TRACE ELEMENTS AND PARASITISM IN NILE TILAPIA FARMED IN THE SOUTHERN BRAZIL

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ABSTRACT

This study evaluated the trace elements and parasitological indices in Nile tilapia examined from two different facilities, named as swine-consorted, using pig manure, and monoculture. For trace element analysis, the fish muscle tissue was collected individually in each facility. Each portion was weighed, dried in a stove at 60°C for 48 h and analyzed by Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF). Parasitological analysis followed the routine method for parasites collection, quantification and identification. The predominant element was zinc (Zn) followed by iron (Fe) and arsenic (As) in fish from both facilities. Fish from swine-consorted system had greater values of abundance and mean intensity of trichodinids and fish from monoculture showed higher abundance and mean intensity of monogeneans. Nevertheless, no signs of damage to fish production was observed. The contents of the elements Zn, Fe, As, cooper (Cu) and mercury (Hg) were above of the maximum permitted limits recommended by the Brazilian legislation.

Keywords: fish farming; *Trichodina*; Monogenea; metals; bioaccumulation; limits.

ELEMENTOS TRAÇO E PARASITISMO EM TILÁPIA DO NILO CULTIVADA NO SUL DO BRASIL

RESUMO

Este estudo avaliou os elementos traço e os índices parasitológicos em tilápias do Nilo coletadas de duas diferentes propriedades denominadas consorciada com suínos, utilizando dejetos de suínos, e monocultivo. Para as análises de elementos traço, o músculo dos peixes foi coletado individualmente em cada propriedade. Cada porção foi pesada, seca em estufa a 60°C por 48 h e analisada por Fluorescência de Raios X por Dispersão em Energia (EDXRF). Análises parasitológicas seguiram método de rotina para coleta, quantificação e identificação de parasitos. Os elementos predominantes foram zinco (Zn) seguido do ferro (Fe) e arsênico (As) nos peixes de ambas propriedades. Os peixes consorciados com suínos tiveram maiores valores de abundância e intensidade media de tricodinídeos e os do monocultivo maior abundância e intensidade média de monogenea. Não obstante, nenhum sinal de danos à produção foi observado. Os níveis dos elementos Zn, Fe, As, cobre (Cu) e mercúrio (Hg) foram acima do máximo permitido recomendado pela legislação brasileira.

Palavras-chave: piscicultura; *Trichodina*; Monogenea; metais; bioacumulação; limites.

Artigo Científico: Recebido em 23/05/2015 - Aprovado em 26/07/2016

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INTRODUCTION

In the last decades, the presence of trace elements has been intensely investigated (BARBIERI *et al*., 2010; GUÉRIN *et al.*, 2011), as it causes serious damage to human health and other organisms (DAVID *et al.,* 2010). Fish are excellent source of protein for human feeding (BRÁZOVÁ *et al*., 2012) and trend to concentrate trace elements on their bodies (TAWEEL *et al.*, 2013). As they are possible source of contamination by toxic elements (LING *et al*., 2013), researchs to evaluate the biocumulation in the fillet and organs has been highlighted (YOUNIS *et al.*, 2013).

Due to non distinction between metal and non-metal (GUILHERME *et al.*, 2005), trace elements are classified as essentials, non-essentials and toxics. Essentials are those that even in low concentrations play an important role in the organism such as iodine (I), zinc (Zn), selenium (Se), cooper (Cu), molibdenum (Mo), iron (Fe) and chrome (Cr). The non-essentials are those with no biological function like manganese (Mn), silicon (Si), nickel (Ni), boron (B), vanadium (V); and the toxics are thoose biologicaly non-essentials and considered highly toxic even in low concentrations and also present cummulative potential for humans and animals as plumb (Pb), cadmiun (Cd), mercury (Hg), arsenic (As), aluminum (Al), lithium (Li) and tin (Sn) (OMS, 1998).

