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Effects of sex and season in haematological parameters and cellular composition of spleen and head kidney of pejerrey (*Odontesthes bonariensis*)

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Abstract Phylogenetic diversity in fish determines high interspecific variability in morphology as well as in physiological parameters. Moreover, several haematological variables and the organ composition of haemolymphopoietic sites may vary according to sex or season. The aim of this study was to establish the haematological parameters and the cellular composition of haemolymphopoietic organs in *Odontesthes bonariensis*, a commercially valuable fish species in Argentina, and also to determine gender or seasonal variations. Haematocrit exhibited the highest value in summer, while haemoglobin concentration was greater in summer and autumn. Erythrocyte count was higher in spring than autumn and winter, but did not differ with summer. The increase in these variables in seasons with higher water temperatures might be a compensatory mechanism to compensate the lower level of oxygen in the environment. Leucocyte formula and blast haemolymphopoietic cells in spleen and head kidney also showed annual variations since cells related to specific immune response, i.e., lymphocytes and thrombocytes, decrease in winter, whereas cells of the non-specific immune pathways, such as granulocyte, rise. The elevation of a particular type of circulating leucocyte was preceded by an increase in values of its precursor in blood in the previous season. Both, spleen and head kidney were active in haemolymphopoiesis, although with some differences in their activity during different seasons. Males showed higher values of circulating lymphoblasts and granulocytes than females, whereas females exhibited higher values of thrombocytes. This study corroborates the high interspecific variations in haematological parameters in fish that underlines the needing of basic studies in order to assess fish health status in new promising species for aquaculture.

Keywords Haematology · Blood cell lineages · Seasonal variations · Fish

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Introduction

Pejerrey (*Odontesthes bonariensis*) is one of the most important fish species cultured in Argentina due to the

quality of its flesh, its abundance in lagoons of Pampa Plain, and its high value for recreational fishing (Gómez 1998). Traditionally, the purpose of pejerrey aquaculture in Argentina was the production of embryonated eggs and fry for seeding and reseeding (Reartes 1995; Gómez 1998). The main factors that determine the under-exploitation of this resource are the reproductive seasonality and the slow somatic growth of the species (Reartes 1995; Calvo and Morriconi 1972). However, in the last decade, a growing interest on pejerrey aquaculture was noted and several works were conducted on culture systems (Miranda et al. 2006; Colautti et al. 2010), reproduction (Blasco et al. 2013; Elisio et al. 2012), endocrinology (Vigliano et al. 2011; Elisio et al. 2012; Blasco et al. 2013), immunology (Vigliano et al. 2006b), morphology (Ito et al. 2005; Vigliano et al. 2006a), and potential biotechnological tools applied to pejerrey production (Sciara et al. 2011).

Another drawback to the development of pejerrey culture is the lack of information about diseases affecting this species, and particularly, on immune response against these diseases. As in other forms of livestock rearing, intensive production in aquaculture is directly related to an increase in pathology incidences. Overcrowding and fish handling through management practices, such as grading, reproduction, and vaccination, induce stress in fish impairing their immunological responses which often lead to outbreaks of infectious diseases (Bly et al. 1997; Yada and Nakanishi 2002; Davis 2006; Oliva-Teles 2012). In this sense, several authors have shown the usefulness of haematology to estimate health status in fish (Clifton-Hadley et al. 1987; Steinhagen et al. 1990; Garcia-Abiado et al. 2004; Bermúdez et al. 2005; Remyla et al. 2008). Therefore, the knowledge on normal values of blood parameters as well the cellular composition of haemolymphopoietic organs is essential to interpret the changes observed in the morphology of fish immune system during its ontogeny.

