Parasitological and hematological analysis of Nile tilapia *Oreochromis niloticus* Linnaeus, 1757 from Guarapiranga reservoir, São Paulo State, Brazil

Maria José Tavares Ranzani-Paiva[1]*, Nilza Nunes Felizardo[2] and José Luis Luque[3]

*[1]Instituto de Pesca, Av. Francisco Matarazzo, 455, 05001-900, Cidade de São Paulo, São Paulo, Brasil. [2]Programa de Pós Graduação em Biologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica. [3]Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro. *Author for correspondence. E-mail: mranzanipaiva@uol.com.br, mase@pesca.sp.gov.br*

ABSTRACT. A total of 206 adult specimens of tilapia, *Oreochromis niloticus,* were collected from the Guarapiranga Reservoir, Sao Paulo, from August 1996 to April 1998, to relate their health condition with parasite infestation. Hematocrits and the differential leukocyte counts were analyzed. Scrapings of skin and gills were performed and this last removed and fixed for the identification of monogeneans. In the gills, *Trichodina*, *Ichthyophtirius multifiliis*, *Cryptobia*, amoebas and monogeneans (*Cichlidogyrus* sp.) were found, and in the skin, *Trichodina*, *Cryptobia* and *Henneguya*. The prevalence of some parasites seemed to be associated with water temperature and the level of dissolved oxygen. The hematocrit and leukocyte cells percentage showed little variation during the sampling period. Only basophils demonstrated a significant difference between monthly mean values. The eosinophil percentage was higher in fish parasitized with *I. multifiliis* and *Cichlidogyrus* sp. and in non-parasitized animals. There was no detection of fish mortality. It could be concluded that the fish were in good health even though the condition of the Reservoir water was not ideal.

Key words: *Oreochromis niloticus*, hematology, *Trichodina*, *Ichthyophtirius multifiliis*, *Cryptobia,* Monogenea.

RESUMO. Análise parasitológica e hematológica em tilápia do Nilo, *Oreochromis niloticus* Linnaeus, 1757, da represa de Guarapiranga, Estado de São Paulo, Brasil. Foram coletadas na represa de Guarapiranga, São Paulo, entre agosto de 1996 e abril de1998, 206 tilápias, *Oreochromis niloticus,* com o objetivo de relacionar a condição de saúde desses animais à ocorrência de parasitos. Foram analisados: hematócrito e contagem diferencial de leucócitos. Foram feitos raspados de pele e brânquias, sendo estas removidas e fixadas para a identificação de Monogenoidea. Nas brânquias, foram encontrados: *Trichodina* Ehrenberg, 1838, *Ichthyophtirius multifiliis* (Fouquet, 1876), *Cryptobia*, amebas e Monogenoidea (*Cichlidogyrus* sp.), e na pele: *Trichodina* sp., *Cryptobia* sp. e *Henneguya* sp. A prevalência de alguns parasitos parece estar associada à temperatura e ao nível de oxigênio dissolvido da água. O hematócrito e a porcentagem dos leucócitos apresentaram pouca variação. Apenas basófilos demonstram diferença significativa entre os valores médios mensais. A porcentagem de eosinófilos foi mais alta nos peixes parasitados por *I. multifiliis* e *Cichlidogyrus* sp. e nos não parasitados. Durante esse período, não houve mortalidade de peixes. Conclui-se que os peixes estavam em boas condições de saúde, embora as condições da água da represa não estivessem ideais.

Palavras-chave: *Oreochromis niloticus*, hematologia, *Trichodina*, *Ichthyophtirius multifiliis*, *Cryptobia*, Monogenea.

Introduction

Guarapiranga Reservoir is one of the main water sources of São Paulo city and it is located within the districts of São Paulo, Itapecerica da Serra, Embu-Guaçu, Embu and Cotia. It has a volume of 1,946,430 m^3, a surface area of 33 km^2, an average depth of 5.7 m and a perimeter of 85 km (Beyruth *et al.*, 1996).

