2$  the highest levels were recorded in October and December (Figure 2B).



**Figure 2.** Pejerrey sampling in Cochicó Lake (2014 and 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:** 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.  $P < 0.05$ ).

In the case of males, only 1 was caught at ISP stage in August of 2014, while in October most of them were at SP and FSP stages. In December, 50% were at FSP, and the rest were arrested or at SP stages showing the end of spermiation. In 2015, males caught in August were initiating the spermiation. In October, all males were easily releasing sperm (90% SP stage) and in December, most of them were at FSP stage and the rest were arrested (Figure 3A). The GSI and T plasma values were high in August 2014 (1.87%, 2049.0 pg/ml, respectively) and in October (1.55±0.1 %, 588.8±133.3pg/ml) meanwhile a decreased was observed in December (1.3±0.1 %, 401.1±57.6 pg/ml,). In 2015, the highest GSI value was recorded in October (2.0±0.1 %) being statistically different (ANOVA, P<0.05) with respect to the minor value recorded in December samples. For T, the highest level was found in August (2042.1±70.3 pg/ml) meanwhile minor and statistically significant values (ANOVA, P<0.05) were observed in October and December (Figure 3B).

Unexpectedly, the histological gonadal analysis revealed that one male of August and one male of October belonging to 2015 samplings, had oocytes without follicles cells scattered among and within testicular lobules (testis-ova; Figure 4).

