Aquaculture 479 (2017) 38–44

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Antiparasitic efficacy and blood effects of formalin on *Arapaima gigas* (Pisces: Arapaimidae)

Sanny Maria Andrade-Porto[a], Elizabeth Gusmão Affonso[b], Daiani Kochhann[c], José Celso Oliveira Malta[b], Rosemary Roque[b], Eduardo Akifumi Ono[b], Cleusa Suzana Oliveira Araújo[e], Marcos Tavares-Dias[d,*]

[a] *Fisheries Science Department, Federal University of Amazonas (UFAM), Manaus, AM, Brazil*
[b] *National Institute for Research in the Amazon (INPA), Manaus, AM, Brazil*
[c] *State University Vale do Acaraú, Sobral, CE, Brazil*
[d] *Embrapa Amapá, Macapá, AP, Brazil*
[e] *State University of Amazonas (UEA), Manaus, AM, Brazil*

ARTICLE INFO

Keywords:
Physiology
Monogeneans
Parasites
Pirarucu
Residue
Treatment

ABSTRACT

This study evaluated the *in vitro* and *in vivo* antiparasitic efficacy of formalin against *Dawestrema cycloancistrium*, the effects on the physiological response of *Arapaima gigas* and the residual action on fish muscle after 96 h of exposure. As regards the *in vitro* assay, 0, 22, 44, 66, 88, 110, 330, 660 and 880 mg L^{-1} formalin were tested. After 1 h of exposure to 660 and 880 mg L^{-1} formalin, there was a 100% mortality of *D. cycloancistrium* as well as after 2 h of exposure at 330 and 110 mg L^{-1} and 3 h of exposure at 44, 66 and 88 mg L^{-1}. Concerning the *in vivo* test, when fish were exposed to formalin at 0, 220, 330, 440 and 550 mg L^{-1}, there was 100% survival at all concentrations and exposure times evaluated. Baths of 1 h with 440 and 550 mg L^{-1} formalin showed 93.3% and 99.3% efficacy respectively. However, the baths of 12 h with 55 and 66 mg L^{-1} formalin had the efficacy of 44.5% and 55.5% respectively. In 1 h baths with 220, 330, 440 and 550 mg L^{-1} formalin, hematocrit, hemoglobin, number of total erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, plasma glucose levels, cortisol, total proteins, chloride, calcium, sodium, potassium and magnesium of the fish presented no differences in relation to the control values. However, in baths of 12 h with 33, 44, 55 and 66 mg L^{-1} formalin, there was a decrease in hematocrit, plasma levels of calcium and chloride, and increased levels of glucose and cortisol, depending on the concentration of formalin used. In the fish muscle, the formalin residue decreased after 96 h in all concentrations and periods evaluated, returning to values close to the control ones. The results indicate that formalin had its efficacy successfully proved in the treatment against *D. cycloancistrium* at higher concentrations such as 440 and 550 mg L^{-1} formalin and shorter exposure time (1 h) without compromising fish homeostasis and consumer food safety.

Statement of relevance: The manuscript represents original research on use of formalin in vitro and in vivo for treating infection by monogenoidean *Dawestrema cycloancistrium in Arapaima gigas*, the giant fish from Amazon. In the fish, muscle the residue levels of formalin after exposure was also investigated. Formalin have efficacy in the treatment against *D. cycloancistrium* at higher concentrations (440 and 550 mg.L^{-1}) of formalin and shorter exposure time (1 h) and without compromising *A. gigas* homeostasis and consumer food safety.

1. Introduction

With the intensification of *Arapaima gigas* farming (pirarucu), diseases caused by ectoparasites are limiting factors for the development of the aquaculture activity of this fish, once it has caused epizootic outbreaks with losses of 80% during the nursery stage (Araújo et al., 2009a, 2009b; Marinho et al., 2013). Studies have shown that monogeneans *Dawestrema cycloancistrium* Price & Nowlin, 1967 and *Dawestrema cycloancistrioides* Kritsky, Boeger & Thatcher, 1985 are the main ones that cause diseases in *A. gigas* fish farmed in the Amazon (Araújo et al., 2009a, 2009b; Marinho et al., 2013). In addition to that, monogeneans can directly affect the zootechnical performance of the fish during the productive cycle, causing anemia, reduced growth and inappetence (Araújo et al., 2009a, 2009b; Marinho et al., 2013).

