Cyrilia sp. (Apicomplexa: Haemogregarinidae) in the Amazonian freshwater stingray *Potamotrygon wallacei* (cururu stingray) in different hydrological phases of the Rio Negro

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Abstract

Intraerythrocytic parasites are frequently found in fish, including elasmobranchs. The Amazonian rivers present well defined annual hydrological cycles that results in drastic modifications of the environmental conditions with deep implications in the life cycle of the whole associated biota in those fluvial systems. The freshwater stingray *Potamotrygon wallacei* (stingray cururu) is a new species restricted to the Middle Rio Negro basin and it is subject to strong alterations in their natural habitats (*igapós*) a result of the constant variations in the water level of Rio Negro. This work demonstrates the occurrence of intraerythrocytic parasite *Cyrilia* sp. in this stingray species. Additionally, the prevalence and quantification of hemoparasites in different phases of Rio Negro were also established. Field sampling was carried in the Archipelago of Mariuá, Middle Rio Negro, involving different stages of the water cycle. The intraerythrocytic parasites were quantified by direct counting in blood smears using a total counting of 2000 erythrocytes in each blood smear. The presence of parasites intraerythrocytic generates changes in the morphology of blood cell. The largest amount of the hemoparasites was recorded in the drought period. We observed a decreasing tendency in the number of parasites in the blood between the drought periods and inundation. We concluded that the level of Negro River influences the incidence of intraerythrocytic parasites in the cururu stingray and the drought represents the period of larger susceptibility to the infestation.

Keywords: Amazon basin, prevalence, intraerythrocytic, seasonal variation, freshwater stingray.

Cyrilia sp. (Apicomplexa: Haemogregarinidae) na arraia de água doce Amazônica *Potamotrygon wallacei* (arraia cururu) em diferentes fases do ciclo hidrológico do Rio Negro

Resumo

Parasitas intraeritrocitários são frequentemente encontrados em peixes, incluindo elasmobrânquios. Os rios Amazônicos possuem ciclos hidrológicos anuais que resultam em modificações drásticas nas condições ambientais, com implicação profunda no ciclo de vida associada a biota dos sistemas fluviais. A arraia de água doce *Potamotrygon wallacei* (arraia cururu) é uma nova espécie restrita a bacia do médio Rio Negro e sujeita a fortes alterações em seus habitats naturais (igapós) resultantes das constantes variações do nível de água do Rio Negro. Este trabalho demonstra a ocorrência de parasita intraeritrocitário *Cyrilia* sp. em espécimes de arraias de água doce. Além disso, a prevalência e quantificação

de hemoparasitas em diferentes fases do Rio Negro foi estabelecida. Coletas de campo foram realizadas no Arquipélago de Mariuá, médio Rio Negro, envolvendo diferentes fases do ciclo hidrológico. Os parasitas intraeritrocitários foram quantificados por contagem direta em esfregaços de sangue usando a contagem total de 2000 eritrócitos em cada esfregaço sanguíneo. A presença de parasitas intraeritrocitários gera alterações na morfologia da célula sanguínea. A maior quantidade dos hemoparasitas foi registrado no período de seca. Observou-se a tendência de diminuição no número de parasitas no sangue entre o período de seca e de cheia. Concluiu-se que o nível de Rio Negro influencia a incidência de parasitas intraeritrocitários na arraia cururu, e a seca representa o período de maior susceptibilidade à infestação.

Palavras-chave: bacia Amazônica, prevalência, intraeritrocitário, variação sazonal, arraia de água doce.

1. Introduction

Parasites are abundant in tropical climates, and are one of the major causes of death and disposal of fish (Tavares-Dias et al., 2001), including elasmobranchs (Clewley et al., 2002; Aragort et al., 2005), highlighting the intraerythrocytic parasites (Davies and Smit, 2001; Hayes et al., 2006; Smit et al., 2006). Recently described in a freshwater stingray (Magro et al., 2015), these parasites can take advantage of temperature fluctuations, reproduction strategies, population genetic patterns, as well as habitat and migratory behavior to reproduce and proliferate in the blood of a diversity of hosts (Davies and Johnston, 2000).

