Short Communication

Some hematology and blood chemistry parameters of the Pacific fat sleeper *Dormitator latifrons* (Richardson, 1844)

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ABSTRACT. *Dormitator latifrons* is an amphidromous fish species distributed on the Pacific coastal region from California to Peru. It has a high potential to be cultured in Mexico. However, there is very little information about its biology, physiology and culture. This research study is a contribution to the hematology and blood chemistry of this native species. Results show hematocrit values of 28%, erythrocytes 2.075×10^6 mm^3, leukocytes 35.035×10^3 mm^3, mean corpuscular volume 161.547 fL, NBT 0.39, glucose 51.467 mg dL^{-1}, protein 3.936 g dL^{-1}, albumin 1.906 g dL^{-1}, globulin 2.391 g dL^{-1} and albumin/globulin ratio 0.686.

Keywords: *Dormitator latifrons*; amphidromous fish; hematology; blood chemistry

The Pacific fat sleeper *Dormitator latifrons* (Richardson, 1844) is distributed on the Pacific slope from California to Peru (Vicuña, 2010). *D. latifrons* is an amphidromous fish, so it spawns in freshwater and larvae migrate to brackish water and then return to freshwater bodies (Milton, 2009), besides, is an omnivorous fish; however, it feeds mainly on detritus particles (Yáñez-Arancibia & Díaz-González, 1977). The species is characterized by its pigmentation from blue-green to green-red in the dorsal area. Bluish sidebands. Slate-colored skull, bluish in the ventral area. Pale gray belly. Gray dorsal fin with black spots and red stripe. Green anal fin at its base with dark spots on edge (Kähsbauer, 1973).

The production of *D. latifrons* represents an alternative source of income, being able to grow in extensive systems with artisanal methodologies, obtaining good growing results, mainly in the monosex male culture (Castro-Rivera *et al.*, 2005). Also, because it is a white meat fish with high protein content, it can be considered as a key product for a developing market (Vicuña, 2010).

In order to properly perform this culture, it is necessary to monitor the health of the organisms constantly, and this could be done through blood tests. Hematology and blood chemistry are an essential tool for the diagnosis of diseases produced from environmental changes, nutritional imbalance, or even the presence of pathogens (Stoskopf, 1993; Hrubec & Smith, 2010).

Although this fish has characteristics that make it an excellent candidate for aquaculture in Mexico and most Latin America, there is little information on different aspects of its physiology. In the case of hematology and blood chemistry, the information is limited, with the exception of the research by Todd (1972), where the hematocrit ($39.1 \pm 6.1\%$), red blood cell count ($3.2 \pm 0.5 \times 10^6$ mm^3), mean corpuscular volume (122.8 ± 6.1 fL), the hemoglobin concentration ($15.5 \pm 2.7\%$), the mean corpuscular hemoglobin (48 ± 61.5 µg) and the

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corpuscular media hemoglobin concentration (40.5 ± 1.8%) were documented. For this reason, the main objective of this research is to contribute with some hematological and blood chemistry parameters of this fish.

D. latifrons (30 fish, 13 ± 1 cm of total size, 33.8 ± 3.7 g weight) were collected from El Quelele Estuary, Banderas Bay, Nayarit, Mexico (20°43'54.2"N, 105°18'07.5"W), with the help of a 3 cm diameter light mesh net. Organisms were kept in quarantine for 30 days. Subsequently, fish were placed in 400 L tanks, five fish per tank, connected to a recirculation system with a bio-filter and a UV light system. The organisms were acclimated for seven days. During the acclimation and the bioassay, fish were exposed to a photoperiod of 12 h light:12 h darkness, at temperatures of 26.5 to 28°C, oxygen with a concentration ≥5 mg L^{-1} and a pH from 7 to 8.

Purina$^®$ brand commercial feed with 35% protein and 8% fat (for tilapia, 3.5 mm particle size) was used. Food was administered in three daily rations at 9, 13 and 17 h, for four weeks, under the conditions described above. The first biometry was performed to adjust the amount of food concerning 4% of the biomass. At the end of week four, the blood samples of fish were collected as described below. All fish used were healthy and without parasites. Besides, fish did not show any sign of sexual maturation, such as changes in the genital papilla or abdomen coloration (Bonifaz *et al.*, 1985).

Organisms were anesthetized with clove oil, previously dissolved in ethanol in a 1:10 ratio (Javahery *et al.*, 2012). Blood samples were taken from the caudal vein, with a 3 mL syringe, without anticoagulant. A minimum sample of 1,000 µL per fish was obtained. Half of the sample (500 µL) was placed in a tube with EDTA-K2 anticoagulant, and the other half in Eppendorf$^®$ vials without anticoagulant, for subsequent analyses.