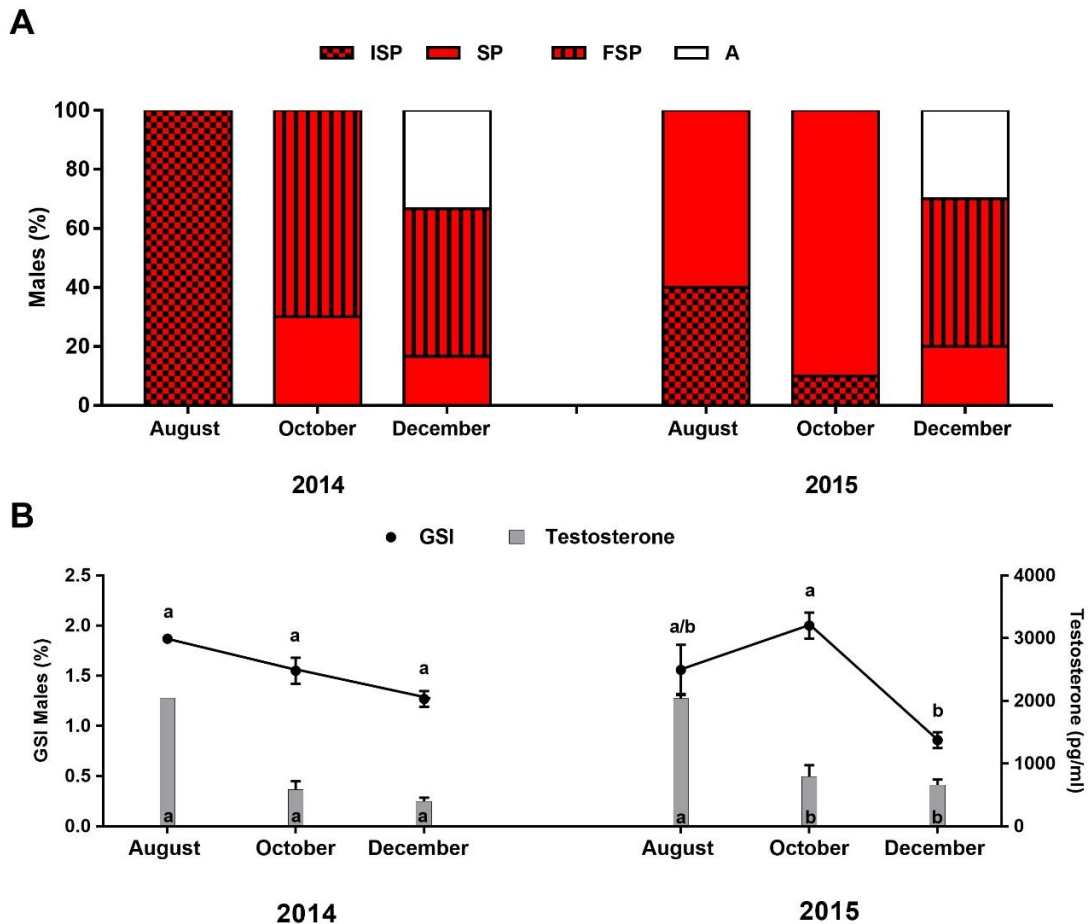
**Environmental Variables**

The air temperatures were summarized in Table 2. In 2014, the mean air temperature ranged from 7.6±3.0°C (July) to 20.2±3.4°C (December). In addition, the absolute minimum varied from - 4.1 to 3.8°C and the absolute maximum from 19.1 to 33.8°C. In the case of 2015, the mean air temperature ranged from 6.2±3.2°C (July) to 20.5±3.6°C (December), showing lower mean temperatures from July to October when compared with 2014 temperatures. Additionally, the absolute minimum varied from -5.5 to 3.7°C and the absolute maximum from 20.7 to 36.0°C.

Annual rainfall varied from 1141.5 mm in 2014 to 925.4 mm in 2015. Water salinity ranged from 1 to 1.7 g/l in 2014 and 1.8 to 2 g/l in 2015.

**Discussion**

This study described for the first time the reproduction status of pejerrey population from Cochicó lake using gonadal histology, GSI, sex steroids plasma levels during two consecutive spawning seasons. In general, it was observed that during the sampling periods the temperature did not present substantial



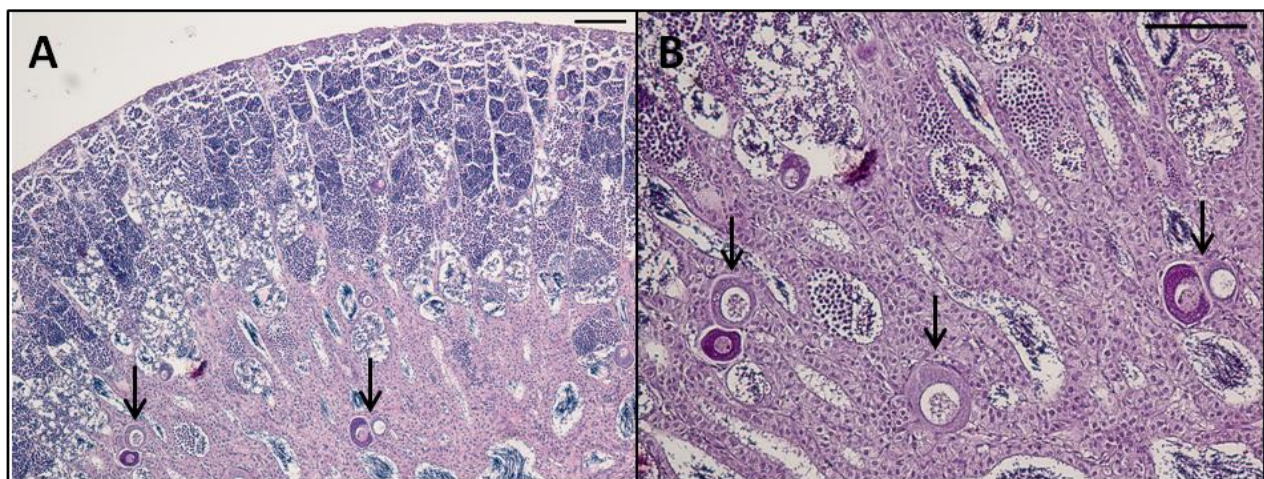
**Figure 3:** Pejerrey sampling in Cochicó Lake (2014 and 2015). **A:** Percentage of males at different gonadal stages. ISP: Initial spermiogenic; SP: spermiogenic; FSP: final spermiogenic; A: arrested. **B:** Male gonadosomatic indexes (GSI: dots) and T plasma levels (bars). Values are mean ± SEM. Different letters represent significant differences between sampling dates (Tukey's multiple comparison test. P<0.05).

variations. However, mean lower temperatures were recorded from July to October in 2015 when compared with 2014. Besides, the rainfall conditions were a little different modifying also the salinity of the water in the lake.

