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RELATIONSHIP BETWEEN WATER TOXICITY AND HEMATOLOGICAL CHANGES IN *OREOCHROMIS NILOTICUS*

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ABSTRACT

Seriani, R.; Abessa, D. M. S.; Kirschbaum, A. A.; Pereira, C. D. S.; Romano, P. & Ranzani-Paiva, M. J. T. 2011. Braz. J. Aquat. Sci. Technol. 15(2): 47-53. eISSN 1983-9057. Hematological analyses were used as biomarkers in *Oreochromis niloticus* from a polluted pond and a fishfarm (negative control) in winter, spring and summer. Water samples from this polluted pond were also taken in order to perform acute toxicity tests with *Daphnia similis*. Hematological data were evaluated in fish: total leukocytes (TLC), trombocytes (TT) and differential leukocytes (neutrophyls, basophils, eosinophils, lymphocytes and monocytes). The pond waters were toxic in spring and winter to *D. similis*, but no toxicity was detected in summer, which presented higher values of hardness, conductivity and ammonia. The fish collected at the polluted pond exhibited increased immature erythrocytes and decreased of lymphocytes, total leukocytes and neutrophyls and trombocytes.

Keywords: Hematological changes, pollution, fish, biomarkers.

INTRODUCTION

Biomarkers in fishes have been used within environmental monitoring programs to estimate the degradation of aquatic ecosystems (Rand, 1995; Seriani et al., 2009). Aquatic contamination cause ecological (biological integrity, biodiversity, ecological processes) and economic (aquaculture, production of potable water, fishing, bathing, recreation) effects. Hematological changes in fish may be used for assessing the effects of contaminants, because blood parameters respond to low doses of pollutants (Ranzani-Paiva et al., 2000, Affonso et al., 2002, Adhikari et al., 2004, França et al., 2007, Seriani et al., 2009, Seriani et al., 2010). Fishes exposed to metals, pesticides and effluents exhibit hematological changes, not only after laboratory exposure, but also when the exposure occurs in the field (Ranzani-Paiva et al., 1997; Oliveira-Ribeiro et al., 2006; Adhokari, 2004; Shah, 2006; França et al., 2007, Seriani et al., 2010).

Blood parameters are considered good physiological indicators of the whole body conditions and therefore can be used in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari et al., 2004, França et al., 2007, Seriani et al., 2009). In addition, haematological studies are procedures frequently and routinely applied in the diagnosis of diseases in aquaculture (Ranzani-Paiva et al., 2000; 2008). Fish blood parameters have been increasingly employed in environmental monitoring programs to indicate physiological changes due to toxicants (França et al., 2007, Seriani et al., 2009; Zutshi et al., 2009). However, the knowledge on the fish hematology still needs to be expanded, to provide data for different species (Blaxhall, 1972; Affonso et al., 2002, França et al., 2007; Ranzani-Paiva et al., 2000, 2008; Zutshi et al., 2009, Seriani et al., 2010).

In addition, toxicity tests with cladocerans have been widely used to estimate responses at population or sub-population level as well as to estimate possible disturbances in aquatic ecosystems (Knie & Lopes, 2004). Planktonic cladocerans have an important role in the aquatic food chain, because they feed on algae and constitute food for secondary consumers. Alterations in the cladocerans natural populations may indicate evidences of ecological disturbance and possible effects on the trophic structure of the aquatic ecosystem (Knie & Lopes, 2004).

Due to their proximity to cities and other man-made structures, reservoirs, creeks, urban ponds and other water bodies commonly receive the inputs of many contaminant sources, which discharge different classes of contaminants into such water bodies, thus their biota can be negatively affected by pollution. In addition to that, aesthetical and recreational attributes are affected by fish mortality, release of sulfides and cyanobacteria blooms (Seriani et al., 2004).

Oreochromis niloticus is an exotic species which was introduced into the Brazilian water bodies and became abundant in reservoirs, representing nowadays

an important fishery resource for the subsistence of artisanal fishermen. In this context, the present study evaluated the quality of the water of Girassol Pond through the analysis of physical-chemical parameters of the water, toxicity tests with *Daphnia similis* and the analysis of blood parameters in *O. niloticus*.

