ORIGINAL ARTICLE

HEMOPARASITES OF RED PIRANHA *Pygocentrus nattereri* (KNER, 1858) (CHARACIFORMES: CHARACIDAE) CAPTURED IN RED RIVER, MIDDLE ARAGUAIA RIVER REGION, STATE OF GOIÁS (GO), BRAZIL

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ABSTRACT

This work aimed to evaluate blood smears of 128 red piranha specimens *Pygocentrus nattereri* (Kner, 1858) (Characiformes: Characidae) as for the presence of hemoparasites and blood cells that were visualized. Samples were collected in Rio Vermelho (Red River) (15°10'44.73"S and 51°09'55.83W"), in the Brazilian state of Goiás, between April 2012 and August 2013, including ebb, full and dry phases. After identificating and numbering the piranhas, we collected approximately 0.5 mL of blood from each animal, from the caudal vein or through intracardiac puncture, and two blood smears were done and stained with May Grünwald-Giemsa and Fast Panoptic stains. After euthanasia, the body mass and biometrics of every fish were measured. Abiotic factors of the river water were also analyzed, such as temperature and transparency. In general, the biometric parameters were higher in the full phase of the river, and the values obtained on water quality were similar in relation to the average temperature of the water, which was around 82,4°F. The average transparency of the water varied, being greater at low tide. Erythrocytes, thrombocytes, neutrophils, eosinophils, monocytes, heterophile and lymphocytes were identified as blood cells of red piranhas and hemogregarines have been found in about 25% (32/128) of the blood smears.

Keywords: *Pygocentrus nattereri*, blood parasites, blood cells, biometrics, abiotic factors.

INTRODUCTION

Pygocentrus nattereri, popularly known as red piranha (Kner, 1858) (Characiformes: Characidae), can be found in abundance in the rivers of Central and South America. It is a carnivorous species with razor-sharp teeth and strong jaws, features that classify them as an essential predator for the aquatic biome balance. Due to its wide distribution and large muscles, it is widely used as food, such as broths and filets (BARROS et al., 2010).

Among the main features of this species are: a convex dorsal profile, round and shortened snout, bulky and prognathous jaw, rounded body with silver-gray color, darker back and anterior-ventral region ranging from orange to reddish (PIORSKI et al., 2005).

Vital (2008) stated that the red piranha fills all criteria to be selected as a host fish. Due to its restricted niche, that is this species has a reduced or well defined area in which it spends most of its life, it is easier to point the place where they acquired the parasitism. The red piranha is also capable of hosting a relatively large number of parasites, especially those whose life cycle has a wide range of hosts. In addition to being a common, abundant fish, easy of sampling and having relatively small size, the parasite collecting becomes less laborious compared to larger fishes.

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In general, like other fish, *P. nattereri* can be infected by a vast amount of pathogens such as bacteria, viruses, fungi and parasites, which can impair their development and, in some cases, endanger consumer's life too (ÂNGELA, 2010).

In this way, the search for parasites in wild animals *in situ* is a tool that assists the study of population health and environmental quality, in order to facilitate the understanding of the relationship between parasites and hosts and the environment in which they live (ALMOSNY; MONTEIRO, 2007). Unlike many intra-erythrocytes parasites of mammals and some birds, it is known that, in fish, a few blood infections are pathogenic (DAVIES; JOHNSTON, 2000).

More specifically, the term hemogregarine describes the set of blood parasites belonging to the suborders Adeleiina and Eimeriina of the phylum Apicomplexa (LEVINE et al., 1980). The hemogregarines may occur in red and white lines of blood cells of a variety of vertebrates and are widely distributed among fish, especially in marine environments (DAVIES; JOHNSTON, 2000). Although they have much of their life cycles unknown, it is assumed that these parasites have fish as intermediate hosts and leeches and crustaceans as definitive (MOLNÁR, 2006).

In the Eimeriina suborder, sporogony and merogony occur in the vertebrate host, resulting in the transmission of parasites through the invertebrate host, which acts only as a mechanical vector. On the other hand, in Adeleiina, which includes the genera *Hepatozoon* – that probably does not gather fish parasites (O'DWYER, 2013) –, *Haemogregarina* and *Karyolysus*, merogony occurs in the vertebrate host and sporogony occurs in the vector (LANE; MADER, 1996).