The disorganized occupation of its banks and the lack of basic conditions result in continuous discharge of domestic and industrial wastes into this reservoir. Because of the importance of this water resource, various studies have been conducted in it with the purpose of its restoration and preservation.

According to Hylander *et al.* (2000), fish constitute a very useful indicator of the true purity of the water. No water body can be considered in satisfactory condition if fish do not live and proliferate in it. Since many of the agents that cause diseases in fish are frequently found in water, the most important factors for a disease to be established would represent the state of the animals' health. The appearance of an epizooty depends on the contact of the host with the infectious agent and most of the time the existence of stress conditions. Water contamination or alteration of water quality by toxic heavy metals has been considered as one of the factors responsible for individual hematological changes and consequently affecting all the population of an environment.

The knowledge of blood composition is of fundamental importance in the evaluation of the physiologic condition and nutrition state of fish (Chagas and Val, 2003) and in assessing its disturbance by environmental conditions. The latter can include water temperature (Houston and de Wilde, 1968), concentration of O_2 or CO_2 (Soivio and Oikari, 1976), pollution and diseases (Ranzani-Paiva *et al.*, 1997) and even parasitism (Mahajan and Dheer, 1979; Ranzani-Paiva *et al.*, 2000).

Pilarczyk (1986) examined *Cyprinus carpio* (Linnaeus, 1758) raised in tanks and suffering from bacteriosis and parasites, and demonstrated the alteration of normal values for blood parameters and necrosis in the gills. Ranzani-Paiva *et al.* (1997) found in *Mugil platanus* Günther, 1880 a significant difference in the mean porcentage of hematocrit and hemoglobin concentrations between individuals parasitized by trichodinae in the gills and those not infected. Ranzani-Paiva *et al.* (2000) demonstrated alterations in blood composition related to parasitism in fish from the Parana River.

The aim of the present study was to determine the health of fish from this reservoir by identifying and quantifying the pathogenic agents in the gills and skin of Nile tilapia, *Oreochromis niloticus,* and relating them with hematologic analysis.

This species was chosen because it is the most abundant in Guarapiranga Reservoir (Barbieri *et al.*, 2000) and because a project has been developed in this area with tilapia raised in cages to increase productivity and furnish alternatives for the river dwellers to whom fishing is a way of life. Tilapia is a rustic species which grows quickly and has an omnivorous feeding habit. They tolerate considerable limits of temperature and oxygen levels as well as the presence of different types of pollutants. Besides, it is well accepted by the consumer because of its tasty meat.

In the present study, only gill and skin parasites were considered because according to Thatcher (1981), the biological agents that attack the gills, or simply fix in them, can be viewed as pathogenic even in the absence of visible damage. Low levels of dissolved oxygen will affect first those fish that have a disturbance in the gills, in natural environments as well as in captivity.

Material and methods

The fish were collected monthly from the Guarapiranga Reservoir during the period of August, 1996 to April, 1998. The fish were captured with a casting net and maintained in boxes with aerated water until the sampling time.

Blood was withdrawn from the caudal puncture and used for hematocrit determination by the microhematocrit method (Gondenfarb *et al.*, 1971) and differential leukocyte counts on stained smears by the Rosenfeld (1947) method.

Scrapings of skin and gills were taken from each fish and examined on a glass slide with cover slip for parasite identification by microscopy. The gills were totally removed and processed according to Amato *et al.* (1991). The branchial arcs were examined under a stereoscopic microscope for the presence of parasites. The monogeneans found were separated and placed in 4% formalin for further identification.

The means and standard error for blood analysis parameters and the monthly prevalence of parasites were calculated according to Busch *et al.* (1997). The differences between the means were tested by ANOVA and Tukey's test, at 5% probability (Zar, 1999).

Results and discussion

A total of 206 specimens of *O. niloticus* were studied. They had a total length between 11.5 and 28.0 cm and total weight between 25.0 and 687.9 g.

Fish mortality was not determined in the reservoir during the sampling period.

