

# Triploidy in the hematology of jundia juveniles (Siluriformes: Heptapteridae)

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## Abstract

This study compared the hematological characteristics of diploid and triploid of jundia, *Rhamdia quelen* juveniles, an important freshwater fish cultured in south Brazil. Hematological morphometry of erythrocytes were determined in blood smears under a light microscope. The blood was used to measure the number of red blood cells (RBC) with a hemocytometer Neubauer chamber, and the numbers of white blood cells (WBC) and thrombocytes that were obtained using an indirect method. The results showed that triploidy increased ( $p < 0.01$ ) the size and volume of the erythrocytes. Nevertheless, as expected, triploidy decreased ( $p < 0.01$ ) the number of circulating erythrocytes, leucocytes and thrombocytes in the blood of jundia. Moreover differential cell counts were different in diploids and triploids, suggesting that triploidy affects the number of cells and their proportion in blood. Lymphocytes were the most predominant cells in the differential counting of diploid fish (62.5%) while monocytes were predominant in triploid fish (49.6%). These results suggest performance differences between ploidies of jundia, and require future studies to evaluate the potential of triploid jundia in the culture conditions and resistance to infection.

*Keywords:* *Rhamdia quelen*, triploid, diploid, blood cells.

## Triploidia na hematologia de juvenis de jundiá (Siluriformes: Heptapteridae)

### Resumo

O presente estudo comparou características hematológicas de juvenis de jundiás *Rhamdia quelen* diploides e triploides, um importante peixe de água doce cultivado no sul do Brasil. A morfometria hematológica dos eritrócitos foi determinada em extensões sanguíneas sob microscopia óptica comum. O sangue dos animais foi também utilizado para mensurar o número total de eritrócitos em câmara de Neubauer, bem como os números totais de leucócitos e trombócitos pelo método indireto. Os resultados demonstraram que a triploidia aumentou ( $p < 0,01$ ) o tamanho e volume dos eritrócitos. No entanto, como esperado, a triploidia reduziu ( $p < 0,01$ ) o número de eritrócitos, leucócitos e trombócitos circulantes do jundiá. Além disto, a contagem diferencial de células sanguíneas foi diferente em diploides e triploides, sugerindo que a triploidia afeta o número das células, bem como sua proporção no sangue. Linfócitos foram as células predominantes na contagem de leucócitos de peixes diploides (62,5%), enquanto os monócitos foram os predominantes nos peixes triploides (49,6%). Estes resultados sugerem desempenhos diferentes entre as ploidias do jundiá e exige estudos futuros para avaliar o potencial dos jundiás triploides sob condições de cultivo e frente à infecção.

*Palavras-chave:* *Rhamdia quelen*, triploide, diploide, células sanguíneas.

## 1. Introduction

The jundia *Rhamdia quelen* (Siluriformes: Heptapteridae) is a native species reared in south Brazil due to zootechnic and organoleptic characteristics favourable to its development and tolerance to living in low temperatures that provide continual growth in winter (Fracalossi et al., 2004). However, the precocious sexual maturation has been identified as a problem that results in decreased fish growth. It occurs by the fact that the energy used for somatic growth is deviated to gonadal tissue. This condition induces the heterogeneous growth of the fish increasing the rearing time and the production costs.

The technique of the induction of triploidy has been widely diffused as an effective solution to the impediment produced for precocious sexual maturation of fish in the modern aquaculture industry. One of the benefits of triploidy concerns the warranted fish sterility that avoids energetic waste with sexual maturation, resulting in a continuous growth in triploid fish. Moreover, several studies show that triploid animals can attain better survival rates, growth, feed conversion and greater resistance against diseases when compared to diploid (Kerby et al., 2002). In this way, triploid has been used in aquaculture as a way to improve the income of the traditional rearing of sundry species of fish that present economic importance, including Siluriformes (Beaumont and Hoare, 2003). One of the most important aims of fish triploidy is to reduce the escape of reared fish to natural fish populations (Lutz, 2001).