Trace elements are found in the environment as consequence of natural and antropogenic processes (LOW *et al.*, 2015), and are susceptible to environmental and seasonal variations as a result of artificial and natural contamination (YOUNIS *et al.*, 2014). Despite the fact that they are not degradable they can accumulate in the ecosystem components in which reveal their toxicity (BAIRD, 2002). Bioaccumulation is a natural process (KEBEDE and WONDIMU, 2004) and trace elements are absorbed by the fish from the gills and skin, food and water particles (ALQUEZAR *et al.*, 2008). The most important inorganic elements in fish diet are macrominerals (Ca, P, Mg, Na, K, Cl) and microminerals (Fe, Cu, I, Mn, Zn, Se) (LOVELL, 1998). Macrominerals are needed in great amounts and in contrary, the microminerals are biologically needed in lower concentrations (WATANABE *et al.*, 1997) being present necessarily in formulated feed for fish. After ingestion and/or absorption these elements are transfered to the gills or intestine to the circulating blood and then distributed to other organs (HOGSTRAND and HAUX, 1991). On this view, fish are used as biological indicators to evaluate the ecological risks either of essential trace elements or non-essential (KEBEDE and WOONDIMU, 2004).

Intensive aquaculture has experienced economical losses leading in some cases to disease outbreaks mostly associated with inadequate management of farmed fish, feed and water being responsible for parasitic or secondary bacterial infections (MARTINS *et al.*, 2015). On the other hand, parasitic diseases have become more frequent in the culture system with inestimable economic losses (PAVANELLI *et al.*, 2013) and mortality outbreaks (JOHANSEN *et al.*, 2011) as a result of decreased susceptibility of fish (BOWDEN *et al.*, 2007). Specific information on the relationship host-parasite-environment constitutes important strategy in controlling fish diseases and implementation of prophylactic methods (AKOLL *et al.*, 2012).

The most common parasites found in the Brazilian tilapia culture (*Oreochomis niloticus*) are the Monogenea helminthes (VARGAS *et al.*, 2000; LIZAMA *et al.*, 2007; ZAGO *et al.*, 2014), ciliated protozoan trichodinids (VARGAS *et al.*, 2000; AZEVEDO *et al.*, 2006; MARTINS *et al.*, 2010; JERÔNIMO *et al.*, 2011; PANTOJA *et al.*, 2012; VALLADÃO *et al.*, 2013) and the dinoflagellate *Piscinoodinium pillulare* (MARTINS *et al.*, 2001; JERÔNIMO *et al.*, 2011; ZAGO *et al.*, 2014).

This study aimed to evaluate the trace elements in the muscle and parasitic levels in Nile tilapia farmed in two facilities using swine-consorted and semi-intensive production systems in southern Brazil.

MATERIAL AND METHODS

Two facilities were selected for samples collection, one that possesses a fattening fish area and termination of swines disposed in small stalls above the ponds named “swine-consorted” and the other with the traditional culture system named “monoculture” (Table 1). Swine-consorted facility uses the solid portion of pig manure daily at 5-10% of the fish biomass in the ponds as mulch to increase the primary productivity of water. The biomass is based on the averages of the fish sampling and calculated from 80-90% of total harvested fingerlings. Both facilities are situated in a rural zone of Vale do Braço do Norte, southern region of the State of Santa Catarina, Brazil (28°16'33"S, 49°09'56"W), and use the river water as source of supply for ponds. The rivers Braço do Norte and São Bernardo supply the swine-consorted and monoculture facilities, respectively. The surrounding activities are composed mainly by agriculture, pig breeding and milk cattle farming responsible for generate residues that can affect the fish farming.

Table 1. Characteristics of the facilities studied in the Southern Brazil according to the farming system.

Characteristics	System	
	Swine-consorted	Monoculture
Water line (m^2)	7,650	4,860
Ponds depth (m^3)	1.5	1.5
Culture system	Semi-intensive	Semi-intensive
Stocking density (fish/m^2)	3.53	4.11
Feeding	Twice a day	Twice a day
Aeration	1 HP/4 ton.	1 HP/4 ton.
Water source	River	River
Water renewal	Yes	Yes
Customer market	Warehouse of fish	Warehouse of fish
Drying of ponds between cycles	Yes	Yes
Source of fingerlings	Commercial hatchery	Commercial hatchery

A total of 60 Nile tilapia from each facility were examined. The fingerlings were harvested in the ponds in March 2014 for sampling in July 2014. Animal management procedures were approved bu the Ethic Committe on Animal Use (CEUA-

UFSC PP00801). In the sampling days, the water temperature and dissolved oxygen were measured with an oxymeter Hannah HI 9146, transparency with Secchi disc, and pH, ammonia, alkalinity, nitrite, nitrate, sulphate, iron, orthophosphate, silica, phenol and aluminum with kit Alfakit.

