# *IN VITRO* AND *IN VIVO* STUDY OF CHLORINE DIOXIDE TO TREAT *Ichthyophthirius multifiliis* IN SILVER CATFISH *Rhamdia quelen*

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**ABSTRACT**

This study evaluated the efficacy of chlorine dioxide in the treatment of *Rhamdia quelen* infected with *Ichthyophthirius multifiliis*. The experiment was divided into two stages: *in vitro* and *in vivo*. The *in vitro* assay was conducted to determine the lowest concentration of chlorine dioxide ($ClO_2$) capable of immobilizing the theronts of *I. multifiliis*. Parasitized fish (degree III severity) with *I. multifiliis* were exposed short-term (0 mg $L^{-1}$, 25 mg $L^{-1}$, 125 mg $L^{-1}$ and 250 mg $L^{-1}$ for 1 h) and long-term baths (0 mg $L^{-1}$, 25 mg $L^{-1}$, 35 mg $L^{-1}$ and 45 mg $L^{-1}$ for 48 h) of chlorine dioxide. Chlorine dioxide was effective in immobilizing theronts at the lowest concentration (25 mg $L^{-1}$) *in vitro*. The same concentration in long-term exposure for 48 h reduced more than 50% of theronts in the gills of fish. Fish exposed to a short-term bath with 125 mg $L^{-1}$ and 250 mg $L^{-1}$. Chlorine dioxide ($ClO_2$) showed reduced parasitism in the gills (62%). It could be suggested the use of chlorine dioxide at 25 mg $L^{-1}$ for up to 48 h to reduce more than 50% of the *I. multifiliis* infestation.

**Keywords**: fish; treatment; Protozoa; disinfectant; fish farming

# ESTUDO *IN VITRO* E *IN VIVO* DO DIÓXIDO DE CLORO PARA TRATAR *Ichthyophthirius multifiliis* EM JUNDIÁ *Rhamdia quelen*

**RESUMO**

Este estudo avaliou a eficácia do dióxido de cloro no tratamento de *Rhamdia quelen* infectado com *Ichthyophthirius multifiliis*. O experimento foi dividido em duas etapas: *in vitro* e *in vivo*. O ensaio *in vitro* foi conduzido para determinar a menor concentração de dióxido de cloro ($ClO_2$) capaz de imobilizar terontes de *I. multifiliis*. Peixes parasitados (grau III de severidade) com *I. multifiliis* foram expostos à banhos de curta (0 mg $L^{-1}$, 25 mg $L^{-1}$, 125 mg $L^{-1}$ e 250 mg $L^{-1}$ por 1 h) e longa (0 mg $L^{-1}$, 25 mg $L^{-1}$, 35 mg $L^{-1}$ e 45 mg $L^{-1}$ por 48 h) duração com dióxido de cloro. Dióxido de cloro foi efetivo em imobilizar terontes na concentração mais baixa (25 mg $L^{-1}$) *in vitro*. A mesma concentração em banho de longa duração por 48 h reduziu mais do que 50% dos terontes nas brânquias dos peixes. Peixes expostos ao banho de curta duração de 125 mg $L^{-1}$ e 250 mg $L^{-1}$ $ClO_2$ apresentaram redução no parasitismo nas brânquias (62%). Pode ser sugerido o uso do dióxido de cloro na concentração de 25 mg $L^{-1}$ por até 48 h para reduzir mais do que 50% da infestação por *I. multifiliis*.

**Palavras chave**: peixe; tratamento; Protozoa; desinfetante; piscicultura

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## INTRODUCTION

The *Rhamdia quelen* has represented in 2000 approximately 1.4% of the fish produced by the Brazilian aquaculture sector (BOMBARDELLI *et al*., 2006). The production of this species in Santa Catarina, southern Brazil, has been stimulated since 2006 by the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI). However, there are barriers to the establishment of the technological package of *R. quelen* farming, such as good quality food that meets the nutritional requirements, culture of single sex animals, and especially the effective treatments against *Ichthyophthirius multifiliis*, the causative agent of ichthyophthiriasis (white spot disease) in fish.