For the genus *Haemogregarina*, it is important to say that sporogony happens in the vector's stomach, oocysts do not have sporocysts and its transmission occurs via saliva, through inoculation of sporozoites. The hematozoa of this genus seems to be very adapted, because they cause little or no clinical-pathological changes in their natural hosts (MOÇO, 2008), although cases of hemolytic anemia can be observed (GARCIA-NAVARRO; PACHALY, 1994).

It is known that intracellular protist parasites of the Haemogregarinidae family are found in the blood of freshwater fish much more rarely than flagellate parasites. In addition, sporozoites, merozoites, microgametocytes and hemogregarines' macrogametocytes can be detected in the examination of stained blood smears (SIDDALL, 2001).

In this context, the objective of this work was to evaluate the biometric parameters, identify the blood cells and the hemoparasitoses that affect free life *Pygocentrus nattereri* piranha.

MATERIAL AND METHOD

Location

The specimens of *P. nattereri* were captured at Rio Vermelho (Red River) (15°10'44.73"S and 51°09'55.83"W), in a region near Britânia, a city in the state of Goiás (GO), in Brazil, between April 30th and May 4th 2012; October 3rd to October 7th 2012 and August 2nd to August 8th 2013, covering three distinct seasons of the hydrological cycle: ebb (May), dry (August) and flood (October), cited by Moreira and Zuanon (2000).

The Ethics Committee

The experimental protocol was submitted and approved by the Committee of Ethics in the Use of Animals/CEUA-UFU (038/12 Protocol), and licensed by Ibama – Brazilian Institute of Environment and Renewable Natural Resources (license SISBIO/ICMBio 17625-number 2).

Experimental procedure

128 specimens of *P. nattereri* were captured with the help of bamboo sticks, nylon yarn and hooks number 10. Pieces of

raw beef were used as bait for fishing. Once hooked in, the piranhas were taken away from the water and the hook was removed with the aid of specific fishing pliers. Then, the specimens were identified with a tag attached to a segment of string tied around the caudal peduncle, and numbered according to the order of collection and analysis.

The observation of the piranhas was performed with the animals out of the water, in a clean and airy environment and under the evaluation of an observer. After this process was done, we collected about 0.5 mL of blood from each animal by puncturing the caudal vein, located within the venous operculum, or through intra-cardiac puncture, using 1 mL syringes and hypodermic needles, 13x4,5 mm. For each piranha two smears were done and stained later in the Animal Pathology Laboratory of the Federal University of Uberlândia, one by the May Grünwald-Giemsa stain and the other with the Fast Panoptic method.

The slides were identified according to the number series assigned to the animal and stored in a proper box.

Following the observation and collection of the blood samples, the piranhas have undergone euthanasia through anesthetic deepening with 60 mg/kg pentobarbital intracardiacally (CARPENTER, 2005).

The body mass of each fish and biometrics were performed with the aid of a metal caliper rule accurate to 0.05 mm. Such procedure covered the following parameters: standard length, maximum height of the body, body width, length of the caudal peduncle, height of the caudal peduncle, caudal peduncle width, maximum length of pectoral fin, pectoral fin width maximum vertical distance of the caudal fin, maximum length of the caudal fin, head height below the middle of the eye, head, mouth width and height of the mouth (Figure 1). Data was recorded on worksheets.