The pejerrey population of Cochicó lake showed a wide range of lengths, a cyclical and annual gonadal pattern of maturation with the presence of early juvenile in spring. In general, this study showed an increase of gonadal development pattern as temperature raises, associated to elevate GSI and  $E_2$  and T plasma levels with a decreased of all these parameters in early summer with higher temperatures. In teleost, gonadal development and spawning are regulated through different hormones which controlled the reproductive axis (hypothalamus-pituitary-gonads; Zohar *et al.*, 2010). Also, photoperiod and temperature are one of the most important factors that regulate fish reproductive cycle (Miranda *et al.*, 2013).

It has been previously reported for Chascomús pejerrey population that spawning period started at the

end of winter with a peak during spring (September-October) and ended in November-December with water temperatures near 23°C (Elisio *et al.*, 2014, 2015a). Similar results were obtained for Cochicó pejerrey population in 2014 samplings. However, in 2015 a marked delay in female maturation was observed since in August no ovulated fish were captured. Moreover, similar proportion of ovulated pejerrey were caught in October and December, with a few atretic animals. This fact may be because of the minors mean temperatures recorded from July to October in comparison with 2014 temperatures. In December samples, atretic females were captured, with a higher proportion in 2014. These observations indicated that the ovulation was ending associated to an increase of temperature during the end of spring, as it was reported by Elisio *et al.* (2014). It is known that in temperate teleost, the increase of water temperature during summer signals the end of reproductive episodes (Pankhurst & Porter, 2003; Miranda *et al.*, 2013).



**Figure 4.** A: Transversal section of pejerrey testicles showing oocytes (black arrow) in testicular lumen. B: High magnification of A. Bar: 100µm.

**Table 2.** Data of air temperature (°C) recorded during pejerrey spawning season. National Meteorological Service (Coronel Suarez, Buenos Aires Province, Argentina).

	Year	Months					
		July	August	September	October	November	December
Mean	2014	7.6±3.0	9.8±4.1	11.9±2.0	14.8±3.7	16.8±3.8	20.2±3.4
	2015	6.2±3.2	8.8±2.6	9.1±2.4	10.4±3.5	17.0±2.5	20.5±3.6
Max. Mean	2014	12.5±3.3	16.5±4.8	18.2±2.8	20.9±4.7	23.9±5.3	28.3±4.3
	2015	13.2±3.6	15.6±3.4	16.6±3.4	16.5±4.0	24.7±2.8	27.6±3.8
Min. Mean	2014	2.6±4.2	3.1±4.9	5.6±3.5	8.8±4.1	9.7±3.0	12.1±3.7
	2015	2.2±4.1	3.9±3.1	1.7±4.1	4.4±4.4	9.3±3.4	13.3±4.3
Abs. Max.	2014	19.1	27.3	22.3	29.3	33.2	33.8
	2015	20.7	23.4	22.8	24.2	29.2	36.0
Abs. Min.	2014	-4.1	-4.0	-0.6	2.3	4.5	3.8
	2015	-5.5	-1.4	-5.6	-4.2	1.6	3.7

Max: maximum; Min: minimum; Abs: absolute.

It was demonstrated that GSI increased proportionally with gonadal development associated with E<sub>2</sub> plasma levels, raising during vitellogenesis and declining before ovulation in several species as the Gilthead bream (*Sparus aurata*; Kadmon *et al.*, 1985), the Channel catfish (*Ictalurus punctatus*; Kumar *et al.*, 2000), Senegalese sole (*Solea senegalensis*; García-López *et al.*, 2007), Ballan wrasse (*Labrus bergylta*; Muncaster *et al.*, 2010), the Waigieu seaperch (*Psammoperca waigiensis*; Pham *et al.*, 2011), the River catfish (*Hemibagrus nemurus*; Adebisi *et al.*, 2013) among others (Lubzens *et al.*, 2010).

In this context, our results showed that in 2015, GSI and E<sub>2</sub> plasma levels increased from August to October and declined in December evidencing the spawning from October. Similar results were obtained by Elisio *et al.* (2014) for pejerrey population in Chascomús lake and in captivity (Miranda *et al.*, 2006; Chalde *et al.*, 2014; Chalde *et al.*, 2016). However, in 2014 the GSI value decreased from August to October (1 atretic female) meanwhile E<sub>2</sub> plasma levels, remained equal but with a significant reduction in December, evidencing the majority of atretic females.

