HEMATOLOGIA DEL CATAN (*Astractosteus spatula*), COLECTADOS EN EL NORESTE DE MEXICO.

HEMATOLOGY OF CATAN (Astractosteus spatula), COLLECTED IN NORTHEASTERN MEXICO.

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Resumen

Las poblaciones de catán (*Atractosteus spatula*), se han visto disminuidas drásticamente durante las últimas dos décadas en el noreste de México, debido principalmente a la captura, así como la perdida de hábitats por la actividad antropogénica y la contaminación. Por lo anterior es importante conocer su Biología y en particular su Hematología, ya que es un tejido que refleja en forma rápida cambios tanto intrínsecos como extrínsecos provocados por el medio ambiente. Los parámetros hematológicos estudiados fueron Hemoglobina (Hb, $\overline{X}$=12,45gr/dl), Microhematocrito (Ht, $\overline{X}$=44.33%) Leucocrito (Lc, $\overline{X}$= 2.76 %), Proteína Total del Plasma (PTP, $\overline{X}$= 5.48 gr/dl), Recuento por Dilución de Glóbulos Rojos (RDR, $\overline{X}$=1 004 000), Recuento por Dilución de Glóbulos Blancos (RDB, $\overline{X}$= 64 471). Las células cuantificadas para el recuento diferencial fueron: Trombocitos ($\overline{X}$=46.5), Neutrofilos ($\overline{X}$=4.5), Eosinofilos (

$\overline{X}$=3.14), Basofilos ($\overline{X}$=3), Linfocitos ($\overline{X}$=26.6), Monocitos ($\overline{X}$=5.42), Células Plasmáticas ($\overline{X}$=6.56), Promielocitos ($\overline{X}$=1.67), Mielocitos (=0.31 ±$\overline{X}$0.66), Células No Identificadas ($\overline{X}$=1.25 ±1.33). Los valores hematológicos reportados concuerdan con los reportados para otros peces, no así la diversidad de los leucocitos.

Palabras clave: Catán, *Atractosteus spatula,* Hematología, Leucocitos.

Abstract

Gar populations (Atractosteus spatula), have been decreased dramatically over the past two decades in northeastern Mexico, mainly due to the capture and loss of habitats by anthropogenic activity and pollution. Therefore it is important to know their biology and hematology in particular because it is a fabric which quickly reflects both intrinsic and extrinsic changes caused by the environment. The hematological parameters studied were hemoglobin (Hb = 12.45 g / dl), Microhematocrit (Ht, = 44.33%) Leucocrito (Lc, = 2.76%), Total Plasma Protein (PTP, = 5.48 g / dl), count by Dilution of Red Blood Cells (RDR = 1.004 million), Count White Blood Cell Dilution (RDB, = 64 471). Quantified cells for the differential count were: Platelets (= 46.5), neutrophils (= 4.5), eosinophils (= 3.14), basophils (= 3), Lymphocytes (= 26.6), Monocytes (= 5.42), plasma cells (= 6.56), Promyelocytes (= 1.67), Myelocytes (= 0.31 ± 0.66), Unidentified cells (= 1.25 ± 1.33). Haematological values reported are consistent with those reported for other fish, but not the diversity of leukocytes.

Key words: Catan, Atractosteus spatula, Hematology, Leukocytes.

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Introduction

Catans or lizard fish are extremely ancient organisms, with primitive structures, which can be considered true living fossils. The first species appeared in the Cretaceous Period, 180 million years ago, and flourished in almost the entire world (except Asia), later decreasing to seven species divided into two genera, which still prevail in North and Central America. The “Catán” (Atractosteus spatula) has an original distribution that goes from the Ohio and Missouri rivers in the United States to the Tamiahua Lagoon in Veracruz, Mexico (Contreras-Balderas and Ruiz-Campos, 2010). It is found in pools of large rivers and in brackish or marine waters along the coast of the Gulf of Mexico.

The populations of alligator gar have been drastically reduced during the last two decades in northeastern Mexico, mainly due to capture, as well as the loss of habitats due to anthropogenic activity and pollution, for which their current conservation status is considered vulnerable (Jelks et al., 2008). Therefore, it is important to increase knowledge about its Biology, in particular its Hematology, since it is a tissue that rapidly reflects both intrinsic and extrinsic changes caused by the environment.

Background.