Due to phylogenetic diversity of fish, high interspecific variability in haematological parameters has been reported (Schütt et al. 1997; Modrá et al. 1998; Burrows et al. 2001; Jerônimo et al. 2011), hindering the extrapolation to species not yet studied. Studies on blood parameters in fish have been made on species of greatest commercial interest worldwide, such as rainbow trout (*Oncorhynchus mykiss*) (Clifton-Hadley et al. 1987), turbot (*Psetta maxima*) (Burrows et al. 2001), common carp (*Cyprinus carpio*) (Svetina et al. 2002), Atlantic salmon (*Salmo salar*) (Sandnes et al. 1988), and tuna (*Thunnus maccoyii*) (Rough et al. 2005) among others. In the last decades, aquaculture in Latin America and the Caribbean showed the world′s highest average annual growth and studies on haematology of native species are increasing (Parma de Croux 1994; Tavares-Dias and Sandrim 1998; Tavares-Dias et al. 1999, 2002; Moraes et al. 2002; Ranzani-Paiva et al. 2003; Tavares-Dias and Mataqueiro 2004; Alesso et al. 2005; Cazenave et al. 2005; Ranzani-Paiva et al. 2005; Oliveira-Ribeiro et al. 2006). To date, there are few studies related to blood tissue in pejerrey. These studies were focused on morphological and enzymecytochemical features of blood cells (Vigliano et al. 2005), but others' significant parameters such as haematocrit, haemoglobin concentration, leucocyte count, and leucocyte formula were not determined. Thus, the aim of this study was to establish haematological parameters in *O. bonariensis*, in order to obtain reference values that can be used in further applied studies on diseases affecting pejerrey culture. In addition, we determined in this species if the haematological variables as well as cellular composition of haemolymphopoietic organs were affected by sex and seasonality.

Materials and methods

Fish, sampling and staining of slides

Adult specimens of pejerrey obtained from natural environment (La María Lagoon, S 34°03′26.0″W 061°37′36.7″) in the southern of Santa Fe (Argentina) were used. Fish were caught with a gill net which was revised every 15 min to remove the specimens in order to minimise stress effects. Four samples were performed, one in each season of the year carried out a month after summer and winter solstice, and a month after spring and autumn equinox. Fifteen to twenty-five specimens in each season were sampled (total fish sampled: 52 females and 27 males; mean weight: 251.4 ± 9.51 g; mean standard length: 269.3 ± 3.20 mm; mean condition factor: 1.28 ± 0.04; no significant differences between sexes for these variables were observed). Fish were euthanised by cervical dislocation since the use of anaesthetic could modify haematological parameters. Afterward, weight, standard length, and sex of fish were registered.

Peripheral blood was collected by puncture of caudal vein with EDTA-coated 23-gauge needles attached to a 1-ml syringe, put in 1.5-ml plastic tubes, and then immediately cooled. Blood smears were performed on precleaned slides in duplicate. After blood sampling was finished, a complete necropsy of fish was carried out in order to discard internal pathologies. For the evaluation of cell composition of spleen and head kidney, both organ were removed and sliced transversally. The cut organ surface was blotted and imprinted gently onto precleaned slides in duplicate. Both, blood smears and organ imprints were air dried and properly stored until staining was performed in the laboratory.

Slides were stained with a panoptic stain (Tinción 15, Biopur Diagnostics, Argentina), air dried, and coverslipped.

The experimental procedure was approved by the Ethics Committee of the School of Veterinary Sciences of the National University of Rosario.

Water quality

During each sampling, several aquatic parameters were measured (Table 1) according to specifications of the Standard Methods for the Examination of Water and Wastewater (APHA 1995).

Haematological parameters

Haematocrit (Ht) was determined using microhaematocrit tubes filled with blood and spinning at 8,700 g for 5 min in a microhaematocrit centrifuge, and expressed as the percentage of the total blood volume.

Haemoglobin (Hb) was determined by cyanomethaemoglobin method using Drabkin reactive and measuring absorption at 540 nm (Drabkin and Austin 1935).

To determine erythrocyte (EC) and leucocyte count (LC), an indirect method was performed. First, the total blood cell count (TBCC) was calculated on diluted blood samples (1:20 dilution in Turk's fluid) using a Neubauer haemocytometer. Blood cells located in the four primary squares of the camera were counted, and TBCC mm^{-3} was calculated using the following formula: TBCC $mm^{-3} = N \times 50$, where N is the number of blood cells observed in the four primary squares of the haemocytometer. After that, differential blood cell counts were done on blood smears. For each smear, 400 blood cells were classified as erythrocytes or leucocytes (including thrombocytes) and expressed as percentage of the total number of blood cell analysed. Finally, the results of differential blood cell count were used to calculate EC and LC mm^{-3} from the TBCC mm^{-3} as determined in the haemocytometer.

Differential leucocyte count was also performed on blood smears, in which 200 leucocytes per slide were counted and classified as lymphocytes, thrombocytes, granulocytes, and monocytes, as described by Vigliano et al. (2005), and expressed as a percentage of the total number of leucocytes observed. Immature stages of circulating leucocytes such as lymphoblasts or progranulocytes were also recorded.