MATERIAL AND METHODS

The study area, Girassol Pond (GP), is located in Santo André City, São Paulo State, Brazil (23º37'53"S, 46º31'58.73"W). Due to its proximity to residential areas, this pond receives discharges of domestic effluents, urban drainage and storm waters. Despite the lack of previous studies on GP, massive fish kill episodes are well known and already reported by the media, and have been attributed to *Microcistys aeruginosa*, *Cylindrospermopsis raciborskii* and *Anabaena flosaquae* (Seriani et al., 2005).

The renewal of the water in the pond is very low and depends exclusively on pluviometric rates. The pond fauna exhibits low diversity, with *O. niloticus* (Cichlidae) as the dominant species. Individuals of *O. niloticus* were artificially introduced into GP along with some smaller fish, such as ornamental guppies *Poecilia reticulata*.

In order to evaluate the pollutants present in GP, healthy organisms obtained from an experimental fish farm were used as the experimental control group. This farm belongs to the State Fisheries Institute. The water conditions in the culture tanks are considered adequate (Boyd, 1990; CONAMA, 2005) and the animals present good health (Seriani et al., 2009). This farm is used in many different research projects. Water samples and individuals of *O. niloticus* were collected in winter, spring and summer in Girassol Pond. Young fish of comparable age and size to the animals analyzed in the other seasons were not available at the Farm (control) in autumn. Therefore, no sampling was performed in this season.

Surface water samples were collected using buckets, transferred to glass flasks and stored frozen till the beginning of toxicity tests. Before freezing and after thawing, the following physical-chemical parameters of the samples were analyzed according to APHA (2005): pH, hardness (mg $CaCo_3$/L), dissolved oxygen content (mg/L), conductivity (μS/), temperature (ºC). The total ammonia concentration (NH_3-NH_4) was measured by the colorimetric method (Koroleff, 1976) Unionized ammonia concentrations were estimated by the method reported by Whitfield (1974), using measured pH, temperature, conductivity and total ammonia data.

In each sampling campaign, seven individuals of *O. niloticus* (Wt= 180 ± 20 g and Lt= 18.0 ± 3 cm) were collected. After anesthesia with clove oil (Delbon, 2006, Kirschbaum, et al., 2009), blood samples were taken by caudal punction using previously heparinized needles and syringes. Blood extensions were prepared in glass slides and colored with May-Grünwald-Giemsa (Rosenfeld, 1947). Two thousand cells were analyzed per slide/animal under a compound microscope (1000x) and the immature erythrocytes (IE), total leukocytes (TLC) and trombocytes (TT) were counted. Additional blood extensions were used for differential counting of leukocytes (neutrophyls, monocytes, lymphocytes, basophyls and eosinophyls). Hematological parameters were expressed as mean ± standard error (S.E.) and statistical analysis was performed using a two-tailed Student's t-test (control x GP).

Acute toxicity tests with *Daphnia similis* were conducted with water samples collected concomitantly to the fish. Neonate individuals of *D. similis* were exposed to the whole samples and immobile organisms were counted after a 48-h exposure. The tests were performed according to the ABNT-12713 protocol (1993). A dilution series (100%, 50%, 25%, 12.5%, and 6.25%) was tested. Culture water was used to dilute the samples.

To analyze the existence of relationships between the hematological responses and water quality, the data of the three collections were jointly submitted to a Multi-Dimensional Scaling (MDS) analysis coupled to a Monte Carlo test. This analysis used two data matrices: the first contained the blood data, and the second consisted of the water quality descriptors (acute toxicity and physical-chemical parameters). The data were logn-transformed before the analysis.

RESULTS

Water quality: physical-chemical parameters

The water physical-chemical parameters are shown in table 1. The pH in GP ranged from 6.6 to 7.6, whereas in the control it ranged from 7.1 to 7.6. In all the sample collections, Hardness of GP waters was higher than the controls in all samples (110-149 and 22-31 mg/L $CaCO_3$, respectively). Dissolved oxygen contents in GP water were variable, ranging from 1.5 to 7.5 mg/L, whereas in the control the values were high (5.5 to 6.7 mg/L). The conductivity of GP waters was much higher than that of control waters, reaching values above 400 μS/cm. In situ temperatures were variable in GP (20.0 to 26.9 ºC) and more homogenous in the controls (20.0 to 22.0 ºC). Total ammonia concentrations in GP ranged from 0.25 to 3.96 mg/L, with unionized ammonia concentration ranging from 0.01

Table 1 - Physical-chemical parameters of water from Girassol Pond and the control group in different seasons.