Triploidy in fish has been accompanied by alterations in their physiology in reducing the fish resistance against acute and chronic stress (Cotter et al., 2000) that can also influence the productive performance during the rearing. This fact is normally characterised by high stocking densities and/or a decrease in dissolved oxygen concentration. Several experiments have been carried out to determine the performance and production of triploid fish (Arai, 2001; Felip et al., 2001). Huergo and Zaniboni-Filho (2006) induced triploidy in jundia in an effective way, enabling the use of triploidy as an alternative to solve problems caused by precocious maturation of jundia.

Hematological parameters have been used to evaluate fish health status, environmental changes and stress of inadequate handling (Ghiraldelli et al., 2006). Comparative studies on the hematology of diploid and triploid fish were observed in Atlantic salmon, *Salmo salar* (Cogswell et al., 2002), sturgeon, *Acipenser sturio* (Flajshans and Vajcová, 2000), channel catfish, *Ictalurus punctatus* (Wolters et al., 1982), perch, *Umbrina cirrosa* (Ballarin et al., 2004) and loach, *Misgurnus aguillicaudatus* (Gao et al., 2007).

In the present assay, hematological and morphological parameters of the circulating blood cells of diploid and triploid juveniles of jundia were compared to provide knowledge to stimulate the rearing of triploids jundia.

## 2. Material and Methods

### 2.1. Biological material and water quality

The present study was conducted at the Biology and Rearing Freshwater Fish Laboratory and at the Study Nucleus in Aquatic Pathology, Aquaculture Department of the Federal University of Santa Catarina, Florianópolis, SC.

Larvae were obtained through hormonal induction from male and female jundia captured from the wild population of the Uruguay River Basin and maintained at the Biology and Rearing Freshwater Fish Laboratory. Eight females and two males of jundia received application of carp pituitary extract and the gametes were obtained by extrusion in the course of 234 degrees/hour after the end of the hormonal treatment. Using a pool of gametes, 4 minutes after gametes activation, a part of the eggs were submitted to a pressure shock, by the action of a hydraulic spoil above a steel chamber with volume of 800 mL with intensity (5000 psi) for 5 minutes (Huergo and Zaniboni-Filho, 2006). Simultaneously to the triploid production, another part of the gametes was used for diploid production, following a similar procedure, except for the pressure shock application.

Diploid and triploid eggs were kept separated in a cylindrical conical incubator with a volume of 56 L supplied by a recirculation water system with movement of water and temperature at 26 °C. The outbreak occurred 36 hours after fertilisation and larvae were maintained in the incubators until the mouth opening and the beginning of the exogenous feed. Subsequently, the animals were stocked and reared in circular tanks of intensive larvae culture (1000 L), maintained at a salinity of 2.5 ppm, 26 °C, fed daily with artemia naupli in the first month. After this period they were fed with dry ration (56% crude protein) twice a day *ad libitum*.

During the experimental period, the water quality was as follows: dissolved oxygen ( $5.9 \pm 0.8$  mg/L), temperature ( $25.0 \pm 0.4$  °C), pH ( $7.3 \pm 0.3$ ) and salinity ( $2.5 \pm 0.4$  ppm). The triploidy in fish was ascertained according to the method of Phillips et al. (1986) slightly modified by Huergo and Zaniboni-Filho (2006). Briefly, the number of nucleolous in the cell nuclei stained with silver nitrate ( $\text{AgNO}_3$ ), was determined. All triploid fish analysed were 3n and a 100% success rate of the triploid technique used was confirmed.

### 2.2. Hematological analyses

For blood collection, 30 juveniles of jundia of each ploidy were selected and stocked in tanks of 50 L capacity. The averages and standard deviation of length and weight for diploids were  $8.7 \pm 0.8$  cm and  $20.3 \pm 1.2$  g, and for triploids  $8.5 \pm 1.3$  cm and  $19.2 \pm 1.4$  g.

Fish were anaesthetised in a benzocaine solution ( $50 \text{ mg.L}^{-1}$ ) and a 1 mL blood sample was taken from the caudal vein using a syringe containing a drop of 10% EDTA solution. The blood was used to measure hematocrit percentage (Goldenfarb et al., 1971), the number of red blood cells (RBC) was determined with a hemocytometer, and

the numbers of white blood cells (WBC) and thrombocytes were obtained using an indirect method (Ishikawa et al., 2008). Leucocytes differential count was performed using a combination of Giemsa/May-Grunwald (Rosenfeld, 1947) staining in which a hundred cells were counted to determine the cell percentage.