Blood samples with EDTA-K2 were used for hematocrit (hct), red blood cell counts (RBC), white blood cell counts (WBC) and respiratory burst analysis. Hct was realized from blood samples with EDTA-K2, glass capillaries were filled with about 60 µL and sealed at one end, centrifuged at 4,000 g for 10 min in Scilogex DM1424 centrifuge. Hct percentage was determined by a circular hematocrit reader.

For the respiratory burst analysis, the Ibrahem *et al.* (2010) method was used, which consisted in placing 100 µL of blood with EDTA-K2 in plastic vials (Eppendorf$^®$), added 100 µL of Nitro-blue Tetrazolium (NBT) solution at 0.2% and incubated for 30 min at room temperature. Subsequently, 50 µL of the mixture was taken and placed in 1 mL of N, N-dimethyl formamide, and then centrifuged at 2,000 *g* for 5 min in Ika mini G 3958000 centrifuge. The supernatant was recovered and analyzed in a Velab VE - 5000V spectrophotometer, in 1 mL glass cells, at 620 nm.

For total cell counts, a sample of 20 µL of blood with EDTA-K2 was placed in 4 mL of Natt-Herrick solution. A Neubauer chamber with 1/400 mm^2 and 1/10 mm deep, was filled with 5 µL of the dilution. The analysis was carried out by observation in a Quasar Qm20 Binocular 2500x Professional Microscope. The RBC was performed in the central grid, from which five squares of 0.0025 mm^2 each were selected, and the WBC in four large squares, from each corner, with an area of 1 mm^2 each. With the RBC and hct, the mean corpuscular volume (MCV) was calculated by the formula: MCV = (hct × 10) / number of erythrocytes (millions mm^3).

The sample with no anticoagulant was centrifuged for 10 min at 2,000 *g* for 5 min in Ika mini G 3958000. The resulting serum was recovered in a plastic vial (Eppendorf$^®$) and used to blood chemical assays. Kits (Mexlab$^®$) were used for albumin (BCG, 620 nm), glucose (God-Pad, 505 nm), and total protein (Biuret, 540 nm), and the absorbance was recorded by spectrophotometry. Globulin was calculated by difference concerning albumin and total protein, and albumin/globulin rate (A/G) was determined.

Shapiro Wilk normality tests were performed for each of the measured parameters. Besides, the reference intervals were calculated by twice the standard deviation (Stoskopf, 1993).

At the end of 30 days under the conditions described above, the organisms reached a size of 14.5 ± 0.6 cm, and a mean weight of 43.1 ± 6.9 g. Table 1 shows the results of the blood chemistry and hematology studies in *D. latifrons*. The reference intervals are established. The results of hematocrit, respiratory burst (NBT), glucose, total proteins, albumins and globulins showed a normal distribution ($P > 0.05$). In contrast, the RBC and WBC did not meet the normality assumptions.

Studies of blood parameters in fish is a widely documented subject with different objectives, taking into account the season of year, physical stress (temperature and salinity), contaminants, transport, nutritional aspects, crop densities and presence of diseases, among others; since they allow us to know the physiological state of the organisms (Stoskopf, 1993; Roche & Bogué, 1996; Hrubec *et al.*, 2000; Kumar *et al.*, 2005; Saravanan *et al.*, 2011; Cengizler *et al.*, 2017).

The hct, RBC and WBC are the hematology parameters most analyzed in blood fish studies. The hct of *D. latifrons* (28.08%) is higher than that of *Sorubim*

Table 1. Hematological parameters, blood chemistry and reference intervals of *Dormitator latifrons*. SD: standard deviation, RBC: red blood cell, WBC: white blood cell, MCV: mean corpuscular volume, NBT: Nitro-blue tetrazolium.

Parameter	Mean ± SD	Reference interval
Hematocrit (%)	28.083 ± 8.490	11.103 - 45.064
RBC×10^6 (cells mm^3)	2.075 ± 0.449	1.177 - 2.973
WBCs×10^3 (cells mm^3)	35.035 ± 7.012	21.011 - 49.059
MCV (fL)	161.547 ± 34.996	91.555 - 231.539
NBT	0.39 ± 0.080	0.230 - 0.550
Glucose (mg dL^{-1})	51.467 ± 8.418	34.630 - 68.304
Total protein (g dL^{-1})	3.936 ± 0.840	2.256 - 5.616
Albumin (g dL^{-1})	1.906 ± 0.784	0.339 - 3.474
Globulins (g dL^{-1})	2.391 ± 0.266	1.859 - 2.922
A/G ratio	0.686 ± 0.191	0.304 - 1.068

cuspicaudus (22.70%) (Correa-Negrete *et al*., 2009). Both hct are lower than those of *O. niloticus* (32.44%) at a mean temperature of 27°C (Hahn-von-Hessberg *et al*., 2014), similar to that of the present study as described by Larsson *et al*. (1976), the low percentages of Hct, corresponding to benthic, sedentary or slow-moving species.