Table 1 Methods employed for testing physicochemical parameters in water and values measured in each season

	Spring	Summer	Autumn	Winter	Methods[a]
Temperature (°C)	19	24	18	6	Digital thermometer
Dissolved oxygen (mg l^{-1})	6.5	5.9	7.3	8.1	Membrane electrode method (4500-O G)
pH	8.6	8.2	7.9	8.0	Electrometric method (4500-H^+ B)
Conductivity (μS cm^{-1})	5,820	4,060	3,080	2,190	Conductimetry (2510 B)
Alkalinity (mg l^{-1})	437	373	335	326	Titration (2320 B)
Hardness (mg l^{-1})	233	201	167	126	EDTA titrimetric method (2340 C)
Nitrite (mg l^{-1})	0.015	0.020	0.020	0.010	colorimetric method (4500-NO_{2-} B)
Nitrate (mg l^{-1})	4.6	3.0	5.2	3.1	UV Spectrophotometric screening method (4500-NO_{3-} B)
Chloride (mg l^{-1})	1,756	414	565	158	Argentometric method (4500-Cl^- B)
Sulphate (mg l^{-1})	813	133	84	112	Turbidimetric method (4500-SO_4^{2-} E)

[a] According to APHA (1995)

Tissue imprints

On each stained head kidney and spleen imprint, 200 blood cell precursors per slide were counted and classified into the erythrocytic, lymphoid, granulocytic, or monocytic lineage in accordance with Vigliano et al. (2009), and expressed as a percentage of the total number of cells recorded.

Statistical analyses

Significant differences ($P < 0.05$) for each variable measured between sexes and among different seasons were assessed by a *t* test and one-way ANOVA with a Tukey post hoc test, respectively. The 95 % confidence interval of the mean for each variable was also calculated. All analyses were done using JMP software, Version 5.1.1 (SAS Institute Inc., Cary, NC).

Results

Water quality

Table 1 summarises the water physicochemical parameters assessed in this study, which were considered among normal values.

Seasonal modifications

All haematological variables considered in this study showed significant differences between seasons with the exception of the percentage of blood monocyte (BMc) (Table 2).

Haematocrit exhibited significantly higher values in summer, whereas haemoglobin concentration (Hb) was greater not only in summer, but also in autumn. Erythrocyte count (EC) in spring was higher than autumn and winter, but did not differ with summer. On the contrary, concerning leucocyte count (LC), significant lower values in spring and winter than in summer and autumn were observed (Table 2).

Regarding differential leucocyte count in blood smears, lymphoblasts (BLb), lymphocytes (BLc), progranulocytes (BPg), granulocytes (BGc), thrombocytes (BTc), and monocytes (BMc) were observed. BLc and BTc were the most abundant cell types throughout the year with the exception of winter. BLc exhibited its lowest mean value in winter while BLb mean value was highest at this season. BTc showed significantly higher values in summer and underwent a statistically significant decline in winter. Finally, in BGc, a significantly higher mean value was observed in winter, whereas BPg exhibited greater mean values in summer and autumn (Table 2).