* Corresponding author at: Embrapa Amapá, Macapá, Rodovia Juscelino Kubitschek, km 5, 2600 Macapá, AP, Brazil.
E-mail address: marcos.tavares@embrapa.br (M. Tavares-Dias).

http://dx.doi.org/10.1016/j.aquaculture.2017.05.009
Received 16 February 2017; Received in revised form 7 May 2017; Accepted 8 May 2017
Available online 09 May 2017

In aquaculture, one of the ways to control and treat diseases so as to minimize economic losses has been the use of chemical products such as formalin (Yildiz and Pulatsu, 1999; Fajer-Ávila et al., 2003; Stephens et al., 2003; Sharp et al., 2004; Sitjà-Bobadilla et al., 2006; Fujimoto et al., 2006; Pahor-Filho et al., 2012; Paixão et al., 2013; Andrade-Porto et al., 2017). Nevertheless, formaldehyde has been classified by the International Agency for Research on Cancer (IARC) as a human carcinogen. Recently, epidemiological studies have suggested that the exposure to this chemical can lead to the development of human hematopoietic cancers, such as leukemia (Lai et al., 2016). Safe concentrations of formalin recommended as a chemotherapeutic drug in baths for *A. gigas* have not been established (Andrade-Porto et al., 2017), and it has thus been used incorrectly and empirically in the aquaculture of this important Amazonian fish. Beyond that, therapeutic concentrations are used without taking the adequate exposure time, toxicity, tissue changes and accumulated residue in the muscle of treated fish into consideration (Jung et al., 2001; Andrade-Porto et al., 2017), as well as changes in homeostasis of animals exposed to it. Therefore, studies on the efficacy of formalin for *A. gigas* as well as their physiological and residual effects on the muscle are required. With this in view, the present study is aimed at evaluating the efficacy of formalin against monogeneans, as well as its effects on the physiology and the residual action on *A. gigas* muscle.

2. Materials and methods

2.1. Fish

Juveniles of *Arapaima gigas* (n = 300), with an average weight of 38.9 ± 9.3 g and an average standard length of 15.8 ± 1.4 cm, were obtained from a commercial fish farm in the municipality of Nova Olinda do Norte, state of Amazonas. The fish were transported to the Laboratory of Physiology Applied to Fish Farming (LAFAP), the Experimental Aquaculture Station of the Coordination of Technology and Innovation (COTI) of the National Institute for Research in the Amazon (INPA), Manaus, state of Amazonas. These fish were acclimatized for 15 days.

This study was approved by the Ethics Comittee on Animal Research (CEUA), of Nilton Lins University (Protocol N° 001/2012).

2.2. In vitro test with formalin and D. cycloancistrium in the gills of A. gigas

To test the sensitivity of *D. cycloancistrium* to formalin (Sigma-Aldrich 37%), five naturally parasitized *A. gigas* fingerlings were used. The experimental design consisted of 9 treatments: 0, 22, 44, 66, 88, 110, 330, 660 and 880 mg L^{-1} formalin, with three replicates, totaling 27 branchial arches. Each branchial arch containing *D. cycloancistrium* was placed in Petri dishes with different concentrations of formalin. A visual field with 20 specimens of *D. cycloancistrium* was chosen for the analysis of the immobilization of the parasites in each branchial arch, using a stereomicroscope (Zeiss). Mortality and time of immobilization of the parasites were recorded every 15 min. The parasites were considered dead when they remained totally immobilized. The *in vitro* test was terminated when there was 100% mortality in the controls (Fajer-Ávila et al., 2003).

The *in vitro* test results were used to determine the average effective concentration or mean effective concentration (EC_{50}), characterized by the nominal concentration of the toxic agent causing an acute effect (lethality or immobility) at 50% of test organisms during the exposure period (Zagatto and Bertoletti, 2006), which was estimated by the Trimmed Spearmann-Karber method (Hamilton et al., 1977).