Wedemeyer (1977), cited a series of analytical methods, along with guidelines for sample collection and interpretation of results, indicating hematological, water, liver, and muscle tests, resulting in a minimum of 10 fish due to the large coefficient of variation that presents sampling for biological monitoring of farmed fish as well as native populations to assess the effect of environmental stress on healthy fish.

Cameron (1970) found that pesticides and other contaminants cause alterations in the tissues and blood of fish. For his part, Christensen (1972), reported that small amounts of heavy metals such as copper, chromium, mercury ions, in the aquatic environment, cause multiple changes in the internal dynamism of organisms, even lethal.

Wydosky (1976) indicated that metabolism and ionic regulation through blood chemistry in fish are means of identifying abnormal conditions in the environment that produce stress in fish. They conclude that fish are indicators of water quality. Likewise, Agrawal (1980) pointed out that manganese is deposited in freshwater along with fertilizers, food additives and fungicides and that freshwater fish show a significant decrease in the total erythrocyte count when they have been exposed to it.

Tomaso et al. (1981), in tests carried out on catfish, found that calcium had a minimal effect of toxicity and sodium showed no effect on fish. They mention that nitrite toxicity is related to the ability to oxidize hemoglobin to methemoglobin, losing the ability to carry oxygen to cells. The toxic effects of ammonia in the environment are due to a non-ionized form; which in a sublethal exposure causes damage to the tissue of the gills and kidneys within 24 hrs.

Garofano (1982) indicated that fish can be used as monitors for water bodies contaminated with cadmium. In his study, he reveals that cadmium chloride in high concentrations produces a decrease in erythrocytes and an increase in leukocytes in Ictalurus nebulosus. Prasad et al., (1987), studied the effect of different concentrations of crude oil extracts on catfish (Heteropneustes fossilis), through hematological analysis, finding low hemoglobin levels, increased hematocrit, hyperglycemia and increased concentration of ascorbic acid, also showing that the effects are reversible when the catfish is returned to its natural environment.

Alvarez-Mendoza (1997), reports for largemouth bass (Micropterus salmoides), under conditions of moderate malnutrition, a microhematocrit (Ht) of 33.99%, hemoglobin (Hb) of 7.76 gr/100ml, and total plasma protein (PTP) of 4.56 gr/dl, and severe malnutrition Ht of 22%, Hb of 4.35 gr/100 ml and PTP of 3.72 gr/dl, while the control presented Ht of 28.26%, Hb of 5.10 gr/100 ml and PTP of 4.31 gr/dl, concluding that the hematological parameters are altered from the incipient stages of malnutrition.

Lohner et al. (2001) evaluated sunfish populations (Lepomis sp.), collected in the Ohio River and tributaries that receive coal ash discharges, and the effect of low Se concentrations. Finding that the concentration of Se, Cu and As were statistically high in the exposed sampled fish tissues with respect to the reference fish. Leukopenia, lymphocytosis and neutropenia were evident in exposed fish. The white blood cell count values by dilution and the percentage of lymphocytes were significantly correlated with the concentration of Ser in the liver. Plasma protein levels were significantly lower in exposed fish indicating that nutritional stress may be present. The condition factor and growth range did not present significant differences between the exposed fish and the reference fish, considering the hematological parameters and the analysis of the Se concentration in the liver as diagnostic tools.

Lemly (2002), studied the alterations produced by him Se in the fish communities of Lake Belews in North Carolina, finding: telangiectasia in the gill lamellae, elevated lymphocytes, anemia (reduced hematocrit and hemoglobin), corneal cataracts, exophthalmia, pathological alterations in the liver, kidney, heart and ovaries, reproductive failure (reproduction of viable eggs, due to ovary pathology, and post-

spawning mortality, due to bioaccumulation of selenium in the eggs), teratogenicity of spines, head, mouth and fins. Finding that in eggs a concentration of 10µg/g, or more of selenium, can alter biochemical functions, passing through teratogenic effects, even causing death. On the other hand, adults appear to be healthy but fail to reproduce due to selenium intake through aquatic food chains, which take it from lake sediment.