Table 2 Haematological variables analysed in each season

	Spring ($n = 22$)	Summer ($n = 17$)	Autumn ($n = 15$)	Winter ($n = 25$)	*F*
Ht (%)	42.7 ± 1.42^{a} (39.9–45.6)	48.6 ± 1.62^{b} (45.4–51.9)	41.8 ± 1.72 (38.4–45.3)^{a}	42.0 ± 1.33^{a} (39.4–44.7)	0.0093
Hb (g dl^{-1})	13.0 ± 0.47^{a} (12.1–14.0)	15.9 ± 0.54^{b} (14.8–17.0)	15.8 ± 0.58^{b} (14.7–17.0)	13.3 ± 0.46^{a} (12.4–14.2)	< 0.0001
EC ($\times 10^{6}$ mm^{-3})	1.67 ± 0.07^{a} (1.53–1.81)	$1.44 \pm 0.07^{a,b}$ (1.29–1.59)	1.09 ± 0.08^{c} (0.93–1.24)	$1.29 \pm 0.06^{b,c}$ (1.18–1.42)	< 0.0001
LC ($\times 10^{3}$ mm^{-3})	102.4 ± 17.86^{a} (66.8–138.07)	231.7 ± 18.38^{b} (195.1–268.4)	209.1 ± 19.57^{b} (170.1–248.2)	69.4 ± 15.15^{a} (39.1–99.6)	< 0.0001
BLb (%)	5.9 ± 0.96^{a} (4.0–7.8)	12.4 ± 1.02^{b} (10.4–14.5)	16.2 ± 1.08^{b} (14.0–18.4)	48.0 ± 0.84^{c} (46.3–49.7)	< 0.0001
BLc (%)	46.5 ± 2.15^{a} (42.2–50.8)	21.7 ± 2.28^{b} (17.2–26.2)	25.7 ± 2.42^{b} (20.9–30.5)	10.0 ± 1.88^{c} (6.2–13.7)	< 0.0001
BTc (%)	44.4 ± 2.38^{a} (39.7–49.1)	60.4 ± 2.51^{b} (55.4–65.4)	$52.6 \pm 2.68^{a,b}$ (47.3–58.0)	2.3 ± 2.08^{c} (−1.8–6.5)	< 0.0001
BPg (%)	1.5 ± 0.25^{a} (1.0–2.0)	4.4 ± 0.27^{b} (3.9–4.9)	4.3 ± 0.28^{b} (3.7–4.9)	1.2 ± 0.22^{a} (0.7–1.6)	< 0.0001
BGc (%)	1.7 ± 0.48^{a} (0.8–2.7)	1.1 ± 0.51^{a} (0.7–2.1)	1.2 ± 0.54^{a} (0.9–2.3)	15.9 ± 0.42^{b} (15.1–16.8)	< 0.0001
BMc (%)	1.1 ± 0.14^{a} (0.8–1.4)	0.9 ± 0.16^{a} (0.6–1.2)	1.1 ± 0.17^{a} (0.8–1.5)	1.0 ± 0.13^{a} (0.8–1.3)	0.7371

Mean ± SD (95 % confidence interval of the mean)

Different letters indicate significant differences among stations (ANOVA, Tukey post hoc test)

Ht haematocrit, *Hb* Haemoglobin, *EC* erythrocytes count, *LC* leucocytes count, *BLb* blood lymphoblasts, *BLc* blood lymphocytes, *BTc* blood thrombocytes, *BPg* blood progranulocytes, *BGc* blood granulocytes, *BMc* blood monocytes

Differential cells counts in spleen or head kidney imprints showed the presence of erythroblasts (Eb), proerythrocytes (Pe), lymphoblasts (Lb), granuloblasts (Gb), progranulocytes (Pg), and monoblasts (Mb) in both organs, although with different ratios (Tables 3, 4). Each cell type was named as SEb, SPe, SLb, SGb, SPg, SMb or KEb, KPe, KLb, KGb, KPg, KMb depending on its location, i.e., spleen (S) or head kidney (K).

Erythrocytes precursor cells exhibited a distinctive pattern in each organ since SEb showed higher mean values in winter, whereas KEb presented significant higher mean values from spring to autumn. In addition, a significant increase in SPe during winter and spring was observed while no variations in KPe through the year were seen (Tables 3, 4).

In lymphoid series, SLb displayed significant differences among seasons (Table 3), showing a sustained increase from spring to autumn exhibiting a subsequent decline in winter (Fig. 1a). A similar trend was observed in BLb, although its higher value occurred in winter agreeing with the abrupt decline in SLb (Fig. 1a). On the other hand, KLb showed significant differences along the year, with intermediate values in autumn and winter with respect to summer and spring (Table 4). Furthermore, despite of the particular pattern of cell kinetics for SLb, KLb, or BLb throughout the year, all variables showed their lowest values in spring coinciding with the higher mean value of BLc (Fig. 1a).

In relation to granulocytic lineage, SGb and KGb showed relatively stable values along the year, with a significant increase in spring and autumn, respectively (Tables 3, 4). Regarding the seasonal variations of granulocytic precursors in head kidney, the maximum value registered for KGb occurred in autumn and after that this variable decreased in winter while mean values of KPg significantly increased in winter and