In the case of pejerrey males, spermatogenesis occurs in a larger range of temperatures than in the oogenesis, because it was possible to find spermiating fish from August to December and in March and April in Chascomús lake (Elisio *et al.*, 2015b). Our results showed that in August all pejerrey captured were releasing sperm, with mean air temperatures of  $16.5 \pm 4.8$  and  $15.6 \pm 3.4$  °C respectively. These temperatures were similar to that reported for the starting of spermiation in the pejerrey of Chascomús lake (Elisio *et al.*, 2015b). In October of 2014, the gonadal histology revealed that most of the fish were in FSP stage meanwhile in 2015 were in SP stage, indicating that the peak of spermiation in 2014 was earlier than in 2015. These findings coincide with the presence of ovulated females in this study and with that reported by Elisio *et al.* (2014, 2015b).

In December of both years, in addition to pejerrey at SP and FSP stages, several arrested fish were caught indicating the ending of the reproductive activity. As it was mentioned for females, during the month before the sampling date (November) maximum air temperatures were above 24°C, being these temperatures higher than those that cause reproductive impairment in wild and captive pejerrey (Soria *et al.*, 2008; Elisio *et al.*, 2012; 2015b). Similar results were reported in other teleost species such as: the roach (*Rutilus rutilus*; Gillet & Quéting, 2006), Atlantic salmon (*Salmo salar*; Pankhurst *et al.*, 2011; Anderson *et al.*, 2012), Atlantic cod (*Gadus morhua*; Tveiten, 2008) among others (Miranda *et al.*, 2013).

In general, male GSI values increased proportionally with gonadal development jointly with T and 11-KT plasma levels (Miranda *et al.*, 2007; Schultz *et al.*, 2010). There have been several studies showing that 11-KT is the androgen most active in stimulate the spermatogenesis (Miura *et al.*, 1991; Ohta *et al.*, 2007;

Schultz *et al.*, 2010; Zohar *et al.*, 2010), while T plays an integral role in stimulating both hypothalamic and pituitary functions (Miranda *et al.*, 2007; Schultz *et al.*, 2010; Zohar *et al.*, 2010). Our findings showed that in 2014 the GSI value and T plasma levels decreased from August to December evidencing that the peak of spermiation was in August. In 2015 maximum values of T were measured in August and for GSI were recorded in October meanwhile a decrease of those parameters occurred in December jointly with the presence of arrested males. This trend indicated that the peak of spermiation was in October coincidentally with gonadal histological data.

According our knowledge there are no reports on the presence of pejerrey testis-ova in the wild. As it was mentioned, the lands surrounding “Encadenas del Oeste” lake system are subjected to an exhausted agricultural activity with an intensive use of agrochemicals such as atrazine. It has been demonstrated, that atrazine had negative effects on reproductive functions in fish (Spano *et al.*, 2004; Tillitt *et al.*, 2010; Papuolias *et al.*, 2014). For example, the fathead minnow (*Pimephales promelas*) and cunner (*Tautoglabrus adspersus*) exposed to atrazine produced fewer eggs and testicular oocytes than control fish (Tillitt *et al.*, 2010). However, other endocrine disruptors could cause similar adverse effects on male fish reproduction (Tyler *et al.*, 1996; Dietrich & Krieger, 2009; Sumpter & Jobling, 2013; Gárriz *et al.*, 2017; Norris *et al.*, 2018). In this way it has been recently demonstrated that the exposure to Cadmium produced testis-ova in pejerrey (Gárriz *et al.*, 2019).

In conclusion, it was demonstrated that pejerrey population is vulnerable to climate variability (specially temperature variations) and human pollution, and future investigations are needed to clarify these issues to protect this emblematic fish species and to develop an integrated management of Cochicó lake.

### Ethical Statement

All fish were handled 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.

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### Author Contribution

M.L.A: Conception, design and funding.  
M.L.A., D.C.C., G.E.B., del F.P.S: Acquisition, analysis and interpretation of data.  
M.L.A., del F.P.S., D.C.C: Manuscript writing.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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