2.3. Prior analyses of infection by D. cycloancistrium in the gills of A. gigas

Ten *A. gigas* juveniles were euthanized by cerebral commotion for prior evaluation of parasitic rates by monogeneans before the therapeutic baths. Branchial arches were fixed in 5% formalin, used for parasite counting and, subsequently, for the determination of prevalence, mean intensity and mean abundance (Bush et al., 1997). The identification of monogeneans followed Thatcher's recommendations (Thatcher, 2006). A total of 2.377 *D. cycloancistrium* were found in the gill of the examined *A. gigas*, with a prevalence of 100%, mean intensity of 237.7 ± 37.6 and mean abundance of 237.7 ± 37.6. The remaining fish were subjected to food deprivation for 24 h prior to the start of therapeutic baths for gastrointestinal emptying.

2.4. In vivo tests with A. gigas exposed to formalin

The experimental design was a completely randomized block with five treatments and three replicates with five fish each. *In vivo* tests consisted of therapeutic baths of 1 h with 0, 220, 330, 440 and 550 mg L^{-1} formalin and therapeutic baths of 2 h with 0, 33, 44, 55 and 66 mg L^{-1} formalin. Seventy-five *A. gigas* fingerlings were used for the 1 h bath and 75 for the 2 h bath. Therapeutic baths were performed in 60 L glass aquaria, with a static water system and constant aeration. These concentrations were based on the lethal concentration ($CL_{50–96h}$) of 36.4 mg L^{-1} (Andrade-Porto et al., 2017).

After the short and long baths, in order to collect the gills, three fish of each replicate were used to evaluate the efficacy of the treatments. The branchial arches of the fish from each treatment were collected and fixed in 5% formalin for the parasite count. The prevalence and mean abundance were determined (Bush et al., 1997) and the efficacy of treatments *in vitro* and *in vivo* was calculated (Martins et al., 2001).

The water from each aquarium was filtered in qualitative Whatman filter papers n° 1, in order to visualize possible monogenean specimens that might have been detached from the gills. The filtered water and the filter itself were examined separately in Petri dishes under a stereoscopic microscope.

After the therapeutic baths, the remaining fish were kept for a recovery period of 96 h in 60 L aquaria, with clean water and constant aeration. At the end of this period, the parasitic load of the fish, the occurrence of mortality and the formalin residue in the fish muscle were evaluated.

2.5. Blood collection and determination of blood parameters in A. gigas exposed to formalin

Blood samples were collected from 3 fish within each replicate of the treatments, by puncture of the caudal vessel using EDTA-containing syringes (10%) and divided into two aliquots. The first aliquot was used to count total erythrocytes with the help of a Neubauer chamber, determination of the hematocrit by the microhematocrit method and hemoglobin concentration by means of the cyanometahemoglobin method. Based on these methods, the hematimetric indexes were calculated: Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC)

The second aliquot was centrifuged at 75G in order to obtain plasma. It was used in the analysis of total plasma proteins, glucose, cortisol and ions Na^+, K^+, Ca^{+2} and Cl^-. The total protein levels were determined by the modified biuret method and glucose by means of the enzymatic colorimetric method (glucose oxidase). Commercial kits were used for both of them (Humam, Brazil). Plasma ions such as Na^+, K^+ and Ca^{+2} were determined with an atomic absorption spectrophotometer (Perclin Elmer, Mod. 1100B). The plasma levels of Cl^- were determined by the colorimetric method using a commercial kit (Labtest®). Cortisol was determined by means of the ELISA method, using a commercial kit (EIA, Kit 55,050, Human®) and spectrophotometer reading with Espectra Max®, at a wavelength of 450 nm.

2.6. Water quality parameters

The levels of dissolved oxygen, pH, electrical conductivity, total ammonia, total alkalinity, nitrite and carbon dioxide in the aquaria water were determined by means of appropriate appliances for each purpose before and after the formalin therapeutic baths. The concentrations of Na^+, K +, Mg^{+2} and Ca^{+2} ions were determined by means of an atomic absorption spectrophotometer (Perclin Elmer (AAS), Mod. 1100B).

