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Short Communication

Some blood parameters of Pacific fat sleepers, *Dormitator latifrons* (Richardson, 1844): a comparative study between male and female in two growth stages

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ABSTRACT. *Dormitator latifrons* is an amphidromous fish widely distributed in the Pacific slope from California to Peru. Although this species has a high potential for aquaculture, there is little information about the blood parameters of sex and growth stage. Eighty specimens of *D. latifrons* (40 males and 40 females in two growth stages) were used. They were placed in 1700 L tanks, one for each group, and fed with a feed for Tilapia diet with 35% protein and 8% fat. At the end of 30 days, blood samples were collected. Some blood parameters were determined (hematocrit, glucose, total protein, albumin, globulin, and A/G), and hematocrit percentage, glucose, albumins, and globulins presented significant differences between stages ($P < 0.05$). Total proteins showed differences only between males and females ($P < 0.05$). The hematocrit registered significant differences between stage and sex ($P < 0.05$). Differences between stages and sex may be related to a higher growth rate, differences in feed conversion, an increase in energy expenditure, and variations in hormonal levels. This study is the first to determine some blood parameters of *D. latifrons* under experimental aquaculture conditions concerning sex and growth stage.

Keywords: *Dormitator latifrons*; hematology; blood chemistry; physiology; native fish

Dormitator latifrons (Richardson, 1844) is an amphidromous fish widely distributed in the Pacific slope from California to Peru. It spawns in freshwater bodies; the larvae migrate to brackish water and later return to freshwater (Milton 2009, Vicuña 2010). Morphologically, this species is characterized by its blue-green to green-red color on the dorsal area, with bluish lateral bands and a pale gray belly. The head is slate gray and bluish on the ventral area. The dorsal fin is gray with black spots and a red stripe; the anal fin is green at the base with dark spots on the edge (Kähsbauer 1973). It is an omnivorous fish, but it feeds mainly on detritus (Yáñez-Arancibia & Díaz-González 1977).

The cultivation of *D. latifrons* is an alternative source of income for people in Latin America, where it is cultured extensively using artisanal methods that have given good results in fattening and growth, mainly in the cultivation of males. It has good commercial potential as a white meat fish with high protein content (Castro-Rivera et al. 2005, Delgado-Morán 2010,

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Vicuña 2010). Recent studies assessed the growth performance of this fish species by evaluating the effect of different concentrations of protein and lipids (Badillo-Zapata et al. 2018) and different stocking densities (Basto-Rosales et al. 2019) on its growth, survival, and nutritional quality (López-Huerta et al. 2018). Proper cultivation of this species requires constant monitoring of the health of the cultured organisms, which can be done by keeping track of some key blood parameters (Fazio 2019, Del Rio-Zaragoza et al. 2021).

Measuring blood parameters is an essential tool for diagnosing diseases caused by environmental changes, nutritional imbalances, and the presence of pathogens (Stoskopf 1993, Hrubrec & Smith 2010). Fish blood parameters have been widely studied to assess their relationship with age, size, time of year, physical stress, feeding condition, presence of contaminants, the effect of transportation, nutritional aspects, culture density, and presence of diseases, among others (McDonald & Milligan 1992, Stoskopf 1993, Roche & Bogué 1996, Hrubrec et al. 2000, Kumar et al. 2005, Del Rio-Zaragoza et al. 2008, 2011, Saravanan et al. 2011, Cengizler et al. 2017, Vivanco-Aranda et al. 2018, Hernández-González et al. 2019).

Although the characteristics of *D. latifrons* make it an excellent candidate for aquaculture in Mexico and much of Latin American countries, little is known about various aspects of its physiology. Only two works have studied this species hematology and blood chemistry (Vega-Villasante et al. 2021). The first, by Todd (1972), determined wild organisms hematocrit, hemoglobin concentration, and mean corpuscular hemoglobin concentration. The second work, by Ruiz-González et al. (2020), analyzed the hematocrit, total cell counts, respiratory burst, glucose, total proteins, albumin, and globulin levels in serum. The present work aimed to expand our knowledge about some blood parameters of *D. latifrons* about sex and two stages of growth. We also estimated the albumin/globulin ratio and the mean corpuscular volume in juvenile fish of *D. latifrons* under laboratory conditions.

Eighty specimens (40 males and 40 females in two growth stages) from the stock of the Laboratory of Water Quality and Experimental Aquaculture of the University Center of the Coast, University of Guadalajara, Jalisco, Mexico, were used. The specimens were classified into four groups: male (34.9 ± 8.7 g and 13.5 ± 0.9 cm) and female (28.6 ± 4.7 g and 13.1 ± 1.1 cm) juveniles; male (109.9 ± 28.4 g and 20.3 ± 1.9 cm) and female (101.7 ± 29.7 g and 19.1 ± 1.2 cm) adults. The fish were placed in circular plastic tanks (2.13 m in diameter of 1700 L capacity) equipped with a bio-filter. The fish were exposed to a photoperiod of 12:12 h light:darkness, temperature ranging from 26.5 to 28°C, oxygen concentration greater than 5 mg L^{-1} and pH of 7.5 ± 0.5.