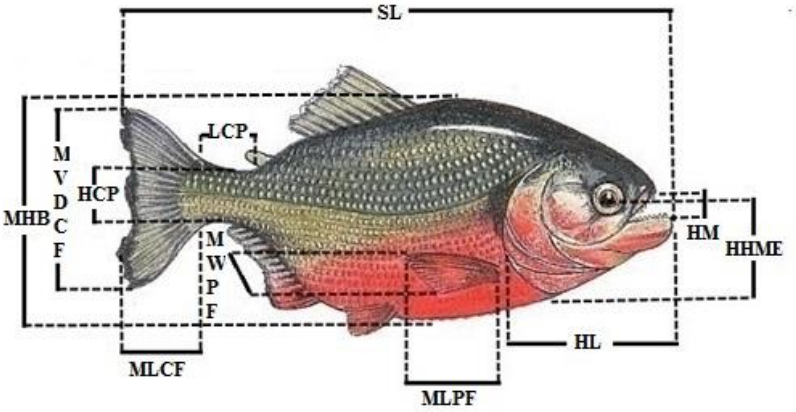

Figure 1. Representation of the parameters analyzed in the biometrics of Piranha *Pygocentrus nattereri*. Caption: SL = standard length; MHB = maximum height of the body; LCP = length of the caudal peduncle; HCP = height of the caudal peduncle; MVDCF = maximum vertical distance of caudal fin; MLCF = maximum length of the caudal fin; MLPF = maximum length of pectoral fin; MWPF = maximum width of pectoral fin; HHME = head height below the middle of the eye; HL = head length; HM = height of the mouth. Note: the width of the body (WB), width of the caudal peduncle (WCP) and width of mouth (WM) were not represented. Adapted from: http://www.pescasemfronteiras.com.br/peixe-interna/piranha-vermelha/51/

Smears readings were performed in the Animal Reproduction Laboratory at the Federal University of Uberlândia, with the aid of a light microscope, immersion oil, and 100x objective, in 20 fields per slide. The blood cells were identified and hemoparasites' family was determined

based on dichotomous keys and compared to reference material.

Some abiotic factors of the water at the site of capture were also analyzed, as well as the temperature and transparency.

The temperature was measured with a Mercury thermometer in three periods of the day (8:00 am, 12:00 pm and 5:00 pm). The average of these values was calculated and this procedure was repeated in three different days during the collection period. Then, the mean and standard deviation were calculated.

Transparency was measured using a Secchi disk. It was submerged in the water and, at the time of its disappearance, the measuring of the tape adhered to it was recorded. Three readings were carried out in sequence, and then the mean of these values was calculated. The transparency measure of the water was performed around noon to avoid shadow over the disk, which could interfere on the reading. This procedure was repeated for three days during the collection period. The mean and standard deviation of the values for the three days were calculated.

RESULTS AND DISCUSSION

The values obtained on the quality of water on the three periods of the hydrological cycle analyzed were similar in relation to the average temperature of the water, 86°F (30°C) in ebb and flood, and 78,8°F (26°C) in drought. However, the values of transparency average varied, being larger on ebb and decreasing in the flood and dry seasons, respectively. This can be justified by the time of the year when the analyses were performed, since in Brazil from June to August we have a dry season and lower temperatures due to the winter.

In general, the biometric parameters were higher in the flood, if compared with other periods, due to the association of the life cycle of the fish to the presence of favorable environmental parameters such as the type of habitat (or dryland-covered area), temperature, photoperiod, and rainfall fluviometry (AGOSTINHO et al., 2004). In this context, we can conclude that during the period of flooding there were sufficient favorable factors to ensure the reproductive success of the piranhas analyzed, in addition to its growth and development (VAZZOLER and MENEZES, 1992), since the rising of the water level may have increased the availability of food and new microhabitats (JUNK; BAYLEY; SPARKS, 1989).

Table 1. Abiotic Factors (water analysis) and biometrics of *Pygocentrus nattereri* piranhas (Kner 1858): means and standard deviations - Red River, GO, Brazil

Phase of the hydrological cycle	Ebb	Flood	Drought
Data collection and analysis	April 30th – to May 5th 2012	October 1st - to 10th 2012	August 2nd - to 8th 2013
ABIOTIC FACTORS			
Average temperature H2O (^{0}C)	30	30	26
Average transparency H2O (cm)	101.25	67	49.6
BODY			
Weight (g)	66.30 ± 46.84	184.40 ± 81.94	88.94 ± 24.75
Standard length (mm)	122.62 ± 23.50	178.68 ± 39.16	139.60 ± 26.52
Maximum height (mm)	51.96 ± 9.15	74.63 ± 13.62	56.59 ± 5.15
Width (mm)	17.20 ± 3.83	25.49 ± 4.17	20.70 ± 2.22
CAUDAL PEDUNCLE			