The control of parasitism caused by *I. multifiliis* has been a major challenge to boost the development of *R. quelen* farming. The damage caused by these mortalities cause economic losses (SHOEMAKER and KLESIUS, 2010).

In the last decade, research on this parasite has been carried out, mainly due to the intensification in crops that can cause imbalances in the breeding systems, therefore increasing the emergence of infectious and/or parasitic diseases (MORAES and MARTINS, 2004). Several studies have tested the use of different chemical substances to control the ichthyophthiriasis in various fish species. Due to widespread use, studies are needed on the changes in fish health, as well as the prevention of risks to the environment (KLEIN *et al.,* 2004).

Chlorine dioxide ($ClO_2$) is an oxidative chlorine disinfectant used in water treatment, and is safe for human use (BERG *et al*., 1980). It has been studied intensively showing its biocide action against viruses, bacteria, algae, zooplankton, fungi and protozoans and its ability to increase the shelf life of fish by reduced their bacterial load (JUNLI *et al*., 1997; KIM *et al*., 1999). Its advantage compared to other products is the permission to be used as a food additive for human consumption by the Food and Drug Administration (FDA, 1999).

Besides presenting a lower toxicity level compared to chlorinated products, the chlorine dioxide has a higher efficiency in various uses and even generates less toxic residue to the environment, which has suggested the substitution of chlorine for chlorine dioxide. This study evaluated the effectiveness *in vitro* of chlorine dioxide and *in vivo* to treat *R. quelen* parasitized by *I. multifiliis*.

## MATERIAL AND METHODS

To evaluate the effectiveness of $ClO_2$ as a disinfectant for fish infected with *I. multifilis*, both *in vivo* and *in vitro* studies have been performed. The *in vitro* assay for immobilizing the *I. multifiliis* theronts was performed to determine the lowest concentration able to stop the movement of the parasites (Test 1). After the *in vitro* assay, the *in vivo* experiments were conducted to determine doses for short and long time exposure of chemical agent feasible to use in fish.

### *Biological material*

A total of 300 fish were captured in commercial fish farming in the municipality of Pomerode, Santa Catarina State (Southern Brazil), and transported to Aquatic Organisms Health Laboratory (AQUOS) of Federal University of Santa Catarina (UFSC). Fish were acclimated for three days in tank of 500 L capacity, under aeration, controlled temperature, and a daily renewal of 30% of the water volume. This study has the approval of the Ethic Committee of Animal Use, CEUA/UFSC PP00870).

Fish were fed twice a day with a diet containing 45% of crude protein *ad libitum* during the experimental period. Water quality parameters were measured daily, such as dissolved oxygen (DO) and the temperature, with an oximeter (YSI 550A, YSI Incorporated); and the pH was determined with the pH meter (YSI pH 100, EcoSense).

To obtain the parasite *I. multifiliis*, parasitized silver catfish from fish farm in the municipality of Pomerode (SC) were collected. The method for obtaining the theronts from the fish mucus was according to XU *et al.* (2009). The visual control of the infestation was carried out with the elaboration of a protocol to determine and standardize the beginning of the experiments *in vivo*.

### *Chemical agent*

The chlorine dioxide ($ClO_2$) used in all the experimental steps was supplied by *Dioxide*

*Chemical Industry*®, with a concentration of 7% of the active principle, in a liquid stabilized formulation. For *in vitro* testing, the solutions used in different concentrations (10000 mg $L^{-1}$, 5000 mg $L^{-1}$, 2500 mg $L^{-1}$, 1000 mg $L^{-1}$, 500 mg $L^{-1}$, 250 mg $L^{-1}$, 100 mg $L^{-1}$, 50 mg $L^{-1}$, 25 mg $L^{-1}$, 10 mg $L^{-1}$, 5 mg $L^{-1}$, 2.5 mg $L^{-1}$) were directly prepared by dilution in 20 µM phosphate buffer pH 7.0 solution to ensure the stability and action of the product.