The fish were fed to satiety for 30 days with a Purina® feed for Tilapia in the development stage that contained 35% protein and 8% lipids. The feed consisted of floating extruded pellets with a particle size of 3.5 mm. The feeding was carried out under the conditions described above. At the end of 30 days, blood samples were collected from apparently healthy fish without parasites. No signs of sexual maturation (changes in the color of the genital papilla and abdomen) were observed in the juvenile fish (Bonifaz et al. 1985).

The specimens were anesthetized with clove oil previously dissolved in ethanol (1:10 v/v) to collect blood samples (Javahery et al. 2012). Blood was collected from the caudal vein using a 3 mL syringe without anticoagulant. A minimum sample of 500 µL was obtained per fish. Half of the sample (250 µL) was placed in a Microtainer® tube (pediatric) with EDTA-K2 anticoagulant (Becton Dickinson®); the other half was placed in 1.5 mL plastic vials (Eppendorf®) without anticoagulant.

In the blood samples with EDTA-K2, the hematocrit (Hct) percentage was determined by the microhematocrit technique, in which glass capillaries filled with approximately 60 µL of blood and sealed at one end were centrifuged at 4000 g for 10 min in a Scilogex DM1424 centrifuge. The Hct was determined with the help of the hematocrit reader of the centrifuge.

The samples in plastic vials (Eppendorf®) without anticoagulant were centrifuged for 10 min at 2000 g in an Ika 3958000 mini G centrifuge. Serum was recovered and placed in a 1.5 mL plastic vial (Eppendorf®). The serum was used for blood chemistry analysis using kits (Mexlab®) to determine albumin (BCG, 620 nm), glucose (God-Pad, 505 nm), and total protein (Biuret, 540 nm). Absorbance values were determined with a Velab VE-5000V spectrophotometer. The serum globulin concentration was calculated from the difference between total protein concentration and albumin. The albumin/globulin (A/G) ratio was also determined.

Shapiro-Wilk normality and Bartlett homoscedasticity tests were performed for each measured parameter. When the aforementioned statistical assumptions were met, a two-way analysis of variance (two-way ANOVA) differences was carried out

between the independent variables of sex and growth stage for each of the blood parameters under study. In all cases $\alpha = 0.05$ was used. The data expressed as percentages were transformed using the arcsine of the square root before analyses, but the results are reported as untransformed means. A Tukey's test was applied *a posteriori* in cases with significant differences.

The results of the Hct and blood chemistry analysis of female and male juvenile and adult specimens of *D. latifrons* are shown (Table 1). Hct, glucose, albumins, and globulins, presented significant differences between stages ($P < 0.05$). Total proteins showed differences only between males and females ($P < 0.05$). The Hct registered significant differences between stage and sex ($P < 0.05$). Particularly, the lowest Hct value was recorded for juvenile females (35%), with significant differences ($P < 0.05$). Juvenile (males and females) fish showed lower glucose values than adults ($P < 0.05$).

Regarding albumin concentration, juvenile males had a significantly lower concentration than juvenile females and adult males. Adult males showed the lower value of globulins and the highest value of the A/G ratio, which was significantly different compared to both groups of juveniles and adult females. No significant differences were found in total proteins between stages, and these results do not represent a significative interaction between stage and sex.

Previously, Hct values for wild fishes of *D. latifrons* were documented (39.1%), that fishes weighing 150-350 g, longer than those used in this study (Todd 1972). Reference intervals under laboratory conditions were recently established for some of the blood parameters of *D. latifrons* (Ruiz-González et al. 2020). For Hct, the authors calculated a range of 11.103-45.064%. In the present study, the juvenile females had an Hct value within the same range. In contrast, the three remaining groups showed Hct values above that range and above the results of Todd (1972), who found Hct values of 150-350 g in adult wild organisms. It is worth remembering that the present study was conducted under laboratory conditions using a balanced diet (35% protein and 8% lipids) that had no negative effects on the survival and growth of *D. latifrons* (Badillo-Zapata et al. 2018). Therefore, the differences between the results of the present study and those of Todd (1972) could be due to different factors such as abiotic and nutritional conditions.

Regarding blood chemistry parameters, Ruiz-González et al. (2020) calculated reference ranges for glucose (34.630-68.304 mg dL^{-1}), total proteins (2.256-5.616 g dL^{-1}), albumin (0.339-3.474 g dL^{-1}), globulins (1.859-2.922 g dL^{-1}), and A/G (0.304-1.068) in *D. latifrons*. The total proteins, albumins and A/G ratio values in males and females of *D. latifrons* in both growth stages were within the reference ranges proposed by those authors. Adult males showed globulin levels below the reference range. In contrast, the glucose concentration in the adult groups was significantly higher than in the groups of juveniles ($P < 0.05$) and higher than the reference ranges. The results for juveniles coincide with what has been reported by Ruiz-González et al. (2020) for juvenile organisms of *D. latifrons*.