Length (mm)	12.96 ± 4.56	17.67 ± 2.75	14.35 ± 2.33
Height (mm)	10.57 ± 1.08	14.73 ± 2.76	11.91 ± 1.38
Width (mm)	4.65 ± 2.17	10.43 ± 7.91	6.94 ± 2.15
PECTORAL FIN			
Maximum length (mm)	22.32 ± 4.55	33.69 ± 5.57	23.97 ± 3.24
Maximum width (mm)	18.12 ± 5.37	27.29 ± 12.15	19.93 ± 3.83
CAUDAL FIN			
Maximum vertical distance (mm)	48.09 ± 12.4	67.41 ± 14.88	51.18 ± 8.33
Maximum length (mm)	19.25 ± 4.41	26.17 ± 3.49	25.43 ± 6.37
HEAD			
Down the middle of the eye height (mm)	21.28 ± 9.34	27.34 ± 8.78	22.27 ± 2.37
Length (mm)	38.94 ± 7.93	54.33 ± 10.44	42.61 ± 4.22
MOUTH			
Height (mm)	13.84 ± 2.87	20.84 ± 3.4	16.80 ± 2.20
Width (mm)	16.29 ± 3.87	27.20 ± 3.54	19.10 ± 2.70
Total number of samples analysed	N = 73	N = 20	N = 35

The cells identified in the peripheral blood of *Pygocentrus nattereri* piranhas were: erythrocytes, thrombocytes, neutrophils, eosinophils, monocytes, heterophiles and lymphocytes (Figure 2). It is important to highlight that, in fish, the identification of leucocyte series cells is more complex, in comparison with those of the erythrocyte and thrombocyte series. This is due to the difficulty in differentiating the thrombocytes from lymphocytes and monocytes from neutrophils, especially when it comes to young cells of different lineages (RANZANI-PAIVA; SILVA-SOUZA, 2004).

The mature erythrocytes have a morphology similar to that described by Thrall et al. (2007), ranging from oval to elliptical shapes, with a centralized nucleus, occupying a quarter of the cellular volume, and a homogeneous clear eosinophilic cytoplasm, containing clear dots and vacuoles.

The thrombocytes analyzed, as well as in Tavares-Dias et al. (2002), had elliptical shape with fusiform nucleus and intense vacuolization.

The lymphocytes found, also similarly to findings of Tavares-Dias et al. (2002), presented rounded shape and nucleus and varying sizes. Nucleus-cytoplasm ratio was observed, with scarce cytoplasm, heavily basophilic and no visible granulation.

It was noted that monocytes are usually the largest peripheral blood leukocyte of red piranhas, reaching twice the size of red blood cells, as well as quoted by Ranzani-Paiva and Silva-Souza (2004). In addition, they have rounded shape, weakly basophilic cytoplasm stained by the dye and eccentric spherical nucleus (TAVARES-DIAS et al., 2002).

Neutrophils were presented as rounded cells with size similar to the monocytes, as described by Tavares-Dias et al. (2002). These cells, because of the wide variation of their eccentric nucleus, are referred to as polymorphonuclear, and they are more commonly found in rod shape (RANZANI-PAIVA; SILVA-SOUZA, 2004), which was confirmed in the present study.

It was also noted that the red piranha is a fish that has both neutrophils as heterophiles, a term used for a

granulocyte with intracytoplasmic inclusions which are seen in the form of fusiform granules or rods, which are intermediary between eosinophil and basophil coloring (TAVARES-DIAS; MORAES, 2004).

Ranzani-Paiva (1995) reported that eosinophils and basophils are found less frequently in the blood of fish, what is due to the small percentage of these cells. Nevertheless, eosinophils were visualized in this work.