Physical-chemical parameters	Winter	Spring	Summer
pH			
Girassol Pond	7,6	6,6	7,0
Control	7,1	7,6	7,3
Hardness ($CaCo_3$) mg/l			
Girassol Pond	149	110	120
Control	22	31	27
Dissolved oxygen (mg/l)			
Girassol Pond	7,5	1,5	1,5
Control	5,5	6,7	6,0
Condutivity (μS/cm)			
Girassol Pond	427	495	400
Control	61	21	68
Temperatue (ºC)			
Girassol Pond	20,0	24,1	26,9
Control	20,0	21,0	22,0
Total ammonia (mg/l)			
Girassol Pond	3,96	0,25	1,50
Control	0,01	0,01	0,01
Unionized ammonia (NH_3)			
Girassol Pond	0,07	0,05	0,01
Control	0,01	0,001	0,001

to 0.07 mg/L. In the controls, total and unionized ammonia concentrations were low.

Water toxicity

The toxicity test with *D. similis* exhibited acute toxicity in GP waters in the winter and spring: acute toxicity was not observed in the summer samples. The conditions in the spring (Effect Concentration - EC_{50-48} h = 77.1%; 70.0 – 84.9%) were slightly more toxic than in the winter (EC_{50-48} h = 84.1%; 69.7 – 101.4%). In the summer the samples were not toxic.

Hematological parameters

The results of hematological analyses are presented in tables 2 and 3. Blood cell counts exhibited significant differences between control and GP fish for total leukocytes in winter, immature erythrocytes and leukocytes in spring and total thrombocytes and immature erythrocytes in summer .

The differential counting of leukocytes indicated differences in the winter, when the fish from GP presented less neutrophils and increased of lymphocytes than the controls. In the other seasons, no differences were observed. No differences were observed between GP and control fish for lymphocytes and monocytes.

Multivariate analysis

The Monte Carlo test showed that the stress in real data was smaller than the stress in randomized data, thus blood parameters are associated to water conditions. Water hardness (-0.62), conductivity (-0.52), total and unionized ammonia (-0.63 and -0.83) and water toxicity (-0.81) were negatively associated to axis 1, as well as the number of leukocytes (-0.96) and lymphocytes (-0.99), as shown in Figure 1 and Table 4. Axis 1 was positively correlated to the number of neutrophyls. Axis 2 correlated positively to the percent of monocytes (0.70) and negatively to the number of immature erythrocytes (-0.82). All the stations-campaigns were associated to axis 1, and only the spring and summer controls were also associated to the axis 2 of the MDS.

These results show the existence of a strong association of the biological responses in fishes and the water physical-chemical parameters (ammonia, conductivity, carbonates).

When the MDS is interpreted together with the result of each analyzed variable, it is possible to understand that the summer GP was placed close to the winter and summer controls because in this campaign, the biological patterns were similar and the water was also not toxic to *D. similis*. This also explains why the spring control was placed isolated, i.e., due to the results obtained for monocytes and immature erythrocytes, which differed from the summer and winter results.

DISCUSSION

In environmental monitoring, the use of biological methods that are easy, cheap and strongly related to contaminant levels is desirable. However, these biological methods exhibit a wide natural variability and are influenced by several natural factors, which may difficult the interpretation of data. In such cases, it

Table 2 - Mean number of leukocytes, thrombocytes, immature erythrocytes in 2000 cells of *O. niloticus* from Girassol Pond and the control group, in different seasons. (* = p<0,05).

Seasons	Leukocytes	Control Mean in 2000 cels	Girassol Pond Mean in 2000 cels
Winter	leukocytes	60.0 ± 23.0	**23.8 ± 9.0***
	trombocytes	9.8 ± 4.3	8.0 ± 5.0
	Immature erythrocytes	0	1.3 ± 1
Spring	leukocytes	47 ± 5	**19 ± 7**
	trombocytes	16 ± 6	14 ± 12
	Immature erythrocytes	5 ± 1.7	**15 ± 5***
Summer	leukocytes	45 ± 15	16 ± 12
	trombocytes	35 ± 10	**9 ± 8***
	Immature erythrocytes	1 ± 0.5	**4 ± 1.5***

is important not only to compare each set of data, but if possible, apply mathematic or statistical techniques that allow an overall analysis of the whole dataset (Chapman, 1983).

In the present study a strong association between the water quality and hematological changes and acute toxicity for *D. similis* was observed.