*Immobilization assay of theronts*

For *in vitro* immobilization assay against theronts, 15 fish (mean ± standard deviation [SD] weight 10.1 ± 4.2 g and an average [± SD] length of 8.7 ± 5.8 cm) with signs of parasites were used. Fish were kept in an aquarium (50 x 25 x 40 $cm^3$) with a volume of 50 L, with constant aeration and controlled temperature at 24 °C. The process of obtaining theronts of *I. multifiliis* was according XU *et al.* (2009). Briefly, 15 parasitized fish were rapidly anesthetized with Eugenol 50 mg $L^{-1}$ for skin scraping and collection of the parasites with the aid of a glass slide. The content was filtered in a 150 µm sieve to remove skin and possible debris, placed in petri dishes (90 x 15 mm) so that the parasites would stick to the bottom of the dishes. After 1 h the water on the dish was exchanged, and the tomonts were incubated for 18 h at 24 °C, to get the theronts.

After incubation, the material was filtered again through a sieve of 52 µm to remove debris and mucus; the filtered material containing the theronts was counted in three 1 mL samples of the Sedgewick-Rafter chamber (Coleman). The concentration was adjusted to 1000 theronts $mL^{-1}$; then they were used for the *in vitro* immobilization assay. The immobilization assay was performed in 96 flat-bottom wells and 50 µL of the different $ClO_2$ solutions (10000 mg $L^{-1}$, 5000 mg $L^{-1}$, 2500 mg $L^{-1}$, 1000 mg $L^{-1}$, 500 mg $L^{-1}$, 250 mg $L^{-1}$, 100 mg $L^{-1}$, 50 mg $L^{-1}$, 25 mg $L^{-1}$, 10 mg $L^{-1}$, 5 mg $L^{-1}$, 2.5 mg $L^{-1}$) were added to each well (MARTINS *et al.,* 2011). After, 50 µL of the theronts solution (1000 theronts $mL^{-1}$) was added to each well and then incubated at room temperature (25 °C) for 1 h. Only the water with the theronts was used as a positive control and the test was performed in triplicate. The immobilization was verified by observation of parasites in the wells of the microplates in the microscope at 40x magnification after 1 h.

*Determining the degree of infestation*

A protocol to visually control the infestation was prepared for the *in vivo* assay, similar to that used by LING *et al.* (2010) when assessing his results of infestation in *Carassius auratus*. During this experiment, on hundred fish were acquired from a fish farming that were contaminated with parasite. Fish that have one to five white spots on their body surface were considered degree I of infestation. Fish having six to 20 white spots were considered as degree II. Fish with more than 20 white spots were considered degree III.

After fish collection and on the 4$^{th}$ day after acclimation, the thermostats were turned off and ice water was added to decrease the temperature from 25.8 °C to 21 °C, in order to cause stress. The degree of infestation was monitored and accompanied for two days after the temperature was altered. All fish presented degree III of infestation.

*Parasitological analysis*

All fishes were euthanized by concussion for the *I. multifiliis* count, the body surface was scrapped, and the mucus and the gills collected and fixed in 70% alcohol. The parasites present in the samples were counted directly in a stereomicroscope.

*Long-term exposure of parasitized **Rhamdia quelen** to chlorine dioxide for 48 h - Test 1*

Ninety-six fish (11.6 ± 2.8 g and 11.1 ± 0.9 cm) parasitized by *I. multifiliis* were exposed to chlorine dioxide for 48 h using three concentrations defined in the toxicity assay to evaluate the effect of the $ClO_2$ oxidant. When all fish were in degree III, they were distributed in the experimental units (40 L), with the concentrations already adjusted in the water (0 mg $L^{-1}$, 25 mg $L^{-1}$, 35 mg $L^{-1}$ and 45 mg $L^{-1}$); eight fish were used for each one of the three replicates. The fish were observed daily and the water from each replicate was renewed by a corresponding solution to each treatment of chlorine dioxide after 24 and 48 h. The mucus and the gills were collected from two fish from each experimental unit after 48 h of exposure for the *I. multifiliis* count.