The following parasites were found in gills: Trichodina sp., Ichthyophthirius multifiliis, Cryptobia Leidy, 1846, amoebas and monogeneans belonging to the genus Cichlidogyrus sp. Paperna, 1960. In the skin, Trichodina sp., Cryptobia sp. and in one fish only Henneguya Thélohan, 1892 were found. The monthly and total prevalences, as well as the frequencies of these parasites on gills and skin, are presented in Tables 1, 2 and 3 and described in Figures 1 and 2.

Table 1. Prevalence of Protozoans and Monogeneans in the gills and skin of *O. niloticus* from Guarapiranga Reservoir, São Paulo, São Paulo State, Brazil.

	Tr (%)	Cr (%)	Ic (%)	He (%)	Am (%)	Mo (%)	NP (%)
Gills							
n=206	22.31	14.97	2.38	0.00	2.26	12.39	54.92
Skin							
n=206	6.50	5.98	0.00	0.53	0.00	0.00	88.57

Tr = *Trichodina* sp.; Cr = *Cryptobia* sp.; Ic = *Ichthyophtirius multifiliis*; He = *Henneguya* sp.; Am = Amoeba; Mo = Monogenea; NP = no parasitized

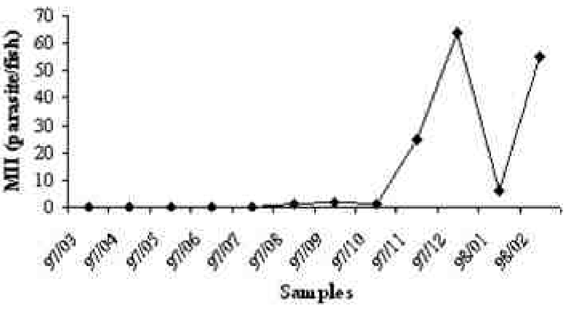

Figure 1. Mean intensity of infestation (MII) with Monogenea in *O. niloticus* from the Guarapiranga Reservoir, São Paulo, São Paulo State, Brazil.

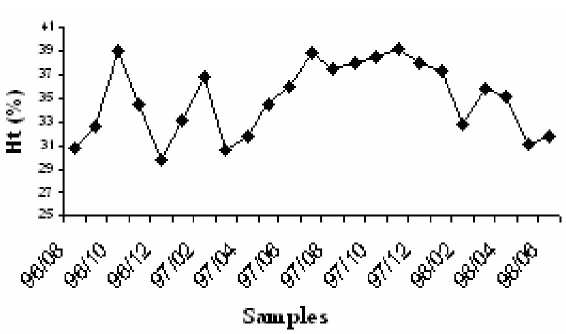

Figure 2. Hematocrit porcentage in *O. niloticus* from Guarapiranga Reservoir, São Paulo, São Paulo State, Brazil

Trichodinids were found in the gills in almost every month and with high prevalence, between 10 and 50% (Table 2). The highest prevalences were found in August and December 1996 and March and April 1997 (with 50% in each period). A large amount of algae in the skin and gills were also observed during these months. In April, 1997, the higher prevalence of this ciliate coincided with increased algae in the water, decreased water temperature and elevated levels of dissolved oxygen (Beyruth *et al.,* 1998). In skin, the trichodinids were rare, occurring with high prevalence only in February, March and April 1997.

Trichodinids are essentially commensal. They feed on waterborne particles and detritus particles from the fish surface as well (Lom and Dyková, 1992). Trichodinids never occur in large numbers in healthy fish, and in these cases, the irritation caused by the attachment of their adhesive disc is negligible. In debilitated fish, or in fish larvae or young fry, the natural repellent ability of the fish surface is impaired and the trichodinids can proliferate massively, causing excess secretion of mucous and lesions in skin and gills (Lom and Haldar, 1977). They can be indicators of water with excess organic material. The higher rate of infection by these protozoans in *O. niloticus* in this study may be explained by a decrease in host condition due to the low temperature (23°C) and low water quality of the Guarapiranga Reservoir (Beyruth *et al.*, 1998).