The relationship between water toxicity, low oxygen dissolved, ammonia and conductivity suggests that contaminants associated to sewage affected the water quality. Conductivity was higher in the dry season and lower in the wet season, which suggests that temporal variations in this variable can be attributed to pluviometric precipitation rates: the higher the precipitation rate, the higher the pond capability to dilute ions. A similar phenomenon may have occurred for ammonia, for which higher concentrations were observed in the dry season. Moreover, the association of toxicity and biological responses to water hardness suggests that natural factors can influence such responses or that $CaCO_3$ is discharged into the lake with the sewage. Therefore, further studies are required to explain this.

The low dissolved oxygen concentrations found in the GP waters, in spring and summer is probably due to the intense decomposition of organic matter, which reduces oxygen concentrations (Esteves, 1988; Seriani et al., 2004). Persistent hypoxia is a negative factor in water quality because, under such conditions, some toxic gases and compounds tend to be released into the water.

Environmental hypoxia can induce many compensatory processes in fish (Fritsche & Nilsson 1993). In spring and summer, together with low levels of dissolved oxygen, a reduced number of leukocytes were observed in fishes from GP.

This kind of response has been described as a result of environmental stress (Pickering, 1984), being initiated by primary endocrine metabolism (Fritsche &

Table 3 - Percent of neutrophyls, monocytes and lymphocytes of *O. niloticus* from Girassol Pond and the control group, in different seasons (* = p<0,05).

Seasons	Leukocytes	Control (percent)	Girassol Pond (percent)
Winter	neutrophils	30 ± 9	**4 ± 3***
	lymphocytes	69 ± 20	**93 ± 4***
	monocytes	2 ± 0.8	3 ± 2
Spring	neutrophils	16 ± 20	16 ± 8
	lymphocytes	83 ± 6	83 ± 8
	monocytes	1 ± 5	1 ± 0.5
Summer	neutrophils	27 ± 25	27 ± 11
	lymphocytes	74 ± 10	74 ± 13
	monocytes	2	2 ± 1

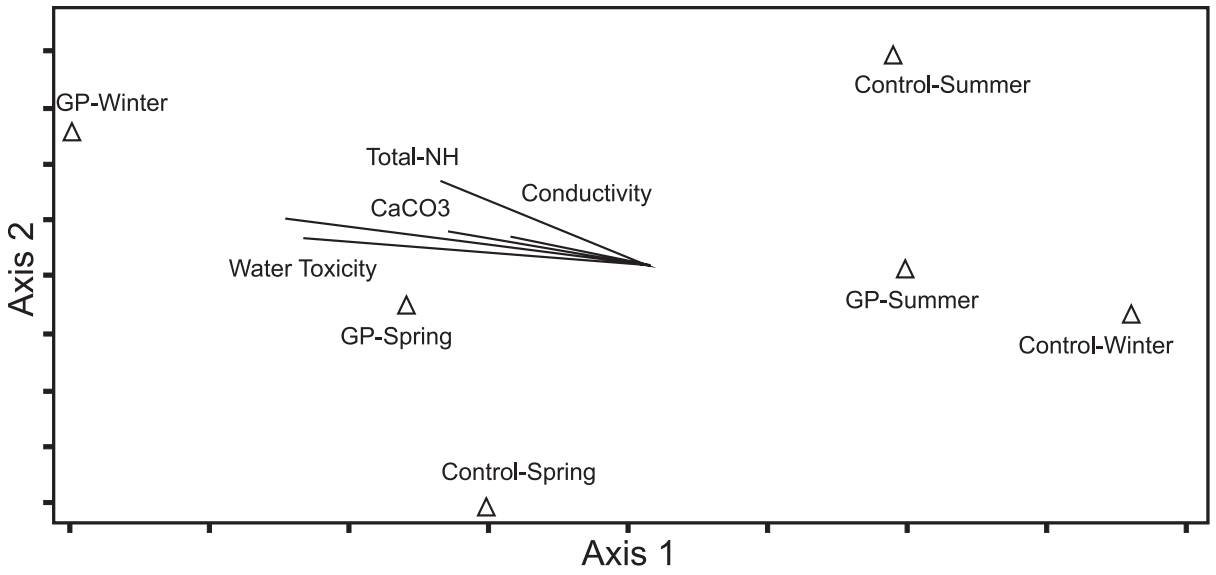

Figure 1 - Multi dimensional scaling, reduced to 2 dimensions, with physical-chemical data, water toxicity and hematological responses of *O. niloticus* from Girassol Pond and control.