Table 3 Variables analysed in spleen imprints in each season

	Spring ($n = 22$)	Summer ($n = 17$)	Autumn ($n = 15$)	Winter ($n = 25$)	F
SEb (%)	5.0 ± 0.70^{a} (3.9–6.8)	$6.8 \pm 0.70^{a,b}$ (5.3–8.1)	4.6 ± 0.80^{a} (3.1–6.2)	8.5 ± 0.60^{b} (7.2–9.8)	0.0008
SPe (%)	20.1 ± 1.20^{a} (17.7–22.4)	7 ± 1.20^{b} (4.7–9.4)	7.6 ± 1.30^{b} (5.1–10.2)	15.5 ± 1.00^{c} (13.4–17.6)	<0.0001
SLb (%)	23.0 ± 1.30^{a} (20.4–25.8)	39.0 ± 1.30^{b} (36.6–41.6)	46.0 ± 1.40^{c} (43.2–48.9)	31.0 ± 1.20^{d} (28.5–33.2)	<0.0001
SGb (%)	16.3 ± 1.00^{a} (14.4–18.2)	8.6 ± 1.00^{b} (6.9–10.8)	7.2 ± 1.00^{b} (5.2–9.3)	10.4 ± 0.80^{b} (8.7–12.1)	<0.0001
SPg (%)	$34.3 \pm 1.30^{a,b}$ (31.6–36.9)	38 ± 1.30^{a} (35.3–40.6)	33 ± 1.40^{b} (30.1–35.7)	33 ± 1.20^{b} (30.8–35.5)	0.0325
SMb (%)	$1.3 \pm 0.20^{a,b}$ (0.8–1.8)	0.6 ± 0.20^{a} (0.1–1.2)	$1.5 \pm 0.30^{a,b}$ (0.9–2.0)	2.1 ± 0.20^{b} (1.7–2.5)	0.0003

Mean ± SD (95 % confidence interval of the mean)

Different letters indicate significant differences among stations (ANOVA, Tukey post hoc test)

SEb splenic erythroblasts, *SPe* splenic proerythrocytes, *SLb* splenic lymphoblasts, *SGb* splenic granuloblasts, *SPg* splenic progranulocytes, *SMb* splenic monoblasts

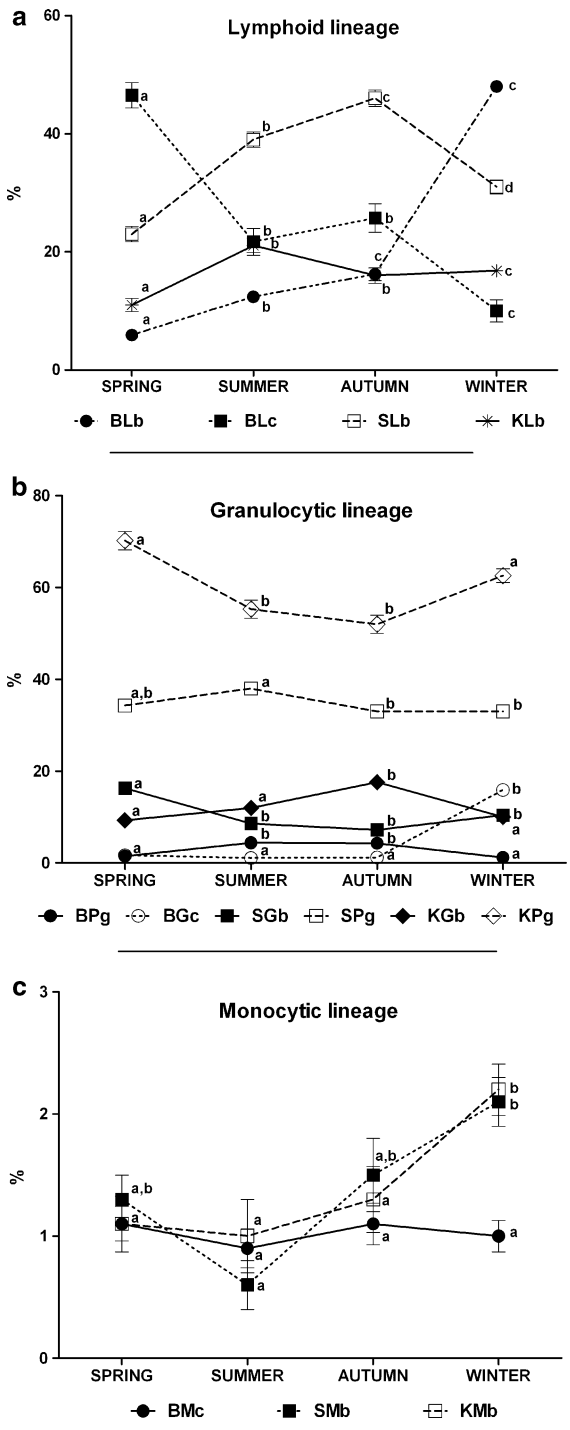
Table 4 Variables analysed in head kidney imprints in each season