Cryptobia sp. was also found to be highly prevalent in *O. niloticus* gills (between 10 and 100%) during several months (Table 2). The maximum incidence of *Cryptobia* sp. was in September 1996, on gills (100%) and skin (30%). In October 1997, the prevalence of *Cryptobia* sp. was 55.6% on skin and 44.4% on gills. In September 1996, the water temperature was low (23.6°C) and oxygen was increased (5.4). In October 1997, on the contrary, increased temperature and decreased oxygen was observed. The dissolved oxygen level was never above normal. During these months, there was an increase in blue-green algal density (Beyruth *et al.*, 1998). Despite the fact that many studies have shown that there is a correlation between the occurrence of *Cryptobia* sp. and mortality, this was not noted in this study.

Ichthyophthirius multifiliis is a cosmopolitan protozoan that probably causes considerable damage to fish populations (Rogers and Gaines, 1975), and may cause high mortality rates even in wild populations. When it occurs excessively in gills, it causes excess mucous, making breathing difficult for the host. On the epidermis they produce secondary infections, dermatitis, hyperplasias and hemorrhage in extreme cases (Pavanelli *et al.*, 1998). Water temperature and stress are critical factors for the spread of *I. multifiliis*. In *O. niloticus* from the Guarapiranga Reservoir, *I. multifiliis* occurred on gills in the months in which the water temperature was around 20 to 22°C and dissolved oxygen around 2.82 mg/L (Beyruth *et al.*, 1996).

Table 2. Prevalence (%) of gill parasites in *O. niloticus* from Guarapiranga Reservoir, São Paulo, São Paulo State, Brazil.

Months	EF	Tr	Cr	Ic	Am	Mo	TrAm	TrCr	TrMo	CrMo	CrTrMo	NP
Aug/96	10	12.5	0.0	0.0	12.5	0.0	25.0	0.0	0.0	0.0	0.0	50.0
Sep/96	10	0.0	70.0	0.0	0.0	0.0	0.0	30.0	0.0	0.0	0.0	0.0
Oct/96	10	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	90.0
Nov/96	10	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	88.9
Dec/96	11	36.4	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	54.5
Jan/97	10	30.0	10.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	50.0
Feb/97	10	10.0	0.0	10.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	70.0
Mar/97	9	44.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	55.6
Apr/97	10	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0
May/97	10	30.0	0.0	10.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	50.0
Jun/97	10	20.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	70.0
Jul/97	10	40.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	60.0
Aug/97	10	10.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	80.0
Sep/97	10	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	10.0	0.0	80.0
Oct/97	9	0.0	44.4	0.0	0.0	11.1	0.0	0.0	0.0	0.0	0.0	44.4
Nov/97	10	10.0	10.0	0.0	0.0	50.0	0.0	0.0	0.0	20.0	0.0	10.0
Feb/98	10	0.0	0.0	0.0	0.0	30.0	0.0	0.0	20.0	40.0	10.0	0.0
Mar/98	10	20.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	70.0
Apr/98	10	10.0	30.0	20.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	30.0
May/98	10	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	90.0
Jun/98	10	10.0	0.0	10.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0	70.0
Total Mean		16.4	8.3	2.4	1.1	7.2	1.2	2.9	1.4	3.3	0.5	55.4

EF = number of examined fish; Tr = *Trichodina* sp.; Cr = *Cryptobia* sp.; Ic = *Ichthyophthirius multifiliis*; He = *Henneguya* sp.; Am = Amoeba; Mo = Monogeneans; NP = no parasitized.

Amoebas are for the most part free-living. Under specific conditions, they grow in a large number of fish, mainly on their surface, where they can be considered as parasites. These conditions include a high abundance of bacteria and mucous on the fish surface and in the water, constituting a food source for these protozoans. In the most serious cases, it causes the death of the host (Pavanelli *et al.*, 1998; Eiras, 1994). This protozoan occurred in August and September of 1996 with 37.15 and 10.0% prevalence, in gills and skin, respectively (Tables 2 and 3).

Table 3. Prevalence skin parasites in *O. niloticus* from Guarapiranga Reservoir, São Paulo, São Paulo State, Brazil.