Nilsson, 1993), and in fish it is generally characterized by elevations in cortisol and catecholamine levels in blood (Pickering, 1981). These hormones are involved in the maintenance of homeostasis in the presence of any stress, but they also may have a marked effect on numerous immunological functions (Heath, 1995).

Hypoxia is also reported to promote erythropoiesis (Nussey et al., 1995). The production of new cells can be interpreted as an adaptation mechanism to the low contents of dissolved oxygen, aiming to increase the oxygen transporting system. The present data corroborate this hypothesis, because fish from GP exhibited higher frequencies of immature erythrocytes in spring and summer.

The MDS indicated that the neutrophyls percentage correlated negatively to the ammonia contents, indicating that this compound (or a sewage related contaminant) affects the fish blood, causing a decrease in the percent of these defense cells in winter.

The presence of immature erythrocytes and monocytes were negatively correlated, but possibly this was caused by a mathematical artifact, since immature cells tended to occur in poor water conditions whereas monocytes were more frequent only in the winter. Thus, the relationship between frequency of monocytes and environmental quality could not be established clearly and should be further studied.

The results showed that the use of immunological biomarkers, together with toxicity tests, can be useful to evaluate the health of fish populations exposed to contaminated waters.

The blood immunological responses tend to be fast and may weaken the defense system, causing the animals to be more susceptible to opportunistic diseases. On the long term, the persistence of a stress condition may lead to the suppression of the leukopoietic centers, replacing the initial leukocytosis by leukopenia.

Since *O. niloticus* is frequently cultured in Brazilian rivers, reservoirs and artificial tanks ("floating-cage" systems), biomarker studies with this species can be easily integrated with toxicity bioassays in wide programs for monitoring water quality.

Table 4 - Correlations of hematological parameters of *O. niloticus* and water variables of Girassol Pond and Fish Farm (control) to the MDS axes.

Variables	Axis 1	Axis 2
Leukocytes	-0.962	0.233
Trombocytes	0.248	0.422
Immature erythrocytes	-0.323	-0.821
Neutrophyls	0.988	-0.011
Limphocytes	-0.995	0.033
Monocytes	-0.117	0.701
pH	-0,298	0,028
Hardness ($CaCO_3$)	-0,617	0,256
O.D.	-0,262	0,132
Conductivity	-0,519	0,228
Temperature	0,132	-0,095
Total Ammonia (NH_3-NH_4^+)	-0,628	0,402
Unionized Ammonia (NH_3)	-0,829	0,296
Toxicity	-0,806	0,224

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REFERENCES

ABNT - NBR 12713 – Água- Ensaio de toxicidade aguda com *Daphnia similis* Claus,1876 (Cladocera, Crustacea). Método de ensaio. 16p. 1993.

Adhikari, S.; Sarkar, B.; Chatterjee, A.; Mahapatra, C.T. & Ayyappan, S. 2004. Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost; *Labeo rohita* (Hamilton). Ecotoxicol. Environ. Safety. 58: 220–226

Affonso, E.G.; Polez, V.L.P.; Corrêa, C.F.; Mazon, A.F.; Araujo.; M.R.R.; Moraes, G. & Ratin, F.T. 2002. Blood parameters and metabolites in the teleosts fish *Colossoma macropomum* exposed to sulfide or hypoxia. Comp. Biochem. Physiol. C 133: 375–382.

APHA; 2005. Standard Methods for the examination of Water and Wastewater. 21a Ed., Washington, DC., APHA - American Public Health Association, AWWA - American Water Works Association and WPCF - Water Pollution Control Federation.

Blaxhall, P.C. 1972. The haematological assessment of the health of freshwater fish: a review of selected literature. J. Fish Biology. 4: 593–604.

Boyd, C. 1990. Water quality in ponds for aquaculture. London: Birmingham, 482p.

Chapman, P.M. 1990 The sediment quality triad approach to determining pollution-induced degradation. The Sc. Total Environ., 97/98: 815-825.

Chapman, P.M. & Long, E.R. 1983. The use of bioassays as part of a comprehensive approach to marine pollution assessment. Marine Poll. Bull. 14(3): 81-84.

Delbon, M. C. 2006. Ação da benzocaína e do óleo de cravo sobre os parâmetros fisiológicos de tilápia, *Oreochromis niloticus*. Dissertação (mestrado em aqüicultura) Centro de Aqüicultura da Unesp/ Universidade Estadual Paulista Júlio de Mesquita Filho. Jaboticabal. São Paulo.