	Spring	Summer	Autumn	Winter	F
KEb (%)	10.0 ± 0.84^{a} (7.91–11.3)	$8.1 \pm 1.01^{a,b}$ (6.3–10.0)	10.3 ± 0.96^{a} (8.4–12.3)	5.2 ± 0.76^{b} (3.6–6.7)	0.0001
KPe (%)	5.2 ± 1.00^{a} (3.9–6.5)	5.1 ± 0.71^{a}(3.7–7.0)	5.2 ± 0.80^{a} (3.6–6.7)	6.0 ± 0.61^{a} (4.3–6.7)	0.9733
KLb (%)	11.0 ± 1.10^{a} (9.0–13.0)	21.1 ± 1.20^{b} (19.0–23.5)	16.0 ± 1.30^{c} (13.4–18.4)	16.8 ± 1.01^{c} (15.0–19.0)	<0.0001
KGb (%)	7.3 ± 1.00^{a} (6.0–9.0)	10.0 ± 1.04^{a} (8.0–11.4)	15.6 ± 1.00^{b} (13.6–17.6)	8.0 ± 0.80^{a} (6.2–9.3)	<0.0001
KPg (%)	70.2 ± 2.00^{a} (64.1–70.4)	55.3 ± 2.00^{b} (52.0–61.0)	52.0 ± 2.00^{b} (48.0–54.0)	62.6 ± 1.50^{a} (60.0–70.0)	<0.0001
KMb (%)	1.1 ± 0.23^{a} (1.0–1.4)	1.0 ± 0.30^{a} (0.3–1.2)	1.3 ± 0.27^{a} (1.1–2.0)	2.2 ± 0.21^{b} (2.0–2.6)	<0.0001

Mean ± SD (95 % confidence interval of the mean)

Different letters indicate significant differences among stations (ANOVA, Tukey post hoc test)

KEb kidney erythroblasts, *KPe*, kidney proerythrocytes, *KLb* kidney lymphoblasts, *KGb* kidney granuloblasts, *KPg* kidney progranulocytes, *KMb* kidney monoblasts



◀ **Fig. 1** Variations in circulating leucocytes and their cell precursors throughout the year. **a** Lymphoid lineage. *BLb* blood lymphoblasts, *BLc* blood lymphocytes, *SLb* splenic lymphoblasts, *KLb* kidney lymphoblasts. **b** Granulocytic lineage. *BPg* blood progranulocytes, *BGc* blood granulocytes, *SGb* splenic granuloblasts, *SPg* splenic progranulocytes, *KGb* kidney granuloblasts, *KPg* kidney progranulocytes. **c** Monocytic lineage. *BMc* blood monocytes, *SMb* splenic monoblasts, *KMb* kidney monoblasts. *Different letters* indicate significant differences among stations (ANOVA, Tukey post hoc test)

spring (Fig. 1b). On the other hand, the higher mean value described for BGc in winter was associated only with an increase KPg, since no significant variations in splenic granulocytes precursor cells were observed in this season (Fig. 1b).

With regard to monocytic lineage, a significant increase in mean values for SMb and KMb in winter was seen, which statistically differed with summer and the rest of seasons, respectively (Tables 3, 4). However, the increase in monopoiesis at both organs observed in winter was not related to levels of BMc, which were stable through the year (Fig. 1c).

Sex variations

Of all haematological parameters analysed, only few variables of the leucocyte formula showed significant differences between males and females. Males exhibited higher mean values of BLb and BGc than females, whereas females presented higher mean values in BTc than males (Table 5). There were no significant differences between sexes for any of the variables assessed in imprints of head kidney and spleen.

Discussion

Even though pejerrey (*O. bonariensis*) is the main native species cultured in lagoons of Pampa Plain (Gómez 1998), there are actually few published studies on their physiological parameters. This is the first study that determines haematological parameters, as well as their gender and seasonal variations, establishing reference values that can be used for the control of fish health status. In addition, the quantification of cell composition in spleen and head kidney of adult pejerrey will allow to analyse variations throughout the ontogenetic development of these organs.