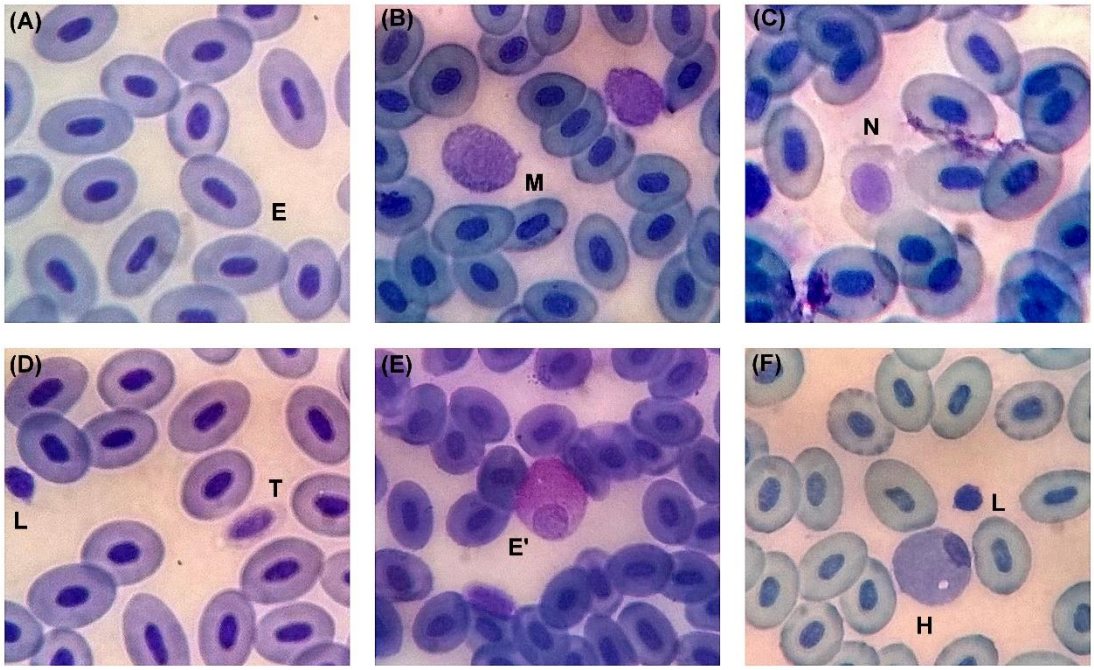

Figure 2. Blood cells of *Pygocentrus nattereri* piranha. E-(erythrocyte); M-(monocyte); N-(neutrophils); T-(thrombocytes); L-(lymphocyte); E'-(eosinophil); H-(heterophile) (x 1000). With the exception of the F blade, stained through the Panoptic method, the rest were stained with May Grünwald-Giemsa.

Hemogregarines were found in 25% (32/128) of the blood smears analyzed (Figure 3). In Brazil, only three species of hemogregarines have been described as parasites of freshwater fish, from a total of 4.035 fish species, which is equivalent to about 31% of the known species in the world (LÉVÊQUE et al., 2008). However, in this study it was not possible to define the species, because of the need for molecular methods such as polymerase chain reaction (PCR).