Fish were collected with nets, quickly anesthetized in a clove oil solution (75 $mg.L^{-1}$) to posterior scraping of the body surface mucus. Part of this mucus was used to make slides for trichodinid examination and the other fixed in formalin 5%. After that, the fish were measured, weighed and transported to the Laboratory of Diagnosis in Aquaculture (LADA) of Santa Catarina State Agricultural Research and Rural Extension Agency (EPAGRI), municipality of Tubarão (SC) for collection of muscle and internal organs according to JERÔNIMO *et al.* (2011) method.

Protozoan quantification was performed by sampling three aliquots of 1 mL of the mucus and gills contents, counting the number of protozoan on the aliquot using a Sedgwick-Rafter chamber and estimating the number of possible protozoan by the total homogenate content (JERÔNIMO *et al.*, 2011). The monogenean helminthes were counted in Petri dishes in stereomicroscope (GHIRALDELLI *et al.*, 2006). From these data were calculated the prevalence, mean abundance and mean intensity of infection (BUSH *et al.*, 1997).

For trichodinid identification, the air-dried slides were impregnated with silver nitrate 2% using Klein's method to posterior measurements and identification (LOM, 1958; VAN AS and BASSON, 1989). Monogeneans were mounted in Hoyer ´s medium for clarification and identification of the attachment and reproductive structures (PAPERNA and THURSTON, 1969; ERGENS *et al.*, 1981; DOUËLLOU, 1993; PARISELLE and EUZET, 1995).

For trace elements analysis, a portion of dorsal muscle of each fish was collected in a microcentrifuge tubes, identified individually and maintained at -18°C. After defrosting the material was stove-dried at 60°C for 48 h and measured in a precision scale with three decimal places. The material was then macerated and stove-dryed for the second time at the same temperature and time. After drying the material was weighed and kept in glass vials for sending to the Laboratory of Tropical Biology of the Institute of Techonology, Aracaju (SE), Brazil, for analysis.

The samples were previously sieved in 0.088 mm mesh sieve to obtain samples between 0.5 and 0.6 g and kept in a cylinder with 3.0 g of boric acid (H_3BO_3 p.a.), pressed for 40 s with the aid of 20 ton. power press in order to obtain double layer tablets (shell sample and boric acid) with 30 mm diameter. The tablets were maintained in a stove at 60^{o}C for 48 h for drying and analyzed by the Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF) (FERREIRA *et al.*, 2012). Analytical curves for determining the chemical elements of the shells were adjusted by linear regression and used the Fundamental Parameter method for matrix correction. This method allow to obtain the sensitivity curve by relating the calculated teoric fluorescence intensity and the measurement of each element to determine the composition of the analyzed material (BONA *et al.*, 2007).

The precision of the technique reading of trace elements in the muscle was verified using a Certified Reference Materials (CRM), certified by the Institute for Reference Materials and Measurements (IRMM), European Reference Materials (code ERM - BB422). It constitutes data for validation of sampling reading by the equipment and is used during the whole period of sample analysis. Each muscle sample was analyzed three times and the fourth sample was obtained from the average of three analysed samples using the equipment EDX – 720 (Shimadzu Corporation). Operating parameters of the spectrophotometer were: X-ray tubes of Rhodium, tension 15 KV; collimator of 10 mm; silicium detector; cooling with liquid nitrogen; measure time 100 s.

For statistical analysis the Kolmogorov-Smirnov (K.S.) test was firstly performed to verify the normality of the data. After that was used the U test of Mann-Whitney at 5% significance level ($\alpha = 0.05$) for comparison the values between the facilities and weight and length of fish.

RESULTS

Fish from semi-intensive conventional system showed significantly higher ($P<0.05$) total mean length (25.20±2.88 cm) and weight (327.93±116.68 g) than that found in those from swine-consorted system (18.37±6.19 cm and 169.60±145.80 g).

The Table 2 presents the trace elements found in the facilities, the maximum value permissible for human consumption by the resolutions 269/2005 (Daily Recomended Ingestion of protein, vitamins and minerals), and 42/2013 (Maximum Limits of Inorganic Contamination in Food) for As, Hg e Cd, beyond the ministerial order nº 2.914/2011 (Procedures for controlling and surveillance of the water quality for human consumption and its standard of drinking water, all todos regulated by the Brazilian Health Surveillance Agency (ANVISA) (ANVISA, 2005, 2011, 2013). These legislations regulate the maximum limit permissible in food in milligrams for an adult person after youth age, and in water in $mg.L^{-1}$ for human consumption. These regulated data by the ministerial orders were used to compare the present values found in the facilities. The concentration of Cd, even showing 1 $mg.Kg^{-1}$, must be disregarded because of the equipment reading provides this value even its absence.

