

EFFECT OF THE INFESTATION BY *Lernaea cyprinacea*
Linnaeus, 1758 (Copepoda, Lernaeidae) ON THE LEUCOCYTES
OF *Schizodon intermedius* GARAVELLO & BRITSKI, 1990
(Osteichthyes, Anostomidae)

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Received November 25, 1998 – Accepted February 18, 1999 – Distributed May 31, 2000

ABSTRACT

Differential white blood cell counts from *Schizodon intermedius* infested by *Lernaea cyprinacea* were carried out and compared using the Wilcoxon matched pairs test. The observations were performed in fish infested by 16-77 adult *Lernaea*, fish with parasitic lesions but without attached crustaceans, and non parasitized fish (control group). The specimens of *Schizodon intermedius* were obtained from the University of Londrina fish farming facilities. The following leucocytes were observed: lymphocytes, neutrophils, monocytes, basophils, eosinophils and immature leucocytes. Intense lymphocytopenia and neutrophilia were observed in the infested hosts. Consistent increasing of monocyte percentage values occurred in the infested fish. The highest values for immature leucocytes counts were recorded from infested fish specimens.

Key words: leucocytes, *Lernaea cyprinacea*, *Schizodon intermedius*, lymphocytopenia, neutrophilia.

RESUMO

Efeito da infestação por *Lernaea cyprinacea* Linnaeus, 1758 (Copepoda, Lernaeidae) sobre o número de leucócitos de *Schizodon intermedius* Garavello & Britski, 1990 (Osteichthyes, Anostomidae)

A contagem diferencial dos leucócitos de *Schizodon intermedius* infestados por *Lernaea cyprinacea* foi realizada e os resultados foram comparados usando-se o teste não-paramétrico de Wilcoxon. As análises foram realizadas em peixes infestados por 16-77 *Lernaea* adultas, em peixes com lesões causadas pelo parasito mas sem a presença dos crustáceos e peixes não parasitados (grupo controle). Os espécimes de *Schizodon intermedius* foram provenientes da estação de piscicultura da Universidade Estadual de Londrina. Os seguintes leucócitos foram encontrados: linfócitos, neutrófilos, monócitos, basófilos, eosinófilos e leucócitos imaturos. Intensas linfocitopenia e neutrofilia foram encontradas nos peixes infestados, assim como um consistente aumento dos valores percentuais de monócitos. Os valores mais altos de número de leucócitos imaturos foram também verificados nos espécimes parasitados.

Palavras-chave: leucócitos, *Lernaea cyprinacea*, *Schizodon intermedius*, linfocitopenia, neutrofilia.

INTRODUCTION

Lernaea cyprinacea Linnaeus, 1758 (Lernaeidae) is an important freshwater fish parasite which has a wide geographical range. It was introduced to Brazil some years ago with Hungarian

carp (Pavanelli *et al.*, 1998) and, currently, it can be found throughout the country, both in wild and farmed fishes. Its economic importance in Brazilian fish farming is increasing due to the numerous epizootics occurring among the most important farmed fish species. The influence of parasitic

crustacea inhabiting fish upon the haematological parameters of the host was mentioned by Nair & Nair (1983), Guillaume *et al.* (1985) and Natarajan & Felix (1987). Despite these studies, limited information about leucocyte responses to fish parasites is available.

In this paper we report on the effect of *Lernaea cyprinacea* infestation upon the leucocytes of *Schizodon intermedius* Garavello & Britski, 1990 (Anostomidae), a Brazilian fish native to the Tibagi river, in the State of Parana, Brazil. This fish have been cultivated in experiment ponds at the University of Londrina, State of Parana, Brazil. The specimens were accidentally infested with *L. cyprinacea*.

MATERIAL AND METHODS

Pond reared fish were obtained from the University of Londrina fish farming facilities in

August 1994. The blood was taken immediately after capture from the caudal vein, using heparinized syringes. Blood smears were air dried and stained with May Grünwald and Giemsa. For each fish a differential count of 200 leucocytes was completed. After blood sampling, each fish was measured (total length), weighed (total weight) and inspected to detect anchor worms (*Lernaea cyprinacea*) and, or, lesions due to recent infestations. The specimens were divided into three groups according to the presence or absence of anchor worms and parasitic lesions: (group I) fish without parasites or lesions (control group); (group II) fish with lesions but without parasites; and (group III) fish with parasites. Sample size, length and weight ranges for each fish group are reported in Table 1. A statistical comparison ($p < 0.05$) for the blood characteristics among the three fish groups was performed with a non parametric test, the Wilcoxon matched pairs test.

TABLE 1

Length, weight and number of *Schizodon intermedius* from the University of Londrina fish farming sampled for *Lernaea cyprinacea* in August 1994. Specimens of *Schizodon intermedius* without both parasites and lesions (group I or control group), specimens with lesions but without parasites (group II) and fish specimens parasitized by *Lernaea cyprinacea* (group III).

Group of Fish	Number of fish	Range	
		Length (cm)	Weight (g)
I	10	29.7-34.6	340.4-545.5
II	16	31.6-36.4	375.4-654.0
III	20	27.6-37.0	297.2-513.5

RESULTS

The white blood cells of fish were identified according to the criteria used by Boyar (1962), Sarasquete (1984), Burrows & Fletcher (1987) and Ranzani-Paiva (1995). The following white blood cells were present: lymphocytes, monocytes, neutrophils, basophils, eosinophils and immature leucocytes.