*Short-term exposure of parasitized **Rhamdia quelen** to chlorine dioxide for 1 h - Test 2*

To investigate the effect of a short exposure to chlorine dioxide on the parasitism by *I. multifiliis*

in silver catfish (5.9 ± 0.44 cm and 9.07 ± 0.18 g), it was necessary to test the exposure to the product in less time and a higher concentration, which is a common practice in fish farming (PAVANELLI *et al.,* 2008). The concentrations used were 0 mg $L^{-1}$, 25 mg $L^{-1}$, 125 mg $L^{-1}$ and 250 mg $L^{-1}$, depending on the results obtained in the *in vitro* tests. After temperature shock, all fish (n = 72) were in the degree III of infestation and then, they were exposed to the three different solutions (25 mg $L^{-1}$, 125 mg $L^{-1}$ and 250 mg $L^{-1}$) of chlorine dioxide in tanks with the volume of 25 L for 1 h. After bath, fish returned to the circular experimental units with a volume of 40 L, with six fish per tank in quadruplicate to observe possible mortalities and for posterior zoothecnical and parasitological evaluation.

*Statistical analysis*

All data and counts obtained in the immobilization assay and the *in vivo* tests were subjected to Bartlett's test to verify the homocedasticity. When the data did not present itself to be homoscedastic, were transformed to Log 10 and Log (x + 1). After the verification of the homocedasticity, the data of the parasitological parameters were analyzed using one-factor ANOVA, with a significance of 5%. When significant differences were found, the means were compared with the 5% probability Tukey test (ZAR *et al*., 2011).

## RESULTS

*Immobilization assay of theronts*

In the immobilization assay (*in vitro*), the theronts were used as a parameter to measure the parasitic efficacy of chlorine dioxide. It was considered a positive result when all theronts showed no movement and negative when more than 5 out of 50 theronts placed in the well showed movement. At the 50 mg $L^{-1}$ $ClO_2$ treatment, theronts were totally immobilized and at the 25 mg $L^{-1}$ concentration there were some theronts with movement and many with no movement. At the 10 mg $L^{-1}$ concentration all of the theronts had normal movement as well as in control (0 mg $L^{-1}$).

*Long-term exposure of parasitized Rhamdia quelen to chlorine dioxide for 48 h - Test 1*

No mortalities were observed during the exposition and the fish collected after 48 h exposure had an average weight (± SD) and length (± SD) of 11.6 ± 2.89 g and 11.1 ± 0.99 cm respectively, and there was no difference between the weight of the fish ($P$ = 0.295). The fish treated with 250 mg $L^{-1}$ $ClO_2$ ($P$ = 0.037) differed from other treatments.

The water quality parameters during the long-term exposure did not differ ($P$>0.05) significantly among the groups tested. Dissolved oxygen was kept in 7.34 ± 0.30 mg $L^{-1}$, pH in 7.28 ± 0.05 and water temperature in 24.6 ± 0.38 °C, suitable for fish species according to BRAUN *et al.* (2006).

The count of *I. multifiliis* in mucus (Figure 1) showed that the infestation was really high with mean intensity of the 2,624.6 ± 1,167.9 theronts, but there was no difference between the treated and untreated fish ($P$ = 0.499).

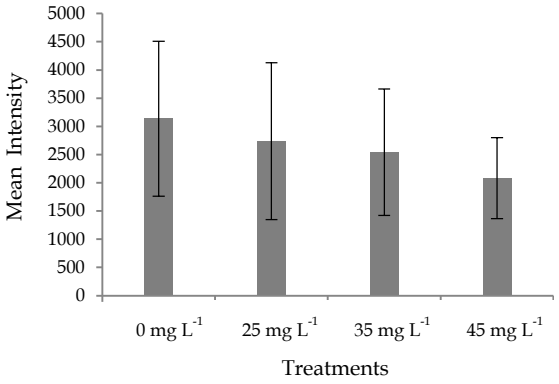


**Figure 1.** Mean intensity of infestation by *Ichthyophthirius multifiliis* in the mucus of *Rhamdia quelen* treated with chlorine dioxide at 0 mg $L^{-1}$, 25 mg $L^{-1}$, 35 mg $L^{-1}$ and 45 mg $L^{-1}$ for 48 h.