There were identified three trichodinid species: *Trichodina compacta* Van As and Basson, 1989, *Trichodina magna* Van As and Basson, 1989 and *Trichodina centrostrigeata* Basson, Van As and Paperna, 1983 in fish from both facilities, the monogeneans *Cichlidogyrus sclerosus* Price and Kirk, 1967 and *Cichlidogyrus tilapiae* Paperna, 1960 in fish from both facilities and *Scutogyrus* sp. Pariselle and Euzet, 1995 only in fish from monoculture. The dinoflagellate *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981 was found in fish from both swine-consorted and monoculture systems with no difference between them. Fish from swine-consorted system showed higher ($P<0.05$) prevalence rate, mean abundance and mean intensity of infection by trichodinids than those maintained in monoculture system. On the other hand, higher values of mean abundance and mean intensity ($P<0.05$) of monogeneans were observed in fish from monoculture system (Table 3).

Table 2. Mean values and standard deviation of trace elements values (in $mg.Kg^{-1}$) of the samples, comparing the facilities and the maximum limit allowed for human consumption (MLHuman) and water (MLWater) by the Brazilian legislation.

Elements	Swine-consorted	Monoculture	MLHuman(1)	MLWater(2)
Zn	37.879 ± 0.590	31.457 ± 0.513	15.00	5.00
Fe	34.523 ± 0.511	23.468 ± 0.421	14.00	0.30
Cu	3.183 ± 0.142	2.817 ± 0.124	3.00	2.00
Se	2.684 ± 0.035	2.316 ± 0.031	70.00	0.01
I	2.107 ± 0.038	2.020 ± 0.036	150.00	-
Mn	1.029 ± 0.035	1.000 ± 0.036	5.00	0.10
As	25.433 ± 0.310	22.136 ± 0.274	1.00	0.01
Hg	1.197 ± 0.135	1.222 ± 0.128	0.50	0.001
Cd	1.000 ± 0.039	1.000 ± 0.039	0.05	0.005

(1) $mg.day^{-1}$; (2) $mg.L^{-1}$

Table 3. Parasitological indices (means±standard deviation and minimum and maximum values in parenthesis) in both Nile tilapia semi-intensive systems (swine-consorted and monoculture) in Southern Brazil.

Facilities	Trichodinids		
	Mean abundance	Mean intensity	Prevalence(%)
Swine-consorted	669.02 ± 1,036.84 (0.00-4335.67)*	771.94 ± 1,078.27 (1.00-4,335.67)*	86.67
Monoculture	45.61 ± 98.77 (0.00-578.33)	70.17 ± 115.66 (1.00-578.33)	65.00
	Monogenea		
Swine-consorted	1.60 ± 1.50 (0.00-5.00)	2.40 ± 1.19 (1.00-5.00)	66.67
Monoculture	23.98 ± 19.90 (1.00-87.00)*	23.98 ± 19.90 (1.00-87.00)*	100.00
	Piscinoodinium pillulare		
Swine-consorted	0.73 ± 5.68 (0.00-44.00)	44.00 ± 0.00 (44.00-44.00)	1.67
Monoculture	0.24 ± 1.32 (0.00-7.30)	7.30 ± 0.24 (7.30-7.30)	3.33

*Significant difference between facilities by the U test of Mann-Whitney

DISCUSSION

No significant difference was found in trace elements values between the facilities. Nevertheless, Zn showed the highest concentration followed by Fe and As, being these last above of the permitted limit as well as Hg levels. Other elements like Mn, I and Se were found to be below the maximum permitted limit.