The differential counts for leucocytes are reported in Table 2, and the results of the Wilcoxon matched pairs test (Z values) are indicated in Table 3.

In both the control (group I) and the specimens with lesions (group II) the lymphocytes were the most common leucocytes, followed by neutrophils, monocytes, basophils and immature leucocytes. On the other hand, the neutrophils were the most abundant in the blood of infested fish (group III).

Basophils were rare in all the specimens, the greatest value (3.7%) occurring in the parasitized fish (group III). Eosinophils were rare in both the control and the fish with lesions (group II), and were not observed in the infested fish (group III).

TABLE 2

Differential leucocyte counts of specimens of *Schizodon intermedius* without both parasites and lesions (control group or group I), specimens of *Schizodon intermedius* with lesions but without parasites (group II) and fish specimens parasitized by *Lernaea cyprinacea* (group III), with mean percentage and range (in parentheses) of each cell type.

Group	Lymphocytes	Neutrophils	Monocytes	Basophils	Eosinophils	Immature leucocytes
I	89.0 (83.0-96.5)	6.9 (1.0-11.5)	1.2 (0-4.0)	2.0 (0-5.5)	0.1 (0-0.5)	0.8 (0-1.5)
II	66.7 (47.5-83.5)	28.1 (8.5-45.5)	1.8 (0-7.5)	1.9 (0-5.5)	0.1 (0-0.5)	1.4 (0-4.5)
III	11.2 (2.0-28.0)	52.8 (26.5-79.0)	27.8 (9.5-51.0)	3.7 (0.5-7.5)	0	4.5 (1.0-10.0)

TABLE 3

Results of Wilcoxon matched pairs test (Z values) between specimens of *Schizodon intermedius* without both parasites and lesions (group I), specimens of *Schizodon intermedius* with lesions but without parasites (group II) and fish specimens parasitized by *Lernaea cyprinacea* (group III). Asterisk indicate significant differences ($p < 0.05$).

	group I				group II			
	lymphocytes	Neutrophils	Mono cytes	Immature Leucocyte	Lymphocytes	Neutrophils	Mono cytes	Immature Leucocytes
group II	2.67*	2.67*	1.19	1.54	-	-	-	-
group III	2.67*	2.67*	2.67*	2.52*	3.52*	3.46*	3.52*	2.54*

The number of monocytes was statistically higher in the infested specimens (group III), when compared with the control (group I) and the fish with lesions (group II).

The mean percentage of immature leucocytes varied among the fish groups, being higher in the infested specimens (group III).

DISCUSSION

Lymphocytes have usually been considered the most abundant of the leucocytes in the peripheral blood of healthy fish. On the other hand, neutrophils are scarce, and basophils and eosinophils only occasionally are seen (Weinreb, 1958; Alexander *et al.*, 1980; Ranzani-Paiva, 1995). Our results concerning the control group collaborate these data.

The leucocytic formula can change related to several causes. Lymphocytopenia and neutrophilia were shown to occur in relationship to the spawning period (McCarthy *et al.*, 1975; Ranzani-Paiva & Godinho, 1983; Kavamoto *et al.*, 1985; Pickering, 1986) or stress due to low water temperature (Bennet

& Neville, 1975; Pickering, 1986). Similar changes can occur due to parasitic infections. Neutrophilia was reported in *Channa punctatus* infected by trematodes and *Salmo trutta* infested by ectoparasites (Mahajan *et al.*, 1979; Pickering, 1986); an increase of the percentage values of monocytes and neutrophils was observed in *Channa striatus* parasitized by the isopod *Alitropus typus* after 96 hours of infestation, and declined thereafter (Nair & Nair, 1983). Similar changes were related to bacterial and viral infections (Alvarez-Pellitero & Pintó, 1987).

Our results showed a significant lymphocytopenia in the blood of infested fish when compared to the other fish groups.

A significant increase of the neutrophils was evident in the blood of infested specimens and fish with lesions. The lymphocytopenia and neutrophilia observed in fish infested by *Lernaea cyprinacea* are interpreted as a consequence of the copepod infestation. A possible influence of the spawning period, which was shown to cause lymphocytopenia and neutrophilia by other authors, can not be claimed in this paper because the fish

analysed were not spawning. The same is true for the increase of the monocytes percentage values found in the infested fish.

The white cell picture found in the fish with lesions showed the most similar values when compared to control group. Apparently, the results show a recovery of the fish, indicating the return to normal healthy values.

According to Enomato (1969) the number of immature leucocytes does not modify with growth maturation or temperature changes, increasing only when infectious diseases or inflammations occur. This was found to be true in the present study, the higher number of immature leucocytes being observed in parasitized fish.

Acknowledgments — The authors thank Dr. Jorge C. Eiras from University of Porto, Porto, Portugal and Dr. Richard A. Heckmann from Brigham Young University, Provo, Utah, USA for careful reading and criticism of the manuscript, and Mauro Caetano Filho for providing the fish samples. The work was supported by UEL/COPATI/Klabin and CNPq.

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