Months	EF	Tr	Tr+Cr	Tr+He	Cr	NP
Aug/96	8	0.0	0.0	0.0	0.0	100.0
Sep/96	10	0.0	0.0	0.0	100.0	0.0
Oct/96	10	10.0	0.0	0.0	0.0	90.0
Nov/96	9	0.0	0.0	0.0	0.0	100.0
Dec/96	11	0.0	0.0	0.0	0.0	100.0
Jan/97	10	0.0	0.0	0.0	0.0	100.0
Feb/97	10	20.0	0.0	0.0	0.0	80.0
Mar/97	9	22.2	11.1	11.1	0.0	55.6
Apr/97	10	20.0	0.0	0.0	0.0	80.0
May/97	10	0.0	0.0	0.0	0.0	100.0
Jun/97	10	0.0	0.0	0.0	0.0	100.0
Jul/97	10	0.0	0.0	0.0	0.0	100.0
Aug/97	10	0.0	0.0	0.0	0.0	100.0
Sep/97	10	0.0	0.0	0.0	0.0	100.0
Oct/97	9	11.1	11.1	0.0	55.6	44.4
Nov/97	10	10.0	0.0	0.0	10.0	80.0
Feb/98	10	0.0	0.0	0.0	0.0	100.0
Mar/98	10	0.0	0.0	0.0	0.0	100.0
Apr/98	10	0.0	0.0	0.0	20.0	80.0
May/98	10	0.0	0.0	0.0	10.0	90.0
Jun/98	10	10.0	0.0	0.0	0.0	90.0

EF = number of examined fish; Tr = *Trichodina* sp.; Cr = *Cryptobia* sp.; He = *Henneguya* sp.; NP = non parasite

The gills of 140 fish were processed for the study of monogeneans (*Cichlidogyrus* sp.). The number of parasites by fish varied from 0 to 25. December 1997 was the month with the highest number of parasites/fish (25) and the highest average intensity (9.1). Only one sample showed a skin lesion, and it was found to harbor 5 monogeneans.

Studies by Pool and Chubb (1987) showed that monogeneans cause the death of the host when present on the gills in high numbers. Most monogeneans have a well-defined yearly infection pattern, where there is an increase in prevalence and intensity during the summer and a decline during the cold months until a minimum is reached in the spring. However, *Dactylogyrus vastator* Nybelin, 1924 survives 20 to 25 days at summer temperatures, while in winter it can survive for 6 or 7 months (Buchmann, 1988).

Cichlidogyrus sp. occurred only in gills and at a low means intensity (Tables 3 and 4), and the highest prevalences were in October and November 1997 (50 and 30%, respectively). Table 2 shows that the mean intensity of this parasite infestation was increased with a peak in December 1997. This monogenean is common in different species of tilapia and displays a vast geographic distribution (Pariselle and Euzet, 1995, 2003), where its presence in Brazil was first recorded in *Tilapia mossambica* (Peters, 1852) in the Amazonia region (Thatcher, 1991).

The mean porcentage of hematocrit and leukocyte subpopulations for Nile tilapia from Guarapiranga Reservoir are shown in Figures 2 and 3. Mean hematocrit levels varied between 29.8 and 39.0%, and there was a small variation over the sampling period. These values are higher than those found by Ueda *et al.* (1997) in Nile tilapia grown in captivity and close to the levels reported by Tavares-Dias *et al.* (2000) for red-flowered tilapia in captivity. Ranzani-Paiva *et al.* (2000) found a significant decrease in hematocrit values in *Prochilodus lineatus* (Valenciennes, 1836) from the Parana River which were infested by Dactylogyridae.

However, the percentages of lymphocytes and neutrophils were shown to vary inversely. In the summer, there was a tendency toward lower values for neutrophils and increased levels for lymphocytes, being converse in the winter months. Immature cells showed a high percentage during all the sampling period, with peaks in December 1997 and April 1998, coinciding in some months with an increasing in monocytes. The frequency of other cell types oscillated in the study period, with percentage values between 0 and 0.5.