In this study, Zn concentration in fish varied from 37.879 $mg.Kg^{-1}$ (swine-consorted) to 31.457 $mg.Kg^{-1}$ (monoculture) higher than that found in farming conditions (LIN *et al.*, 2005; ALLISON *et al.*, 2009) and lower than that related for natural marine environment (CHEUNG *et al.*, 2008; YILMÁZ *et al.*, 2010). On the other hand, in wild marine fish were reported values between 46.20 and 49.39 $mg.Kg^{-1}$ (IRWANDI and FARIDA, 2009; DURALI *et al.*, 2010). WONG *et al.* (1984) have studied pig manure mixed to fish diet at 10% and 40% and observed 151.52 and 196.78 $mg.Kg^{-1}$ higher than that found in swine-consorted fish from this study.

Animal manure contains high levels of trace elements especially Cu and Zn (WONG *et al.,* 1984), being this last one provided from the pig diet to balance the Cu that is used as growth promotor. This balance and the relationship between the plasmatic levels of Zn and Cu in pigs was explained by HAUSCHILD *et al.* (2008) who demonstrated a negative effect of ingested Zn on the Cu metabolism and positive relation with high levels released from the animal dejects. The absorption of Zn and Cu is regulated via hepatic by the metallothionein which is responsible for the maintenance of the mineral homeostasis but presents lower chemical affinity with Cu, thereby elevated Zn ingestion could damage the Cu transport to the circulating blood (COUSINS *et al.*, 1985). Although no significant difference, it can be suggested that increased levels of Zn and Cu in fish from swine-consorted system compared to monoculture system was a result of their use as mulch in ponds.

Concentrations of Fe found in this study (34.524 mg.Kg^{-1} in swine-consorted fish and 23.468 mg.Kg^{-1} in monoculture fish) were higher than that related by ALLINSON *et al.*·(2009) (1.9 and 8.9 mg.Kg^{-1}) in Nile tilapia from reservoir in Sri Lanka and lower than that related in tilapia exposed to pig manure (598.17 and 2,255.92 mg.Kg^{-1}) (WONG *et al.*, 1984). Previous study at the same hydrographic basin showed that water concentration of Fe is from the coal industry and was found to be higher than the maximum permitted limit (11.888 and 5.397 mg.L^{-1}) for water·(BRANDELERO *et al.*, 2013).

Higher concentration of Fe observed in fish from swine-consorted system might be related to an increase in the organic matter contents provided possibly from the pig manure, confirmed in previous study (COCATO *et al.*, 2008) where 80% of dietary Fe binds to hemoglobin after birth and the oral supplementation is an alternative to recompose the organic iron. This explains the accumulation of Fe in the pig manure and consequently bioaccumulation in the fish muscle.

Although in this study was not analyzed trace elements in the fish diet, it could be estimated by analyzing the micromineral and vitamin Premix used in the fish diet as mineral matter at 8%. From the elements registered in this study, Fe, Zn and Cu can be found at 13,820, 17,500 and 2,000 mg.kg^{-1} in fish diet respectively, and constitute a source of uptake by feeding.

The presence of As and Hg in fish muscle should be carefully considered once both elements belong to the category of highly toxic metals and even in low concentrations can accumulate in human and animal tisssue (OMS, 1998). However, there are non-toxic chemical forms of inorganic Hg (DUSEK *et al.*, 2005) and organic As (CULIOLI *et al.*, 2009). Hg detected on these samples were found to be above the permitted limit and deserves attention if present in organic form (methylmercury), hence more specific tests must be done for exact detection of chemical form of Hg due to its cumulative potential.

The formation of methylmercury occurs by the bacterial methylation of Hg increased by low dissolved oxygen levels (DUSEK *et al.*, 2005). Hg dissemination can be caused by mining activities or soil erosion containing natural mercury (ROULET and LUCOTTE, 1995). More than 97% of accumulated Hg in the basin of Tapajós river, Pará, Brazil has natural source (DA SILVA *et al.*, 2013). In the present study, the amounts of Hg could be related to natural source of contamination and not by anthropic action. Temporal conditions as for example low dissolved oxygen, flutuations in water pH or dissolved organic carbon also contribute to an increase in the Hg availability for farmed fish by increasing the methylation (DUSEK *et al.*, 2005). On the contrary of Hg, As in inorganic form (As^{+3} and As^{+5}) are more toxic and are associated with carcinogenic effects in humans (KAR *et al.*, 2011). In freshwater ecosystem, studies involving As are scarce and are mostly studied from marine environment (CULIOLI *et al.*, 2009).