The *I. multifiliis* infestation in the gills resulted in differences among the 0 mg $L^{-1}$, 25 mg $L^{-1}$, 35 mg $L^{-1}$ and 45 mg $L^{-1}$ treatments (Figure 2). The concentrations tested caused a reduction up to 62% (25 mg $L^{-1}$ $ClO_2$) of theronts. However, no difference among the different concentrations utilized was observed, indicating that the lowest concentration (25 mg $L^{-1}$) of chlorine dioxide caused minor damage to fish tissue.

*Short-term exposure of parasitized Rhamdia quelen to chlorine dioxide for 1 h - Test 2*

In short-term treatment, *I. multifiliis* infestation in the gills showed a decrease in fish exposed for

1 h (Figure 3). Untreated fish had an average infestation of approximately 234 ± 73.1 theronts. However, the infestation decreased when the concentration of the product increased, resulting in an inversely proportional relationship. Fish treated with 250 mg $L^{-1}$ chlorine dioxide for 1 h showed greater efficacy, reducing more than 50% of infestation.

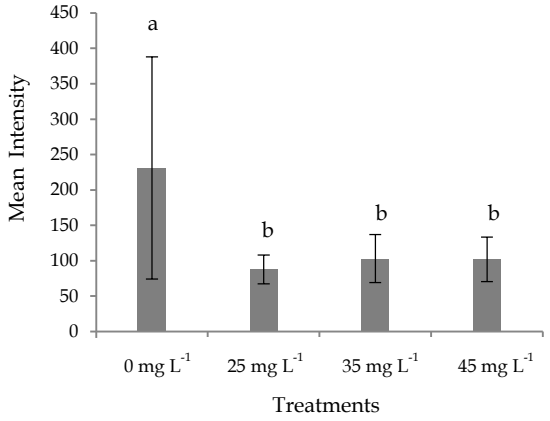


**Figure 2.** Mean intensity of infestation by *Ichthyophthirius multifiliis* in the gills of *Rhamdia quelen* treated with chlorine dioxide at 0 mg $L^{-1}$, 25 mg $L^{-1}$, 35 mg $L^{-1}$ and 45 mg $L^{-1}$ for 48 h. Different letters indicate significant difference by Tukey test ($P$ = 0.002).

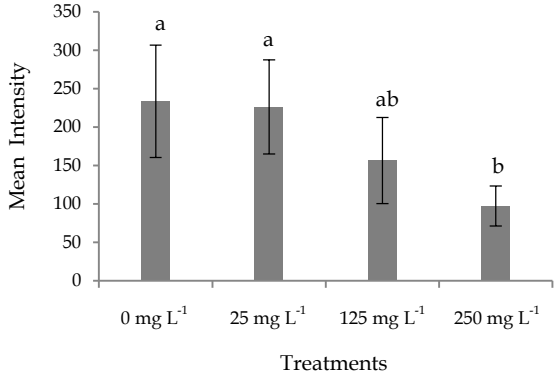


**Figure 3.** Mean intensity of infestation by *Ichthyophthirius multifiliis* in the gills of *Rhamdia quelen* after 1 h baths with chlorine dioxide at 0 mg $L^{-1}$, 25 mg $L^{-1}$, 125 mg $L^{-1}$ and 250 mg $L^{-1}$. Different letters indicate significant difference by Tukey test ($P$ = 0.001).

Water quality during short-term exposure did not differ among treatments ($P$>0.05). Dissolved was kept in 7.98 ± 0.38 mg $L^{-1}$, pH in 7.17 ± 0.17 and water temperature 24.82 ± 0.79 °C.

## DISCUSSION

This *in vitro* assay was first reported for chlorine dioxide in the world. However, when this test was performed with sodium percarbonate (a strong oxidant and disinfectant) after 1 h and at a concentration of 512 mg $L^{-1}$, the death of all theronts was recorded (HEINECKE and BUCHMANN, 2009). Therefore the chlorine dioxide caused theronts immobilization in a lower concentration than other products.