Eosinophils were observed in blood smears from Nile tilapia in this study as reported previously in *Tilapia zilli* (Gervais) by Ezzat *et al.* (1974). However, this cell type has been reported to be absent in blood smears from *O. mossambicus* (Peters, 1852) by Silveira and Rigores (1989) and Tavares-Dias; Faustino (1998). These granulocytes were observed by Pitombeira (1972) and Powel *et al.* (1990) in other fish species, where it was more common in the branchial epithelium and intestinal mesenterium.

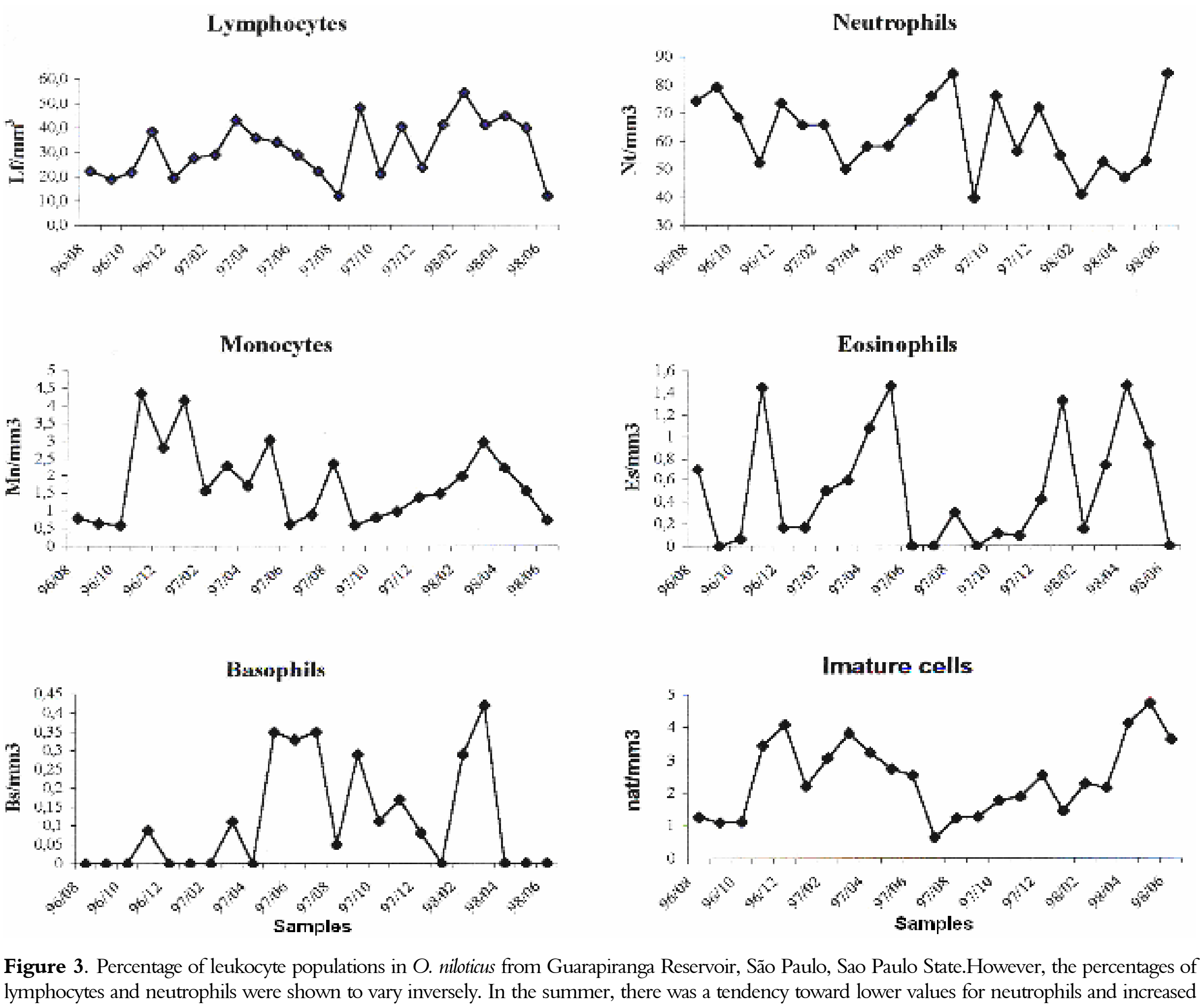

Figure 3. Percentage of leukocyte populations in *O. niloticus* from Guarapiranga Reservoir, São Paulo, Sao Paulo State.However, the percentages of lymphocytes and neutrophils were shown to vary inversely. In the summer, there was a tendency toward lower values for neutrophils and increased levels for lymphocytes, being converse in the winter months. Immature cells showed a high percentage during all the sampling period, with peaks in December 1997 and April 1998, coinciding in some months with an increasing in monocytes. The frequency of other cell types oscillated in the study period, with percentage values between 0 and 0.5.

Table 4 shows the hematologic parameters studied in relation to parasite infections, demonstrating that there was little variation in hematocrit and differential leukocyte count during the sampling period, where only basophils showed significant differences.

The percentage of eosinophils was greater in fish with *I. multifiliis* or Monogenea and in non-parasitized fish.

Table 4. Means ($\bar{x}$) and standard error (SEM) for hematological parameters of *O. niloticus* from Guarapiranga Reservoir, São Paulo, São Paulo State, Brazil, infested with gill parasites.