The pulse system controls the water cycle in the Amazon basin and presents well defined annual hydrological cycles that include drought periods, inundation, flood and ebb tide (Aride et al., 2010). That outstanding fluctuation of the level of the waters, which can reach a difference of over 10 meters results in drastic modifications of the environmental conditions with deep implications in the life cycle of the whole associated biota in those fluvial systems (Sioli 1984; Junk et al., 1989). Most of the relationships maintained by the Amazonian fishes are conditioned to the oscillation of the water level in their natural environment (Soares et al., 2007). That link between the water level and the physiologic properties of the blood was established (Oliveira, 2008) for the Amazonian freshwater stingray *Potamotrygon wallacei* (cururu stingray; Carvalho et al., 2016), a new species with much defined characteristics and appreciated by the international ornamental fish trade. This stingray species is restricted to the Middle Negro River basin and it is subject to strong alterations in their natural habitats (igapós) as a result of the constant variations in the water level of Negro River. This work demonstrates the occurrence, prevalence and quantification of intraerythrocytic parasites *Cyrilia* sp. in this stingray specimen in different phases of Negro River.

2. Material and Methods

Five field collections were accomplished among the years of 2006 and 2007 in the Archipelago of Mariuá, medium Negro River, close to the municipal district of Barcelos, Amazonas (Brazil): December (ebb tide) of 2006, March (dry period), July (flood), October (ebb tide) and December of 2007 (inundation). The procedures for handling and blood collection followed the guidelines established by Oliveira et al. (2012). The intraerythrocytic parasites were quantified through the direct counting in optical microscope in blood extensions stained with May Grunwald-Giemsa-Wright solution (Tavares-Dias and Moraes, 2003). The counting of the parasites was performed in proportion to the total counting of 2000 erythrocytes in each blood extension. The relative monthly water level of the Negro River (registered in the city of Barcelos) was obtained through the Company of Research of Mineral Resources (CPRM), the Brazilian agency responsible for the monitoring of the hydrological levels. One way ANOVA followed by Student "t" test was used to verify the effect of the river level on the amount of the intraerythrocytic parasites ($p<0.05$).

3. Results

A total of 104 *P. wallacei* were captured, 24 during the ebb tide of 2006, 17, 24 and 39, in the drought periods, ebb tide and inundation of 2007, respectively. In the sampling period of July 2007, the peak of the flood season in that area, no animals were captured. In the ebb tide of 2007 no intraerythrocytic parasite infection was observed in the captured stingrays. The prevalence of intraerythrocytic in ebb tide of 2006 was 21%, in the dry period of 2007 was 47% and inundation was 13%.

The presence of gametocytes of a parasite belonging to the *Cyrilia* sp. was observed (Figure 1). This intraerythrocytic parasite occupied a greater part of the cell, dislocating the nucleus to one of the cell extremities. The largest amount of the hemoparasite was recorded in the drought

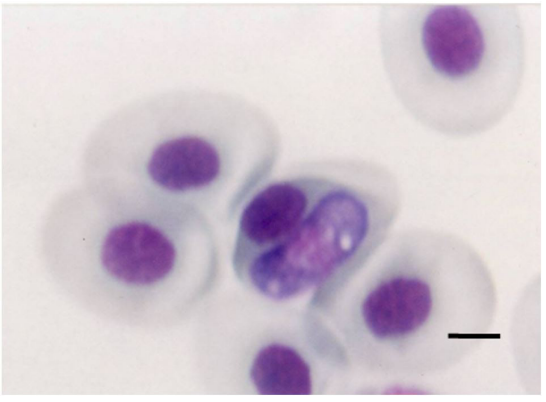

Figure 1. An erythrocyte from *P. wallacei* (stingray cururu) containing a gametocyte of *Cyrilia* sp. Bar=8µm.

period. We observed a decreasing tendency (p=0.073) of the number of parasites in the blood between the drought periods and inundation (Figure 2).

4. Discussion

Intraerythrocytic parasites can modify the amount of leucocytes, the size and the general morphology of the erythrocytes, as well as the capacity of these cells by transporting oxygen (Davies and Johnston, 2000). Differently from the results found by Smit et al. (2006) in which the variation in the morphology of the teleost erythrocytes was not observed by response to the presence of parasites, in the *P. wallacei*, the hemoparasite altered the position of the nucleus, moving it to one of the extremities of the cell. The displacement of the nucleus is probably a result of the greater size that the gametocyte presents in proportion to the volume (>50%) of the red blood cell, these studies were similar to those found by Magro et al. (2015).