The highest mean intensity observed in this study was similar to that found by BORBA *et al.* (2007) also in *R. quelen* (about 400 cysts per fish). However, fish treated with 1% salt (NaCl) and 250 mg $L^{-1}$ formaldehyde did not show efficacy against *I. multifiliis* parasite of surubim catfish *Pseudoplatystoma* spp. (RODRIGUES e CAMPOS, 2011). The reduction of up to 62% (25 mg $L^{-1}$ $ClO_2$ for 48 h) of infestation in gills was similar to that obtained by KLEIN *et al.,* (2004) in *Steindachneridion* sp. parasitized by *I. multifiliis* when exposed to chemicals commonly used in aquaculture.

KLEIN *et al.* (2004) have also related a reduction of approximately 50% of *I. multifiliis* in fish after 1 h baths with different chemical substances used. Further reduction of infestation (49.5%) was achieved after treatment with 25 mg $L^{-1}$ formaldehyde for 7 days followed by a 47.2% parasite reduction after treatment with 250 mg $L^{-1}$ for 1 h.

Decreased of the mean intensity of *I. multifiliis* in the gills of *R. quelen* treated with chlorine dioxide for 1 h revealed satisfactory results similar to those found by YAMABE and YOSHIDA (1990). These authors found reduced parasitism in *C. auratus* treated with 15 and 30 mg $L^{-1}$ chlorine dioxide, reaching the cure of fish seven days after treatment. However, YAMABE and YOSHIDA (1990) did not studied a short-term exposure in those fish.

## CONCLUSIONS

Chlorine dioxide was effective to immobilize *I. multifiliis* theronts at the lowest concentration (25 mg $L^{-1}$ of $ClO_2$) *in vitro*. The same concentration in long-term exposure for 48 h reduced more than 50% of *I. multifiliis* infestation in the gills of *R. quelen*.

After short-term exposure at 125 mg $L^{-1}$ and 250 mg $L^{-1}$ $ClO_2$ chlorine dioxide for 1 h reduced up to 62% parasitism in fish gills. It can be suggested a treatment with chlorine dioxide at 25 mg $L^{-1}$ for up to 48 h to reduce more than 50% *I. multifiliis* infestation. We also recommend for future studies the use of fish in degree II of infestation due to rapid evolution of disease causing the death of the host in short time.

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## REFERENCES

BERG, J.D.; AIETA, E.M.; ROBERTS, P.V. 1980 Comparison of viral and bacterial indicators of disinfection in wastewater with chlorine dioxide and chlorine. *Water Chemical. Environmental Impact and Healthy Effect, 3*: 7-11.

BORBA, M.R.D.; FRACALOSSI, D.M.; FREITAS, F.A. 2007 Efeito da suplementação de vitamina C na dieta sobre asusceptibilidade de alevinos de jundiá *Rhamdia quelen*, ao *Ichthyophthirius multifiliis*. *Acta Scientiarum Animal Sciences*, *29*(1): 93-99.

BOMBARDELLI, R.A.; MÖRSCHBÄCHER, E.F.; CAMPAGNOLO, R.; SANCHES, E.A.; SYPERRECK, M.A. 2006 Dose inseminante para fertilização artificial de ovócitos de jundiá cinza, *Rhamdia quelen* (Quoy & Gaimard, 1824). *Revista Brasileira de Zootecnia*, *35*(4): 1251-1257.

BRAUN, N.; DE LIMA, R.L.; MORAES, B.; LORO, V.L.; BALDISSEROTTO, B. 2006 Survival, growth and biochemical parameters of silver catfish, *Rhamdia quelen* (Quoy & Gaimard, 1824), juveniles exposed to different dissolved oxygen levels. *Aquaculture Research*, *37*(15): 1524-1531.