In most cases, agriculture intensification is associated with the use of great amounts of fertilizers and pesticides containing metals resulting in environmental trace elements accumulation (QIU *et al.*, 2011). On the other hand, husbandry activity such as pig breeding and cattle farming are mostly common surrounding the studied facilities suggesting a source of contamination. Studies showed the importance of the Braço do Norte River that is affected by the mining, its dilution process due to the Basin of Tubarão River and consequently lower environmental critical values (BRANDELERO *et al.*, 2013). It must be highlighted that this river supplies the swine-consorted system herein studied.

Mean abundance and intensity of trichodinids were higher in swine-consorted fish corroborating previous studies in Nile tilapia farmed in this fish production system when compared to feefishing and traditional (monoculture) semi-intensive system (JERÔNIMO *et al.*, 2011). Similarly, trichodinids were more prevalent followed by monogeneans in both Nile tilapia and African catfish (*Clarias gariepinus*) cultured in fish farmings in the Lake Victoria, Uganda (AKOLL *et al.*, 2012). Studying the parasitic fauna of Nile tilapia from swine-consorted system and feefishing in the municipality of Nova Trento, SC, Southern Brazil, MARTINS *et al.* (2010) found trichodinids as the most prevalent parasites in fish from swine-consorted system characterizing their opportunistic presence. In fact, their proliferation is associated with poor water quality, total number of bacteria and ecological aspects of the host (MARTINS *et al.*, 2015).

Fish from swine-consorted system showed increased prevalence, mean abundance and intensity of trichodinids that might be directly related to high organic matter contents, as supported by JERÔNIMO *et al.* (2011) in Nile tilapia from swine-consorted system and OGUT and PALM (2005) in fish examined from polluted sites. They can be safely used as indicators of water quality and eutrophication level (JERÔNIMO *et al.*, 2011) confirming the present results where the highest trichodinid load was found in fish from ponds receiving pig manure mulch.

On the contrary, monogenean helminthes showed higher prevalence, mean abundance and intensity of infection in fish from monoculture system. Stocking density constitutes the key role for monogenean proliferation (AKOLL *et al.*, 2012) probably due to greater area of microenvironment (IBRAHIM, 2012). The stocking densities used in those facilities were 4.11 and 3.66 fish/m^2, respectively in monoculture and swine-consorted system. Moreover, fish from monoculture facility showed greater total length compared to those in swine-consorted system. Both high stocking density and greater length may have favored the proliferation of monogenean parasites. According to IBRAHIM (2012) larger fish offers more surface area to be parasitized explaining the present results.

This study showed the first register on trace elements in Nile tilapia farmed in Southern Brazil and concentrations above the permitted limit regulated by the European Union and Brazil. These results suggest that new studies must be carried out in the region to locate the portal of entry of these elements and their accumulation in the facilities and fish. It must be emphasized that the water can be a source of contamination (BRANDELERO *et al.* 2013) once the gills are one of the main organs for absorption especially in regions of industrial and antropogenic interferences.

In terms of parasitism, the prevalence and mean intensity of infection could be used for prophylactic measures implementation (AKOLL *et al.*, 2012) and fish farmers might be stimulated to use the fish diagnosis and adopt the sanitary management. Although parasitological indices were different between the facilities, they did not damage the fish production and the mean weight was within the expected (1 to 8 g.day^{-1}) during approximately 100 days of culture. However, certainly fish that do not suffer parasitism present more satisfactory weight gain, especially not suffer the stress caused by the presence of parasites. Furthermore, there is a possibility of occurrence of secondary infections by parasites, which apparently did not occur in this study fish.

CONCLUSIONS

In this study, the contents of the elements Zn, Fe, As, Cu and Hg in farmed Nile tilapia were above of the maximum permitted limits recommended by consumption. Low parasite levels herein observed did not compromise the fish production. However, this work can serve as a basis for implementing sanitary practices for preventing future outbreaks.

ACKNOWLEDGMENTS

The authors thank Nacional Council of Scientific and Technological Development (CNPq) for finantial support (CNPq 446072-2014-0) and grant to M.L. Martins (CNPq 305869-2014-8); Post-Doctoral scholarship (CNPq PDJ 506263/2013-4) to G.T. Jerônimo; Coordination for the Improvement of Higher Education Personnel (CAPES) for Master scholarship to G.C. Nunes; Santa Catarina State Agricultural Research and Rural Extension Agency (EPAGRI), Laboratory of Diagnostic in Aquaculture, Tubarão, SC for structures available.

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