The blood chemistry values of *D. latifrons* were similar to those that have been reported for hybrid tilapia (*Oreochromis niloticus O. mossambicus O. aureus*) at a high stocking density (total protein 3.9 g dL^{-1}, albumin 1.8 g dL^{-1}, globulins 2.1 g dL^{-1}) (Hrubrec et al. 2000). However, the glucose concentration shown by adult organisms of *D. latifrons* was almost double that reported for hybrid tilapia, which ranged between 30 and 96 mg dL^{-1} at two stocking densities, high (n = 63, 120 g L^{-1}) and low (n = 15, 4 g L^{-1}), where they reported significant differences in some of the blood parameters between both stocking densities (Hrubrec et al. 2000). In a study was evaluated the growth and survival of *D. latifrons* at four stocking densities (3, 5, 6 and 7 ind m^3). They found no differences in growth between densities (Basto-Rosales et al. 2019). Badillo-Zapata et al. (2022) recently studied the stocking density effect on growth and some blood parameters of *D. latifrons*. They documented that the blood parameters were not affected by the densities tested, perhaps ruling out the possibility that the differences observed in current research are due to density. However, the organisms used in both previous studies were not separated by sex. Further studies of the growth, survival, and other blood parameters of *D. latifrons* should be carried out using higher stocking densities.

It is important to note that although the conditions of density, light, temperature, oxygen, and pH used in the present study are similar to those used in work by Ruiz-González et al. (2020); that work focused only on juvenile organisms and did not consider the sex variable. However, it has been shown that variables such as sex, size, age, nutritional and reproductive condition can be associated with different blood parameter levels such as the number of erythrocytes, hematocrit, total proteins, albumins, globulins, and glucose, among others (McDonald & Milligan 1992, Del Rio-Zaragoza et al. 2008, Percin & Konyalioglu

Table 1. Hematocrit (Hct) and blood chemistry values of male and female *Dormitator latifrons* in two growth stages: juveniles and adults. Superscripts with different letters indicate significant differences. Mean ± standard deviation. A/G: albumin/globulin.

	Juveniles		Adults	
	Females	Males	Females	Males
Hct (%)	35.0 ± 8.0^a	47 ± 7.0^b	45.0 ± 7.0^b	50.0 ± 11.0^b
Glucose (mg dL^{-1})	55.2 ± 13.3^a	67.8 ± 18.0^a	102.3 ± 24.2^b	103.6 ± 18.4^b
Total protein (g dL^{-1})	4.0 ± 0.9	3.5 ± 0.5	3.8 ± 0.6	3.2 ± 0.5
Albumin (g dL^{-1})	1.7 ± 0.3^a	1.3 ± 0.2^b	1.6 ± 0.2^a	1.8 ± 0.3^a
Globulins (g dL^{-1})	2.3 ± 1.0^a	2.2 ± 0.5^a	2.2 ± 0.5^a	1.5 ± 0.5^b
A/G ratio	0.9 ± 0.6^a	0.6 ± 0.2^a	0.8 ± 0.2^a	1.2 ± 0.5^b

2008, Mehdi et al. 2011, Qadir-Charoo et al. 2013, Hernández-González et al. 2019). Under the conditions studied, sex as an independent variable did not affect the parameters studied. However, the lowest globulin concentration was recorded in adult males. The variation in the concentration of globulins is related to the activity of the innate immune system and other factors, such as the stage of gonad maturity (Andreeva 2010, Peres et al. 2014). The adult fish in this study were healthy but were not in the reproductive stage. These results differ from those reported by Qadir-Charoo et al. (2013), who recorded significantly higher concentrations of globulin in males and lower concentrations in females. The authors also found a higher concentration of glucose in males. In the present study, no significant differences in glucose were found between females and males, but some differences were found between juveniles and adults.

The differences in the blood glucose between stages in the present study may be related to a higher growth rate and differences in feed conversion, increase in energy expenditure due to greater activity in the juvenile organisms and variation in hormonal levels (Mehedi et al. 2011, Polakof et al. 2012, Sheikh & Ahmed 2016). They were also reported in other species, such as *Acipenser persicus*, which showed a higher concentration of glucose, total proteins, albumins, phosphorus, and magnesium in males than in females and immature organisms (lower growth stage) (Mehdi et al. 2011). The authors mention that the high values of parameters such as total proteins and glucose found in males of *A. persicus*, compared to females and immature organisms, may be due to a higher growth rate and better feed conversion efficiency. Another study on snow trout (*Schizothorax plagiostomus*) attributed the differences in hematological parameters to the higher metabolic rate and hormonal activity of males compared to females (Sheikh & Ahmed 2016).

The results show that the hematological and blood chemistry parameters (Hct, glucose, albumins, globulins) of *D. latifrons* evaluated in the present study vary more concerning the growth stage than sex. Determining how blood parameters vary according to abiotic, nutritional, stocking density, and stress response variables is still necessary. In the case of adults, it would be interesting to evaluate the variations in the reproductive stage. However, this study contributes to the basic physiology knowledge of *D. latifrons* under experimental culture conditions.

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