In *P. wallacei* from Negro River, the mean prevalence of *Cyrilia* sp. was 20.3%. Aragort et al. (2005) studying species of marine stingrays *Raja microocellata, Raja brachyuran* and *Raja* spp., observed that average 14.3% of the animals were parasitized by *Haemogregarina delagei*. Moreover, in spiny dogfish *Squalus acanthias* the average prevalence of hemogregarinidae was 56.0%, ranging from 22.8% to 75.0% depending on the time that the animals are kept in acclimation (Clewley et al., 2002). These differences may be related to the association between the animal and the environment (marine and freshwater environments). The relationship between behavioral ecology and biology of Amazonian fish and the marked environmental changes that occur in the Amazon basin during the annual cycles (Junk et al., 1983, 1989). Amazonian fishes have developed adaptive strategies to adjust to these environmental pulses of flooding (Almeida-Val et al., 1999). It would not be surprising to expect that hemoparasites have also their biology influenced by the cyclical flood pulses. Smit et al. (2006) studying the parasitic load of several marine fish

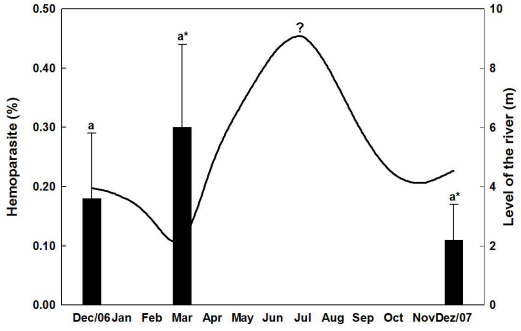

Figure 2. Effect of the variation in water level of Rio Negro on the amount of Intraerythrocytic parasites of the Amazonian stingray, *P. wallacei*. Same letters indicates no significant differences (p<0.05). *indicates a tendency for differences between periods (p=0.073). No parasites were observed in October. **?**=no samples were collected.

observed seasonal variations in the hematozoa prevalence in *Zebrasima scopas* and *Sufflamen chrysopterum*.

For *P. wallacei*, the largest prevalence of *Cyrilia* sp. occurred in the dry season indicates an inverse relationship with the level of the Rio Negro. In this period, the cururu stingrays are confined in small bodies of water with several other species of aquatic animals. That overcrowding scenery provides significant modifications in the water quality such as decreasing oxygen levels, competition for territory and dispute for food. Such factors probably create favorable conditions for the proliferation of the hemoparasites in the cururu stingray.

However, at least for stingray cururu it is not the period of the year but the level of the river that acts as the regulatory agent in the establishment of the intraerythrocytic parasites. Is such statement proven by the results obtained in the ebb tide of 2007 in which all captured animals did not present any indication of parasites differently than the results observed in the ebb tide of the previous year (2006), when the water level was considerably lower. In spite of the difficulty of capturing stingrays when the level of the river is in its maximum and the *igapó* areas are naturally submerged, it is possible to suppose that in that period the stingrays should not be infected by intraerythrocytic parasites due to the "dilution effect" that the flood pulse provokes on the density of fish in those places.

Finally, we concluded that the level of Rio Negro influences the incidence of intraerythrocytic parasites in the cururu stingray and the drought represents the period of larger susceptibility to the infection. As an example of what happens with the reproductive biology (Araújo, 1998) and the properties of the blood oxygen transport capacity (Oliveira, 2008), that are also adjusted according to seasonal variations of the Rio Negro level. Our results indicate that there is a need to expand the erythrocyte cell counts to 2000 in each blood smears, differently from studies developed with marine elasmobranchs that usually count 100 cells per blood extension. Furthermore, further studies are necessary to understand the development cycle of these hemoparasites and to verify eventual effects on the metabolism of the Amazonian stingray in response to the presence of these intraerythrocytic parasites.

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Erratum

In the article "***Cyrilia* sp. (Apicomplexa: Haemogregarinidae) in the Amazonian freshwater stingray *Potamotrygon wallacei* (cururu stingray) in different hydrological phases of the Rio Negro**", DOI http://dx.doi.org/10.1590/1519-6984.00416, published in Brazilian Journal of Biology, ahead of print, Epub Aug 15, 2016,

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