HEINECKE, R.D. and BUCHMANN, K. 2009 Control of *Ichthyophthirius multifiliis* using a combination of water filtration and sodium percarbonate: Dose-response studies. *Aquaculture*, *288*(1-2): 32-35.

FDA - FOOD AND DRUG ADMINISTRATION. 1999 CFR-code of federal regulations title 21. In: *Current good manufacturing practice for finished pharmaceuticals.* U.S. Food and Drug Administration: Washington, DC. 211p.

JUNLI, H.; LI, W.; NENQI, R.; LI, L.X.; FUN, S.R.; GUANLE, Y. 1997 Disinfection effect of chlorine dioxide on viruses, algae and animal planktons in water. *Water Research*, *31*(3): 455-460.

LING, F.; WANG, J.G.; LIU, Q.F.; LI, M.; YE, L.T.; GONG, X.N. 2010 Prevention of *Ichthyophthirius multifiliis* infestation in goldfish (*Carassius auratus*) by potassium ferrate (VI) treatment. *Veterinary Parasitology*, *168*(3-4): 212-216.

MARTINS, M.L.; XU, D.H.; SHOEMAKER, C.A.; KLESIUS, P.H. 2011 Temperature effects on immune response and hematological parameters of channel catfish *Ictalurus punctatus* vaccinated with live theronts of *Ichthyophthirius multifiliis*. *Fish & Shellfish Immunology*, *31*(6): 774-780.

MORAES, F.R. and MARTINS, M.L. 2004 Condições predisponentes e principais enfermidades de teleósteos em piscicultura intensiva. In: CYRINO, J.E.P.; URBINATI, E.C.; FRACALOSSI, D.M.; CASTAGNOLLI, N. (eds) *Tópicos especiais em piscicultura de água doce tropical intensiva. São Paulo:* TecArt, p.343-383.

KIM, J.; HUANG, T.S.; MARSHALL, M.; WEI, C.I. 1999 Chlorine dioxide treatment of seafoods to reduce bacterial loads. *Journal of Food Science*, *64*(6): 1089-1093.

KLEIN, S.; FEIDEN, A.; BOSCOLO, W. R.; REIDEL, A.; SIGNOR, A. 2004 Utilização de produtos químicos no controle de *Ichthyophthirius multifiliis* Fouquet (1876) em alevinos de surubim do Iguaçu *Steindachneridion* sp. Garavello (1991). *Semina: Ciências Agrárias*, *25*(1): 51-58.

PAVANELLI, G.C.; EIRAS, J.D.C.; TAKEMOTO, R.M. 2008 Protozoa. In: PAVANELLI, G.C.; EIRAS, J.D.C.; TAKEMOTO, R.M. (eds) *Doenças de peixes: profilaxia, diagnóstico e tratamento*, 3ª ed., Eduem: Maringá. 311p.

RODRIGUES, R.A. and CAMPOS, C.F.M. 2011 Uso do sal comum no controle de *Ichthyophthirius multifillis* em juvenis de *Pseudoplatystoma* spp. In: ENCONTRO DE INICIAÇÃO CIENTIFÍCA DA

UNIVERSIDADE ESTADUAL DE MATO GROSSO DO SUL, 12., Mato Grosso do Sul, 17/nov./2011. *Anais*.... Mato Grosso do Sul. Resumo número 3.

SHOEMAKER, C.A.K. and KLESIUS, P. 2010 *Keeping Aquaculture Fish Health.* Agricultural Research Report 58. U.S. Department of Agriculture: Beltsville, MD; p.11-13.

XU, D.; KLESIUS, P.; SHOEMAKER, C. 2009 Effect of immunization of channel catfish with inactivated trophonts on serum and cutaneous antibody titers and survival against *Ichthyophthirius multifiliis*. *Fish & Shellfish Immunology*, *26*(4): 614-618.

YAMABE, A. and YOSHIDA, R. 1990 Curative and preventive method for aquarium fish. Patent US 4946690 A.

ZAR, J.H. 2011. *Biostatical Analysis with MyMathLab Student Access Kit (ad Hoc for Valuepacks)***.** In: Pearson College Division, 944p.