For erythrocyte measurement, the major and minor axes of the cell and its nucleus were obtained from 50 cells by using 15 fish of each ploidy. After that, the volume of erythrocytes and its nucleus were calculated considering the Equation 1 proposed by Cal et al. (2005).

$$V = 4/3 \times a \times b^2 \quad (1)$$

where a and b are the major and the minor semi axis.

### 2.3. Statistical analyses

The data were submitted to ANOVA and the Tukey test ( $\alpha = 0.05$ ) to verify the difference between averages among diploid and triploid fish.

## 3. Results

Morphological analysis indicated that triploid RBC had significantly ( $p < 0.01$ ) higher length (major axis), width (minor axis) and volume when compared to diploid fish (Table 1). The RBC of triploid jundia showed an average of 16.5% longer and 30.6% larger than the diploid fish cells (Table 1). Additionally, RBC was the most frequent

cells in the blood smears of diploid fish ( $0.83 \times 10^6$  cells/mL) significantly higher ( $p < 0.05$ ) than that observed in triploid ( $0.63 \times 10^6$  cells/mL) (Table 2).

Total number of thrombocytes and leukocytes were lower in triploids ( $p < 0.01$ ) than in diploids (Table 2). Significant differences were also observed between diploid and triploid for frequencies of differential cell counts, suggesting that triploidy affected the number of cells as well as their proportion in the circulating blood (Table 2). Lymphocytes were the most dominant cells in diploid fish (62.5%), while in triploid, it was the monocytes (49.6%). Moreover, triploid jundia showed an increased number ( $p < 0.05$ ) of eosinophils (35.2%) when compared to diploid (2.9%). On the other hand, the number of basophils, eosinophils and granular leukocyte PAS positive were below 1% in all fish (Table 2).

## 4. Discussion

All variables in triploid jundia showed alterations and a similar hematological profile was observed in triploid catfish (Wolters et al., 1982). The measurement of erythrocytes is well accepted as the most important and fast characteristic to determine the triploidy in teleosts (Benfey, 1999; Ballarin et al., 2004). On this view the results improved the efficacy of the triploidy in jundia.

The number of RBC was lower in triploid jundia than in diploid, similar to other studies that reported an

**Table 1.** Average values ( $\pm$  standard deviation) in micrometres of red blood cell dimensions of diploid and triploid, *Rhamdia quelen*.

Variables	Diploid (n = 30)	Triploid (n = 30)	Significance level
Cell major axis ( $\mu\text{m}$ )	11.45 $\pm$ 0.92	13.33 $\pm$ 1.03	$p < 0.01$
Cell minor axis ( $\mu\text{m}$ )	8.37 $\pm$ 0.77	10.93 $\pm$ 0.99	$p < 0.01$
Cell volume (fL)	421.56 $\pm$ 78.89	838.89 $\pm$ 169.7	$p < 0.01$
Nucleus major axis ( $\mu\text{m}$ )	4.41 $\pm$ 0.54	5.13 $\pm$ 0.48	$p < 0.01$
Nucleus minor axis ( $\mu\text{m}$ )	2.90 $\pm$ 0.29	3.40 $\pm$ 0.36	$p < 0.01$
Nuclear volume (fL)	19.85 $\pm$ 4.86	31.49 $\pm$ 7.59	$p < 0.01$

**Table 2.** Average values ( $\pm$  standard deviation) of total counting of white blood cells (WBC), thrombocytes, and differential counting of leukocytes in diploid and triploid jundia, *Rhamdia quelen*.