Months	Tr	TrCr	TrMo	Cr	CrMo	CrTrMo	Ic	Mo	NP	F	p
n	35	4	31	17	7	3	6	14	106		
Ht - $\bar{x}$	32.8	34.2	31.0	33.8	38.3	41.3	34.8	35.8	35.4		
SEM	0.9	0.7	2.0	1.1	2.5	16.4	1.4	1.3	0.5	1.562	**0.129**
n	33	17	3	17	7	3	5	15	108		
Lf – $\bar{x}$	35.1	26.0	43.3	25.5	49.1	41.3	25.2	34.2	31,4		
SEM	3.4	9.6	13.7	5.2	5.7	16.4	5.3	4.0	1,8	1.382	**0.205**
Nt - $\bar{x}$	59.3	70.0	52.3	70.0	48.0	56.3	70.1	61.6	63.4		
SEM	3.7	10.8	13.6	5.6	5.5	16.7	5.8	4.1	1.9	1.163	**0.324**
Mn - $\bar{x}$	2.2	1.2	0.7	2.0	1.6	2.0	1.1	1.1	2.0		
SEM	0.4	0.9	0.3	1.1	0.6	0.6	0.3	0.3	0.2	0.415	**0.911**
Es - $\bar{x}$	0.4	0.2	0.3	0.3	0.0	0.0	0.6	0.6	0.6		
SEM	0.1	0.2	0.3	0.2	0.0	0.3	0.2	0.5	0.1	0.651	**0.734**
Bs - $\bar{x}$	0.2	0.0	0.3	0.0	0.1	0.0	0.2	0.5	0.1		
SEM	0.1	0.0	0.3	0.0	0.1	0.0	0.2	0.2	0.03	2.023	**0.045***
Imat - $\bar{x}$	2.7	2.0	3.0	2.0	1.6	1.0	2.8	2.5	2.7		
SEM	0.4	1.1	1.0	0.3	0.4	0.0	0.6	0.4	0.2	0.854	**0.557**

*significant at 5% of probability; Ht = hematocrit (%); Lf = lymphocytes (%); Nt = neutrophils (%); Mn = monocytes (%); Es = eosinophils (%); Bs = basophils (%); Imat = immature cells (%); Tr = *Trichodina* sp.; Cr = *Cryptobia* sp.; Mo = Monogenea; Ic = *Ichthyophthirius multifiliis;* NP = no parasitized.

Conclusions

Based on the lack of significant alteration in the hematologic variables determined and the low intensity of parasites, it was possible to conclude that the fish were in good health even when the condition of the Reservoir water was not ideal.

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Received on April 18, 2005.
Accepted on September 14, 2005.