Esteves, F. A. 1988. Fundamentos de Limnologia. Rio de Janeiro: Interciência/FINEP.

Fritsche, R. & Nilsson, S. 1993. Cardiovascular and ventilatory control during hypoxia. In: Rankin, J.C. & Jensen, F.B. (eds.) Fish Ecophysiology. Chapman & Hall, London. 180–206 pp.

Heath, A. G. 1995. Water pollution and fish physiology. London CRC Press, New York, 359 p.

Kirschbaum, A. A.; Seriani, R.; Pereira, C. D. S.; Assunção, A.; Abessa, D. M. S.; Rotundo, M. M. & Ranzani-Paiva, M. J. T. 2009. Cytogenotoxicity biomarkers in fat snook *Centropomus parallelus* from Cananéia and São Vicente estuaries, SP, Brazil. Gen. Mol. Biol., 32(1): 151-154,

Knie, J.L.W. & Lopes, E.W.B. 2004. Testes Ecotoxicológicos: métodos, técnicas e aplicações. FATMA / GTZ, Florianópolis, 289 p.

Koroleff. F. 1976. Determination of ammonia. In: Grasshoff, E.; Kremling, E. (Eds.). Methods of seawater analysis. New York: Verlag Chemie Weinhein, 117-181 pp.

Nussey, G.; Van Vuren.; J. H. J. & Du Preez, H. H. 1995. Effect of copper on haematology and osmoregulation of the Mozambique tilapia, *Orechromis mossambicus* (Cichidae). Comp. Biochem. Physiology (C), 111, 369-380.

Pickering, A.D. 1981. Stress and fish. London: Academic Press, p. 277-293.

Pickering, A.D. 1984. Cortisol-induced linphocytopenia in brow trout, *Salmo trutta* L. Gen.Comp. Endocr., 53: 252-259.

Rand, G.M. 1995. Fundamentals of Aquatic Toxicology: effects, environmental fate and risk assessment. 2nd Edition. Taylor & Francis, Washington D.C., USA. 1125p.

Ranzani-Paiva, M.J.T.; Souza, A.T.S.; Pavanelli, G.C.; Takemoto, R.M. & Eiras, A.C. 2000. Hematological evaluation in commercial fish species from the floodplain of upper Paraná River, Brazil. Acta Scient. 22(2): 507–513.

Ranzani-Paiva, M. J. T. ; Santos, A. A. ; Dias, D. C. ; Seriani, R. & Eganni, M. 2008 . Hematological and phagocytic response of fat snook, *Centropomus parallelus*, reared in net cages, before and after inoculation with *Sacharomyces ceresivisiae*. Bioikos, (22):29-35.

Richards, D.T.; Hoolle, D.; Lewis, J.W.; Ewens, E. & Arme, C. 1994. . Changes in the cellular composition of the spleen and pronephros of carp *Cyprinus carpio* infected with the blood fluke *Sanguinicola inermis* (Trematoda: Sanguinicolidae). Dis. Aquat. Org., Oldendorf, 19: 173-179.

Rosenfeld, G. 1947. Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido. Mem. Inst. Butantan, 20: 329-34.

Seriani, R.; Andrade A.G.; Silveira F.L.; Pinna F.V. & Carvalho L.N.; Rocha M.M. 2004. Aspectos sanitários, ecológicos e de saúde pública decorrente de florações das cianobactérias *Microcystis aeruginosa* e *Cylindrospermopsis raciborskii*. O Mundo da Saúde; 28(4):405-413.

Seriani, R.; Dias, D.C.; Silva, M.R.R.; Villares, E.O.; Abessa, D.M.S.; Moreira, L.B.; Ranzani-Paiva, M.J.T. & Rivero, D.H.R.F. 2009. Hematological parameters of *Oreochromis niloticus* from a polluted site. Annals of World Aquaculture Society. Vera Cruz, México.

Seriani, R.; Moreira, L. B.; Abessa, D. M. S.; Maranho, L. A.; Abujamara, L. D.; Carvalho, N. S. B.; Kirschbaum, A. A. & Ranzani-Paiva, M. J. T. 2009. Haematological analysis in *Micropogonias furnieri* from two estuaries at Baixada Santista, São Paulo, Brazil. Braz. J. Ocean., 58 (special issue IV SOB): 87-92, 2010.

Whitfield, M. 1974. The hydrolysis of ammonia ions in sea water – a theoretical study. Journal Marine Biological Association, UK 54: 565-580.

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