Sepulveda et al. (2004), studied reproductive dysfunction in bass (Micropterus salmoides floridanus), exposed to wastewater from a paper industry, at concentrations of 10%, 20%, 40% and 80%, during periods of 28 to 56 days. , in study ponds, bass were also collected in the St. Johns River. Florida, where the paper mill discharges its wastewater. Blood and plasma tests were performed, in addition to the histopathological study of the liver and spleen. In fish confined in ponds, an increase in albumin concentration and hepatosomatic index was determined for bass exposed to concentrations of 20% or more in a period of 56 days. The bazosomatic index and melanomacrophage centers decreased in bass collected from concentrated current sites (Palataka and Rice Creek) considering also that concentrations of calcium, phosphorus, glucose and creatinine were high, compared to fish from reference rivers. Fish from Rice Creek showed decreased red blood cell count by dilution, and male bass from Palataka lower cholesterol concentration. Plasma albumin concentration and liver glutamic acid concentration were elevated in male bass from Palataka, and female and male bass from Rice Creek had high globulin concentrations. Indicating a complex pattern of the effect of wastewater from the paper industry on various physiological functions, despite being previously treated.

Silveire-Coffigny et al. (2004), studied in Oreochromis aureus the effect of different stress conditions, bacterial infection, nitrite intoxication, excessive dose of malachite green, its effect on hematological indices and its relationship with health condition. The fish showed microcytic anemia under experimental bacterial infection by Corynebacterium sp.; anemia, neutrophilia and erythrocyte deformation due to nitrite intoxication and excessive dose of medication with malachite green.

Becker, et al. (2005), compared the hematological parameters, Hematocrit, Hemoglobin, Mean Hemoglobin Concentration, ionic composition, concentration of

metabolites and Total Plasma Protein, under stress conditions, in Acipenser oxyrinchus and Acipenser brevirostrum, finding differences in plasma osmolality. , concentration of Na+, Cl^-, lactate, cortisol, and total protein, the rest of the parameters did not show significant difference.

Jamalzadeh and Ghomi (2009), carried out a hematological study with Salmon trutta caspius, finding that monocytes, eosinophils and neutrophils increase in winter compared to other seasons of the year. The value of hematocrit, leukocyte dilution count, lymphocytes and large lymphocytes are higher in small organisms than in adults.

Adeyeno et al. (2009), report hematological changes in African catfish (Clarias garipinus), under simulated handling and transport conditions, finding an increase in lymphocytes, but there was no significant difference in microhematocrit, hemoglobin, leukocytes and eosinophils.

Galeano et al. (2010), report the hematological values of Porichthys porosissimus, sampled in Bahía Blanca, Argentina, a place pressured by urban and industrial pollution. The values for erythrocytes were 1.32 ±0.32 x 106/µl, leukocytes 3314.8 ±2058.8 /µl, hemoglobin 8.13 ±1.18g/dl, hematocrit 36.17 ±6.03%, mean corpuscular volume (MCV) 295.14 ±90.02fl, corpuscular hemoglobin mean (HCM) 65.68 ±22.32pg, and a mean corpuscular hemoglobin concentration (MCHC) 23 ±4.92%. Plasma protein in autumn was 4.059 ±0.971 g/dl, and decreased in spring to 2.477 ±0.369g/dl. Six types of blood cells are described, erythrocytes, lymphocytes, eosinophils, neutrophils, thrombocytes, and monocytes.

Akinrotimi et al., (2010), analizaron 60 ejemplares adultos de *Tilapia guineensis* reportando los siguientes valores hematológicos, hematocrito 22.67 ±2.14%, hemoglobina 7.72 ±1.20g/dl, leucocrito 7.81 ±1.14%, conteo por dilución de leucocitos 30.02 ±2.50 células $x10^9$ g/l, conteo por dilución de eritrocitos 2.58 ±0.69 células $x10^9$ g/l, trombocitos 40.65 ±3.14%, neutrofilos 20.45 ±2.21%, linfocitos 35.46 ±4.7% y monocitos 3.12 ±1.00%.

Material and method.

33 adult specimens of alligator gar (Atractosteus spatula) were studied for the parameters of Hemoglobin (Hb,gr/dl), Microhematocrit (Ht,%), Leukocrit (Lc,%), Total Plasma Protein (PTP,gr/dl) , Red Blood Cell Dilution Count (RDR, thousands/mm3), and White Blood Cell Dilution Count (RDB, thousands/mm3). Blood smears of 36 specimens were made, reporting the differential count of leukocytes for each of them.

The length of the fish was measured with a tape measure (Slaymaker 10´ X ½´´), and weighed (Tor-Rey Balance, Model EQ-10/20).