Variables	Diploid (n = 30)	Triploid (n = 30)	Significance level
Total erythrocytes ( $10^6/\mu\text{L}$ )	0.83 $\pm$ 0.45	0.63 $\pm$ 0.23	$p < 0.05$
Total thrombocytes (cell/ $\mu\text{L}$ )	20,872.00 $\pm$ 5,486.20	5,752.00 $\pm$ 3,334.4	$p < 0.01$
Total leukocytes (cell/ $\mu\text{L}$ )	22,686.00 $\pm$ 8,310.6	14,318.00 $\pm$ 5,313.3	$p < 0.01$
Differential counting of WBC			
Lymphocytes (cell/ $\mu\text{L}$ )	1483.29 $\pm$ 4587.11	2157.72 $\pm$ 1877.09	$p < 0.01$
Monocytes (cell/ $\mu\text{L}$ )	8151.08 $\pm$ 4289.92	7104.59 $\pm$ 2495.63	$p < 0.05$
Neutrophils (cell/ $\mu\text{L}$ )	664.70 $\pm$ 1211.43	5041.37 $\pm$ 3132.78	$p < 0.01$
Basophils (cell/ $\mu\text{L}$ )	38.57 $\pm$ 149.73	0.00	$p > 0.05$
Eosinophils (cell/ $\mu\text{L}$ )	63.52 $\pm$ 133.85	0.00	$p < 0.05$
GL PAS (cell/ $\mu\text{L}$ ) <sup>1</sup>	38.57 $\pm$ 106.62	0.00	$p > 0.05$

<sup>1</sup>Granular leukocyte PAS positive.

increase in the size of erythrocytes in triploid teleost and consequently a decrease in its number (Rehulka et al., 2004). In agreement with Benfey (1999), the reduced number of erythrocytes in triploid organisms is a result of a homeostatic mechanism of triploids to compensate the increase of the cell volume, caused by accommodation of extra genetic material.

Several studies have drawn attention to the theoretical impediment caused by the alteration in the relation superficial area/cell volume in the basal metabolism of the cells in the triploid organisms, especially on the physiological processes mediated by the plasmatic membrane of these cells. As the most numerous cells in fish blood, the function of RBC is linked to an oxygen and carbonic transportation made from its component, hemoglobin. Hence, number as much as volume of RBC may indicate the capacity of oxygen transportation in the blood. In comparison to  $CL_{50-96h}$  of dissolved oxygen between diploid and triploid jundia, Weiss and Zaniboni-Filho (2009) have reported the sensibility of triploid 6% higher than the concentration for diploid. In agreement with Benfey (1999), these characteristics can result in a variation of the growth and behaviour of triploid animals when compared to diploids in the same conditions of rearing.

Total and differential counting of WBC and trombocytes are important indexes to determine the non-specific defense, as they are essentials in inflammatory reactions. A decrease in thrombocyte number and WBC in triploid jundia observed in this work corroborated the findings of Budiño et al. (2006) and Benfey and Biron (2000). The induction of triploidy in fish has usually been reported to be accompanied by modification in physiology and reduced resistance to acute stress (Cotter et al., 2000). Further studies must be carried out to evaluate the effects of acute and chronic stress in jundia triploid.

The innate immune system is well developed in fish and is fundamental in the protection against pathogens (Budiño et al., 2006). In rainbow trout, Ranzani-Paiva et al. (1999) observed lymphocytes as the most dominant leucocytes on the circulating blood of both diploids and triploids. Lymphocytes participate in the inflammatory process, acting as immunocompetent cells. In the present study a significant decrease of these cells in the blood of triploid jundia was verified. On the other hand, triploid jundia showed a significant increase in the circulating neutrophils and monocytes, likely to keep its defense system more prepared for adverse rearing conditions. These cells play an important role in the fish immune system against the microorganisms being sensitive to migration to the inflammatory site (Vale et al., 2002; Tavares Dias and Moraes, 2004).

In the present study, the frequency of basophils, eosinophils and granular leukocyte PAS positive were lower than 1% in all fish. Similar results were observed in the circulating blood of teleosts (Tavares Dias and Moraes, 2004). Eosinophils and basophils are frequently absent in fish blood or occur in a low number. The function of the eosinophils in fish is not well known. Some studies

demonstrate positive correlation between the increase in circulating eosinophils in disease situation (Tavares Dias et al. 2000).

In conclusion, this study demonstrated that triploidy is associated with changes in the hematology, such as the cell size and number and their proportion in blood. The erythrocytes of triploid fish were of a larger size and lower number compared to diploid fish, probably due to the higher amount of genetic material in triploid cells. Moreover, triploidy decreased the number of circulating leucocytes and thrombocytes. These differences may affect cell activity which could disturb the physiology of jundia, and the responses of fish to rearing conditions and eventual infection. More studies are necessary considering the performance of triploid jundia submitted to experimental challenges to understand its physiology and adaptative capacity under adverse situations.

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