The values of all physicochemical parameters measured in water were considered normal values since they fall within the confidence intervals of 95 % reported as acceptable for *O. bonariensis* farming (Gómez et al. 2007). Nevertheless, variations in water temperature modify the concentration of dissolved

Table 5 Haematological variables with significant differences between sexes

	Males ($n = 27$)	Females ($n = 52$)	P
BLb (%)	30.1 ± 3.47 (23.2–37.0)	19.7 ± 2.50 (14.7–24.7)	0.0175
BGc (%)	8.8 ± 1.37 (6.1–11.6)	4.7 ± 0.99 (2.8–6.7)	0.0172
BTc (%)	23.6 ± 4.88 (13.9–33.3)	42.1 ± 3.52 (35.1–49.1)	0.0030

Mean ± SD (95 % confidence interval of the mean)

BLb blood lymphoblasts, *BGc* blood granulocytes, *BTc* blood thrombocytes (*t* test)

oxygen, reducing their levels when temperature rises (Noga 2010). Therefore, the significant increase in Ht, Hb, and EC in seasons with higher water temperatures observed in this study, i.e., spring, summer, and autumn, might be a compensatory mechanism to balance the lower oxygen availability in the environment. These results may also be associated with an elevation in metabolic activity as the temperature rises and a consequent increase in oxygen tissular demand. The seasonal influence of temperature and dissolved oxygen on Ht, Hb, and EC values has also been reported in other teleosts such as *Platichthys flesus* (Soldatov 1996), *Tinca tinca* (Guijarro et al. 2003), and *Oreochromis niloticus* (Jerônimo et al. 2011). In *T. Tinca*, a significant increase in Ht and EC in summer and in Hb during spring was observed (Guijarro et al. 2003). Although, in pejerrey, the mean EC values were higher in spring and summer, significant differences were only found among spring and autumn/winter and between summer and autumn. Moreover, Ht was significantly higher in summer while Hb was higher in summer and autumn. These interspecific variations may indicate different adaptive strategies through the year in order to compensate the lesser availability of dissolved oxygen. Thus, pejerrey may achieve this compensation by an increase in EC in spring, by an increase in Hb production in autumn and, by the combination of both mechanisms in summer, the station with the highest water temperature registered. In addition, Guijarro et al. (2003) reported significant differences in *T. tinca* between males and females for Ht, Hb, and EC in summer, a fact that differs with that described for *O. bonariensis* since no variations in these parameters were observed in this study. Regardless of seasonal variations, the mean values of Ht and Hb registered in *O. bonariensis* were high in comparison with others species such as *Corydoras paleatus* (Cazenave et al. 2005), *Prochilodus lineatus* (Parma de Croux 1994), *O. niloticus* (Jerônimo et al. 2011), and *T. tinca* (Guijarro et al. 2003). On the contrary, pejerrey exhibited similar values in Ht, Hb, and EC to those reported in *T. maccoyii* (Rough et al. 2005) or *O. mykiss* (Morgan et al. 2008), which may be associated with the high metabolic rate of these species due to their active swimming habits.

LC also presented differences throughout the year in *O. bonariensis* since mean values were lower in winter and spring than in summer and autumn. These seasonal variations were also described in *T. tinca* (Guijarro et al. 2003) and *O. mykiss* (Morgan et al. 2008). However, absolute values of LC in pejerrey during summer were about five- and tenfold higher than *O. mykiss* (Morgan et al. 2008) and *T. tinca* (Guijarro et al. 2003), respectively.

Leucocyte formula shows interspecific variation and, although lymphocytes and thrombocytes are usually the main cell types, there are species predominantly lymphocytic such as *Xiphophorus helleri* (Schütt et al. 1997), *Salminus brasiliensis* (Ranzani-Paiva et al. 2003), and *T. maccoyii* (Rough et al. 2005) and others like *Pleuronectes platessa* (Ellis 1976), *P. maxima* (Burrows et al. 2001), *Cichlasoma dimerus* (Rey-Vázquez and Guerrero 2007), and *O. niloticus* (Jerônimo et al. 2011) in which thrombocytes are the most important cells in peripheral blood. In *O. bonariensis*, both BLc and BTc were also the most abundant cell types although with a seasonal prevalence of BTc in summer and autumn.