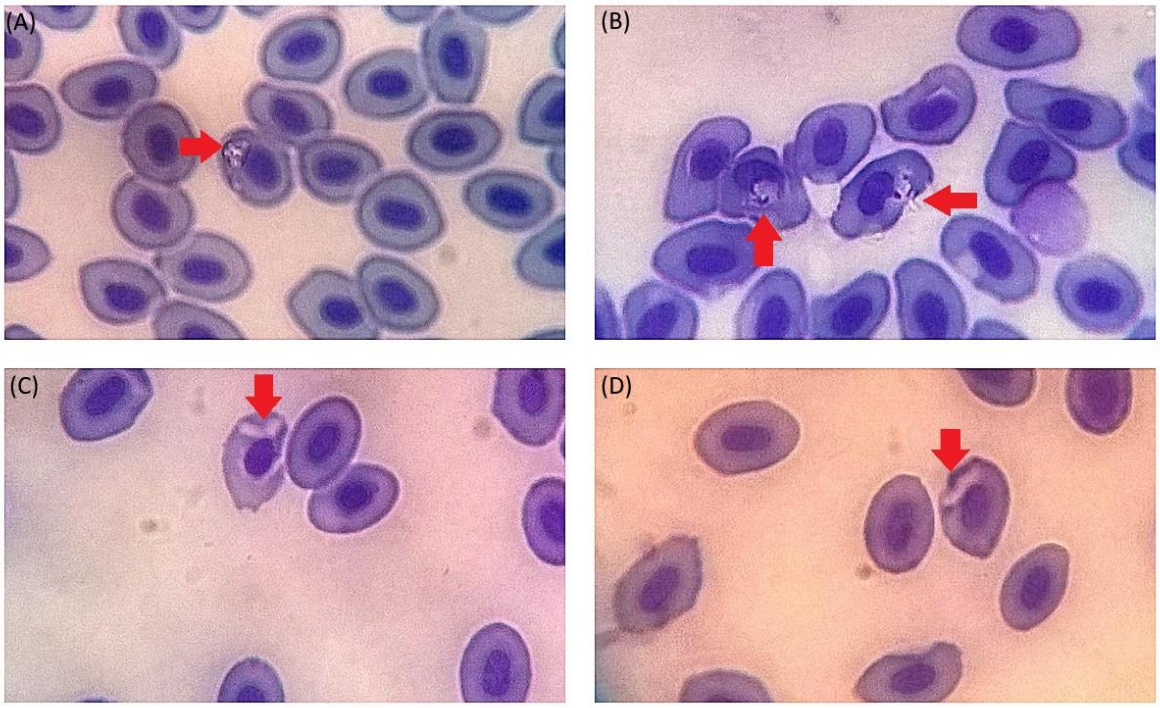

Figure 3. Hemogregarines (red arrows) found in the peripheral blood of piranhas.

It was not possible to correlate if the coloring method had any influence on the identification of hemogregarines and if the parasitism interfered in the biometrics of the piranhas, as well as the phase of the hydrological cycle. To this end, further studies should be carried out with a larger sample.

CONCLUSION

It was possible to determine the presence of hemogregarines in 25% (32) of the *Pygocentrus nattereri* red piranha captured in the Red River, Araguaia River basin, in addition to the visualization and cellular differentiation of erythrocytes, thrombocytes, neutrophils, eosinophils, monocytes, heterophile and lymphocytes.

The values obtained on the quality of water in three periods of the hydrological cycle analyzed (ebb, flood, drought) were similar in relation to the average temperature of the water (28° C). But the values of average transparency varied, being higher on ebb, and then in flood and drought. The biometric parameters, in turn, were superior in the flood, in comparison with other periods analyzed.

HEMOPARASITOS DE PIRANHAS VERMELHAS *Pygocentrus nattereri* (KNER, 1858) (CHARACIFORMES: CHARACIDAE) CAPTURADAS NO RIO VERMELHO, REGIÃO DO MÉDIO RIO ARAGUAIA, ESTADO DE GOIÁS, GO

RESUMO

Este trabalho teve como objetivo avaliar extensões sanguíneas de 128 espécimes de piranha vermelha *Pygocentrus nattereri* (Kner, 1858) (Characiformes: Characidae) quanto à presença de hemoparasitos e células sanguíneas visibilizadas. Foram realizadas coletas no Rio Vermelho (15º10’44.73’’S e 51º09’55.83’’O), no estado de Goiás, no período de abril de 2012 a agosto de 2013, abrangendo as fases vazante, cheia e seca. Após a identificação e numeração das piranhas, coletou-se cerca de 0,5 mL de sangue de cada animal por punção da veia caudal ou intracardíaca e confeccionou-se duas extensões sanguíneas, coradas pelo método

de May Grünwald-Giemsa e Panótico Rápido. Depois de realizar a eutanásia, mediu-se a massa corporal e foi feita a biometria de cada peixe. Também foram analisados fatores abióticos da água no local, como a temperatura e a transparência. De maneira geral, os parâmetros biométricos foram superiores na enchente e os valores obtidos sobre a qualidade da água foram semelhantes em relação à temperatura média da água, que ficou em torno de 28°C. A transparência média da água variou, sendo maior na vazante. Identificou-se eritrócitos, trombócitos, neutrófilos, heterófilos, eosinófilos, monócitos e linfócitos como células sanguíneas de piranhas vermelhas e hemogregarinas foram encontradas em cerca de 25% (32/128) das extensões.

Palavras-chave: *Pygocentrus nattereri***,** parasitos sanguíneos, células sanguíneas, biometria, fatores abióticos.

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