The blood sample was obtained by cardiac puncture, since the fish were sacrificed for a parallel parasitological study. To determine the hemoglobin, I use a Hemoglobinometer (BMS, model AO), where the blood is hemolyzed in a chamber of the same equipment, with applicators that contain saponin for 30 seconds, placing it in the indicated compartment within the device and taking the corresponding reading.

For the microhematocrit and leukocrit test, heparinized capillaries were filled with ¾ of blood, sealing with creatoseal, and centrifuged (Centrifuga Solt-Bat, model PL 16 Scientific Apparatus) at 11,000 rpm for 5 minutes, measuring both microhematocrit in the reader. tests. Once the above was done, the part of the capillary containing the plasma was sectioned, and the sample was placed in a refractometer (model A3000 CL Clinical, Japan), to measure the total plasma protein by gravimetric means.

The count by dilution of Red Blood Cells was performed using a hemocytometer and Thoma pipettes for red blood cells, with Hayem's diluting liquid, following the standard methodology. For the White Blood Cell count, the Hemocytometer, Thoma pipettes for white cells and Turk's diluting liquid were used, following the standard methodology.

Blood smears were made, which were fixed in methanol for a minimum time of 5 minutes, proceeding to stain with rapid blood stain (HYLCEL No. 548) to perform the differential count of leukocytes. The observations were made with a bright field microscope (Leica Model CME), using an immersion objective (100X), counting one hundred leukocytes, separating them into their corresponding percentages.

Results.

The analyzed fish had an average length of 67 cm, with a maximum of 72 cm, and a minimum of 60 cm. The weight presented an average of 2,245 gr, with a maximum of 2,800 gr and a minimum of 1,700 gr. (Table 1).

In the parameters of the red series, Hemoglobin (Hb) presented a mean of 12.45 (±2.86), with a maximum of 17 and a minimum of 5. For Microhematocrit (Ht), a mean of 44.33 (±8.72) is reported. , presenting a minimum of 31 and a maximum of 63. The red blood cell dilution count (RDR), presented an average of 1,004,000 (±562,078), with a maximum of 3,480,000 and a minimum of 410,000 (Table 1).

In the parameters of the white series, leukocrit (Lc), presented a maximum of 4 and a minimum of 1, determining a mean of 2.76 (±0.9). The white blood cell dilution count (WBC) presented a mean of 64,471 (±20,590), with a minimum of 35,400 and a maximum of 105,300. The total plasma protein was determined with a maximum of 8 and a minimum of 2 with an average of 5.48 (±1.22) (Table 1).

The erythrocytes are mainly oval, nucleated, cytoplasm is acidophilic and nucleus is basophilic. Presenting an average of 11.49 µ (±0.75), with a minimum of 7.5 µ, and a maximum of 17.5 µ. (Figure 1).

The cells reported for the leukocyte differential count were: thrombocytes, neutrophils, eosinophils, basophils, lymphocytes, monocytes, plasma cells, myelocyte promyelocytes (Fig. 2). The thrombocytes presented a mean of 46.5% (± 6.06), neutrophils 4.5% (± 7.97), eosinophils 3.14% (± 3.3), basophils 3% (± 3.33), lymphocytes 26.6% (± 6.36), monocytes 5.42% (± 3.24), plasma cells 6.56% (± 1.54), promyelocytes 1.67% (± 1.6), myelocytes 0.31% (± 0.66), there were also cells that were not identified, these presented an average of 1.25% (± 1.33) (Table 2).

Table 1.- Descriptive statistics of the hematological parameters of *Atractosteus spatula.*

Parámetro	N	Media (±SD)	Max.	Min.	Varianza
Longitud (cm)	33	67 (±2.969)	72	60	8.813

Peso (gr)	33	2245 (±298)	1700	2800	8.881
Hb (gr/dl)	33	12.45 (±2.865)	17.00	5.0	8.21
Ht (%)	33	44.33 (±8.72)	63	31	76.042
Lc (%)	33	2.76 (±.902)	4	1	.814
PTP (gr/dl)	33	5.48 (±1.228)	8	2	1.5
RDR (niles/mm$^{3)}$	33	1 004 000 (±562078.064)	3 480 000	410 000	3.159E+11
RDB (niles/mm$^{3)}$	33	64 471.21 (±20590.312)	105 300	35 400	4.240E+08