Interestingly, in winter, the most common leucocyte in blood smears of pejerrey was BLb. At this season, a marked decrease in BLc and BTc as well as almost tenfold increase in BGc were also seen in this study. In line with these results, several studies have shown that temperature also influences the immune system activity, but in a different way in specific and non-specific response pathways (Nakanishi 1986; Collazos et al. 1995; Alcorn et al. 2002). Alcorn et al. (2002) have reported that the immune system of *Oncorhynchus*

nerka was mainly based on non-specific mechanisms at low temperatures, but at higher temperatures, the activity of specific immune response prevailed. As it was stated above, this is consistent with the results obtained in pejerrey since in the coldest season an increase in the number of cells belonging to innate immune system such as granulocytes, and a decrease in those cells involved in specific immunity responses, i.e., lymphocytes and thrombocytes, were observed. On the other hand, decrease in BLc in winter may also be associated with stressful situations such as spawning (Alcorn et al. 2002), since in this season occur several physiological processes for the development of spring spawning of the majority of natural populations of pejerrey (Calvo and Morriconi 1972). Is noteworthy that, in most cases, the increase in a particular type of leucocyte in *O. bonariensis* was preceded by an increase in values of its precursor in blood in the previous season. Thus, the highest values of BLc registered in spring and BGc observed in winter were associated with a significant rise of BLb and BPg in winter and autumn, respectively.

Although several studies indicate seasonal variations in haematological parameters, up to date no published data exist in relation to seasonal fluctuations in blood cell precursors in haemolymphopoietic organs. The presence of the same precursors of blood cells in spleen and head kidney in pejerrey show the capability of both organs in haemopoiesis, a fact that was previously described by Vigliano et al. (2009) in this species. However, their abilities to produce each cell lineage vary throughout the year. Erythrocytes production seems to prevail in head kidney from spring to autumn, while in winter the spleen takes this role. The relationship observed between SLb and BLb along the year appears to indicate that from spring to autumn, there is an increase in lymphopoiesis and storage of lymphoblasts in spleen with a posterior migration of these cells into the bloodstream. KLb mean values suggested that this mechanism was strengthened by head kidney in summer. On the contrary, decrease in SLb and BLc observed in pejerrey in winter, which coincided with an increase in BLb, indicates that in this season there is a high transference of lymphoblasts from spleen to blood, together with a reduction in the process of differentiation of lymphocytic lineage. This impairment of lymphopoiesis might be related to a deleterious effect of low temperature during winter although up to date there are no studies that confirm this hypothesis. Moreover, differentiation from lymphoblasts to lymphocytes reach its top during spring as can be inferred by the low levels of lymphoblasts observed in all compartments (head kidney, spleen, and blood) and the highest value of BLc. In relation to granulocytic precursors, head kidney seems to be the most important organ involved in seasonal variations since although cell precursors were observed in the spleen in an important quantity, little variations through the year were registered. After the peak of KGb in autumn, this value decreased in winter together with a significant increase in KPg that was kept until spring. These results would be associated with an increase in the process of differentiation of granulocytic lineage in these periods. The increase in number of monoblasts observed in spleen and head kidney in winter may be attributed with low temperatures, since as was mentioned above, non-specific defence mechanisms prevail in these environmental conditions (Alcorn et al. 2002). However, these increases in KMb and SMb values were not related with levels of BMc, which remained stable throughout the year. This may be due to the quick migration of monocytes into tissues, after its differentiation, where they develop the immune response.

Sex variations in haematological parameters in fish are less common (Modrá et al. 1998; Gabriel et al. 2004; Rey-Vázquez and Guerrero 2007; Sandblom et al. 2009), although some differences in a few species were reported. In *T. tinca*, males show higher values of EC, Ht, and Hb than females, mainly in summer (Guijarro et al. 2003). On the contrary, no variations in these variables between males and females of *O. bonariensis* were observed. In this study, significant differences between sexes were only seen in some circulating leucocytes. Males exhibited higher mean values of BLb and BGc than females, whereas females presented higher mean values in BTc. In contrast, males of *T. tinca* exhibit higher values of BTc than females (Guijarro et al. 2003). In conclusion, this study determines variations in haematological variables and cellular composition of spleen and head kidney through the year, as well as sex differences in some parameters of leucocyte formula. On the other hand, it also confirms the high interspecific variations of fish haematological parameters. This fact supports the needing of performing basic studies in order to assess fish health status in new promising species for aquaculture.

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