In contrast, *S. cuspicaudus* has a higher value of RBC and WBC than *D. latifrons* (Correa-Negrete *et al*., 2009). Nevertheless, *S. cuspicaudus* showed the lowest MCV (11.5 ± 2.2 fL) than *D. latifrons* (161.54 fL). That is because *S. cuspicaudus* presented small erythrocytes. Additionally, *D. latifrons* showed an erythrocyte number higher than those reported for *O. niloticus* (1.70 ± 0.4×10^6 mm^3) and similar to that of *Rhamdia quelen* 2.11 ± 0.6×10^6 mm^3) (Dal' Bó *et al*., 2015). The MCV of *O. niloticus* and *R. quelen* is similar to that found in this study for *D. latifrons*.

In the case of NBT (Nitroblue Tetrazolium), this is used to evaluate the respiratory burst, in this case, by reducing the NBT by surrounding leukocytes. *D. latifrons*, under the established conditions, showed an absorbance of 0.39, higher than *O. niloticus* with balanced feed (0.173), and even higher than those that received diets with inulin (0.233) and vitamin C (0.277) (Ibrahem *et al*., 2010). The preceding indicates that *D. latifrons* has higher activity of respiratory burst, which could provide greater protection against infectious diseases.

The blood chemistry comparison of hybrid tilapia individuals (*Oreochromis niloticus* × *O. mossambicus* × *O. aureus*) in two culture densities, high (n = 63) and low (n = 15) was performed. Where for the first, a concentration of 3.9 g dL^{-1} of total proteins, 1.8 g dL^{-1} of albumins, 2.1 g dL^{-1} of globulins and 46 mg dL^{-1} of glucose values were found. The hybrid tilapia culti-vated at low density showed lower values of total

proteins, albumins and globulins (2.9, 1.2 and 1.6 g dL^{-1}, respectively); however, a glucose concentration of 52 mg dL^{-1} was found (Hrubec *et al*., 2000). The results of the high-density culture are similar to those found in this research study for *D. latifrons,* although this fish was found in much lower densities, which suggests that differences could be observed about culture densities of *D. latifrons.*

Likewise, species *Carassius auratus* and *Pterois volitans* show blood chemistry values similar to those found for *D. latifrons* in this study; for the first one, glucose values of 34.5 mg dL^{-1}, total proteins 2.84 g dL^{-1}, albumin 2.13 g dL^{-1} and globulins 0.7 g dL^{-1} (Adamovicz *et al*., 2015); for the second one, glucose 26.5 mg dL^{-1}, total proteins 4 g dL^{-1}, albumins 1 g dL^{-1} and globulins 2.9 g dL^{-1} (Anderson *et al*., 2010). However, in both cases, *D. latifrons* shows a higher glucose concentration. It should be noted that orga-nisms studied by Anderson *et al*. (2010) were obtained from a population maintained for reproductive physiology studies. On the other hand, Adamovicz *et al*. (2015) used sexually immature organisms, like the ones in this study.

D. latifrons showed similar characteristics to those of the *Metriaclima greshakei* species, except for the level of albumins (albumin of 0.95 g dL^{-1}) and, therefore, globulins (2.9 g dL^{-1}), where observed that *D. latifrons*, under the conditions established in this study, shows almost double the concentration of albumins (1.906 g dL^{-1}). However, the authors mention that despite reporting a low level of albumins, which may be associated with liver disease, it did not appear in their study. Also, it should be noted that the authors did not anesthetize their organisms and made the sampling only covering the fish's eyes (Snellgrove & Alexander, 2011).

Todd (1972) describes an hct, RCB and MCV higher than the results of this research for *D. latifrons*. However, the fishes used in Todd (1972) were weighing 150-350 g, and the fishes used in this research has less weight (43.1 g). In other species, significant differences have been found in some hematological parameters between different cultivation systems and individuals of the natural environment, as is the case of *Christoma estor estor*, which showed significant differences in the hematocrit and hemoglobin concentration among organisms coming from of the natural environment and three farming systems (Alaye-Rahy & Morales-Palacios, 2013). Likewise, it has been demonstrated that seasonal changes affect the hematology and blood chemistry of fish, such as *O. niloticus* (Cengizler *et al.*, 2017). Also, the sexual differentiation that was found in the blood chemistry of *Oncorhynchus mykiss* (Qadir-Charoo *et al.*, 2013). The above suggests the possibility of observing these same differences in *D. latifrons*.

Results of this research work show that in general, the hematological and blood chemistry parameters found for *D. latifrons* are similar to those of other freshwater species. Also, *D. latifrons* showed higher activity in the respiratory burst, which suggests greater resistance to infections. This first approach to the hematology and blood chemistry of *D. latifrons* is a contribution of importance to the knowledge of the species, so this research lays the basis for further studies.

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