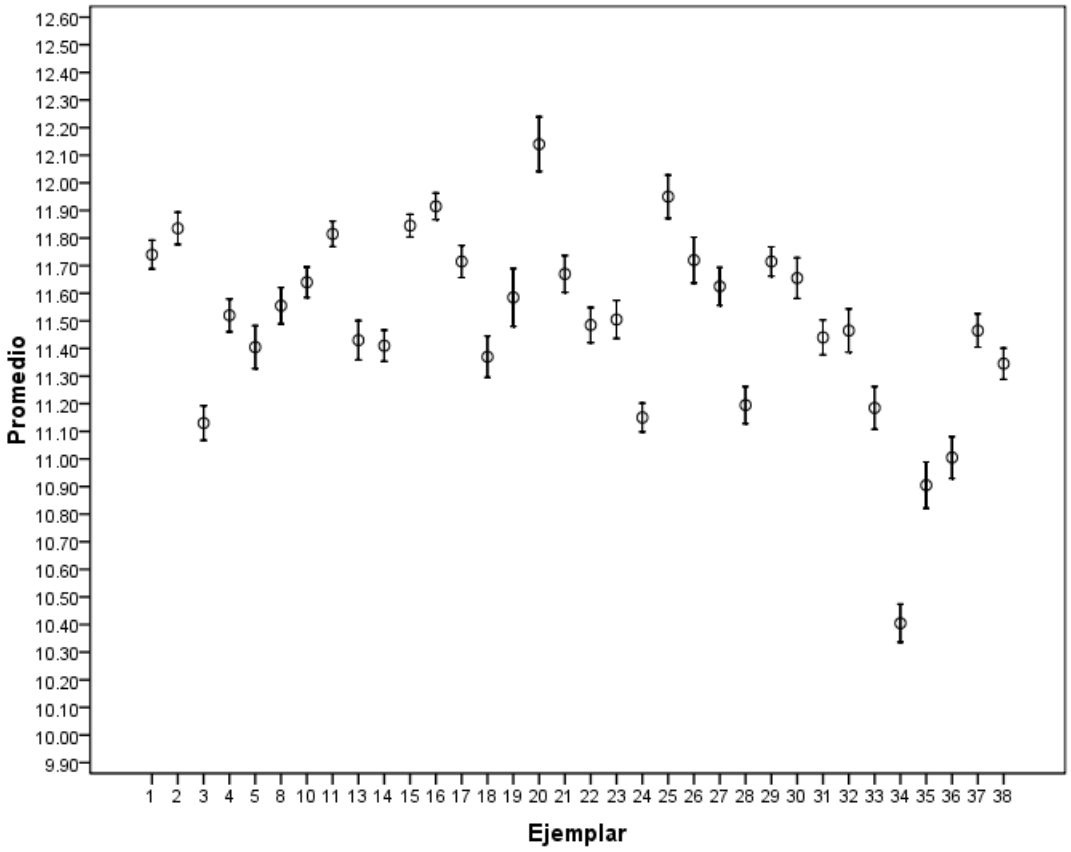

Figure 1.- Behavior of the size of the erythrocytes in each of the specimens of Atractosteus spatula sampled.

Table 2.- Descriptive statistics of the differential leukocyte count in A. spatula

Tipo Celular	Media (±SD)	Max.	Min.	Varianza
Trombocitos	46.5 (±6.064)	62	31	36.771
Neutrofilos	4.5 (±7.977)	28	0	63.629
Eosinofilos	3.14 (±3.305)	16	0	10.923
Basfilos	3.00 (±3.286)	14	0	10.800
Linfocitos	26.61 (±6.366)	41	8	40.530

Monocitos	5.42 (±3.246)	13	0	10.536
Células Plasmáticas	6.56 (±2.455)	11	2	6.025
Primielocitos	1.67 (±1.604)	6	0	2.571
Mielocitos	0.31 ±0.668	3	0	0.447
N.I.	1.25 ±1.339	5	0	1.793

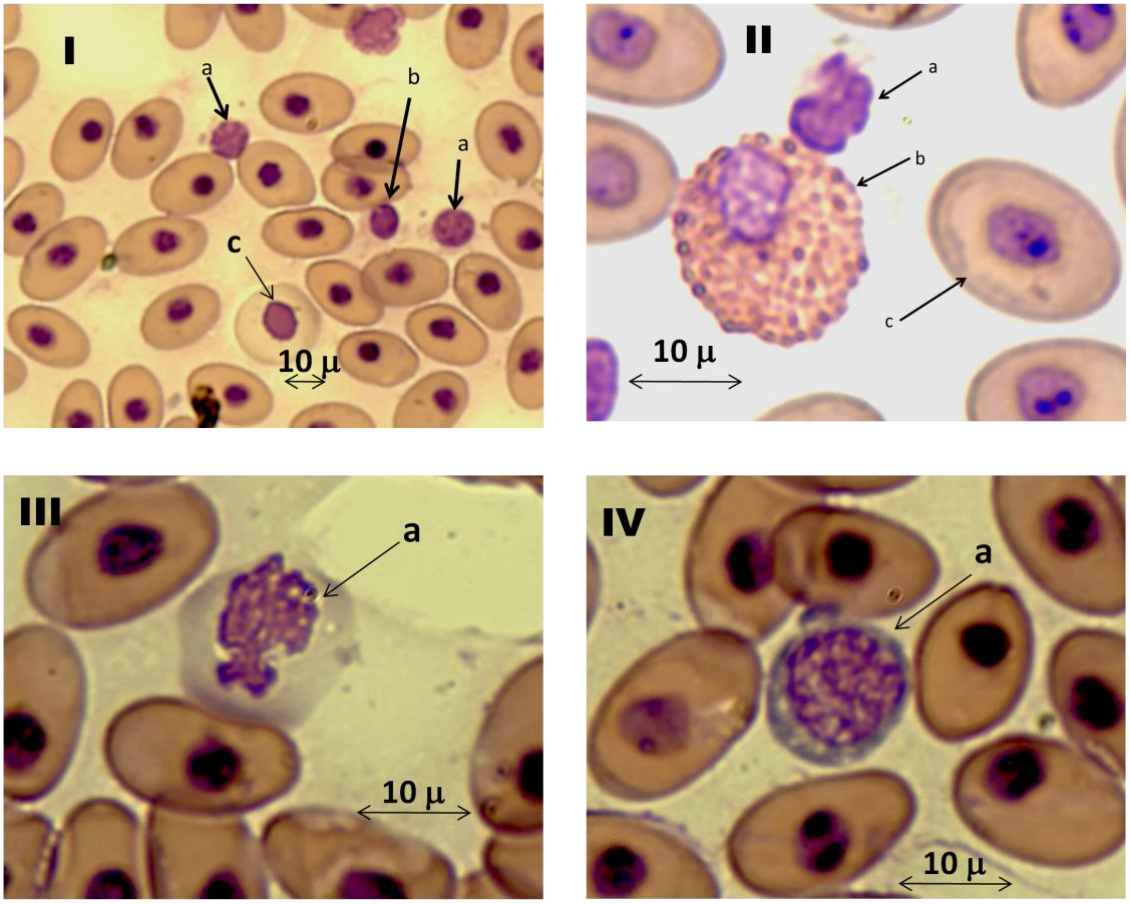

Figure 2.- Blood cells, I a: lymphocyte, b: thrombocyte, c: immature erythrocyte; II a: thrombocyte, b: eocinophil, c: normal erythrocyte; III a: monocyte; IV a: lymphocyte.

conclusion

Hemoglobin values reported in this study for Astractoteus spatula are higher than those reported for other species (Jamalzadeh and Ghomi, 2009; Galeano, 2010; Akinorotimi, 2010), but lower than those reported for two species of Acsipenser (Beker, 2005). For the Hemoglobin parameter, the values of Astractosteus spatula are equal to those reported for Salmon trutta caspius (Jamalzadeh and Ghomi, 2009), but higher for other species (Galeano, 2010; Akinrotimi, 2012; Beker, 2005). Total Plasma Protein is slightly higher for Astractosteus spatula than that reported by Galeano, 2010 and by Álvarez-Mendoza. The value found for the red blood cell count is within those reported for other species (Jamalzadeh and Ghomi, 2009; Galeano, 2010; Akinorotimi, 2010), however the white blood cell count by dilution does not coincide with what was found by the authors. aforementioned.

For the differential leukocyte count, more cell types are reported than for other species, but thrombocytes are the predominant cells, with a difference in the percentage values of lymphocytes and neutrophils (Jamalzadeh and Ghomi, 2009; Akinorotimi, 2010).

It is concluded that the hematological values reported in this study for Astractosteus spatula are within the range of other fish and can be used as a reference for future research in the field of environmental pollution.

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