2.7. Determination of formalin concentration in the water of aquaria and in the muscles of A. gigas

The determination of the levels of formalin in the water and in the *A. gigas* muscle exposed to formalin was performed. The concentration of formalin in the water and tissue was determined by NaOH and $ZnSO_47H_20$ and further reading by the method of 4-ami-3-hydrazine-5-mecapto-12.4-triazole (Jung et al., 2001). For the determination of formalin accumulation in the exposed fish muscle, a total of eight fish from each concentration of therapeutic baths were used.

2.8. Statistical analyses

All data were previously evaluated on the assumptions of normality and homoscedasticity using Shapiro-Wilk and Bartlett respectively. For the data with normal distribution, the analysis of variance was used (ANOVA - One Way), followed by the Tukey test for comparison between means. For data that did not follow this distribution pattern, Kruskal-Wallis was used, followed by the Dunn's test for comparison between means (Zar, 2010).

3. Results

After 1 h of formalin exposure, none of the physical and chemical variables in aquaria water presented differences ($p > 0.05$) when compared to controls (Table 1). After 12 h of formalin exposure, there was an increase in electrical conductivity and ammonia levels in the aquaria with 44, 55 e 66 mg L^{-1} formalin in relation to the controls (Table 2).

After 1 h of exposure, *in vitro*, to 660 and 880 mg L^{-1} formalin, there was 100% efficacy against *D. cycloancistrium*. After 2 h of exposure, 100% efficacy was observed against *D. cycloancistrium* when using 110 and 330 mg L^{-1} formalin, but at the lowest concentrations of formalin, 100% of monogeneans mortality occurred only within 3 to 4 h of exposure. In the control group, mortality started after 4 h of the assay (Fig. 1). The mean effective concentration (50CE) for *D. cycloancistrium* at 1 h and 2 h of exposure to formalin was of 115.3 (91.7–144.9) mg L^{-1} and 54.2 (46.9–62.7) mg L^{-1}, respectively.

There was a high reduction in the abundance of *D. cycloancistrium* after 1 h of exposure to 440 and 550 mg L^{-1} formalin. There was no efficacy against *D. cycloancistrium* at low concentrations of formalin during 12 h baths (Table 3). After the 96 h recovery period of the fish exposed to formalin, there was a reduction in the abundance of *D. cycloancistrium* for the ones in the 1 h baths with 440 and 550 mg L^{-1}, which presented higher efficacy (Table 4).

One-hour baths with different concentrations of formalin did not cause changes in fish blood parameters (Table 5). In fish from the 12 h baths with 55 e 66 mg L^{-1} formalin, glucose levels increased and chloride levels decreased, cortisol levels also increased in those exposed to 44, 55 and 66 mg L^{-1} formalin and calcium levels decreased when compared to controls. Furthermore, there was a reduction in the hematocrit of fish exposed to 33, 44 and 55 mg L^{-1} formalin (Table 6).

Formalin concentrations in the *A. gigas* muscle were higher in fish exposed to 440 and 550 mg L^{-1} formalin, after 1 h baths. In the 12 h baths, formalin concentrations in the *A. gigas* muscle were higher in fish exposed to 44, 55 and 66 mg L^{-1} formalin. In addition to that, there was a reduction in formalin concentrations in the fish muscle after the 96 h recovery period, in the 1 h and 12 h formalin baths (Table 7).

The concentrations of formalin residue in aquaria water after the 1 and 12 h therapeutic baths showed no alteration between the different concentrations used (Table 8).

4. Discussion

The aquaria water quality where *A. gigas* were exposed to formalin remained within the limits considered acceptable for them when they are farmed in the Amazon (Rebelatto-Junior et al., 2015). However, during the 12 h formalin baths, electrical conductivity and total ammonia levels increased in fish exposed to 44, 55 e 66 mg L^{-1}. In contrast, other studies reported that the use of formalin caused increased pH and reduced concentration of dissolved O_2 in the water (Treves-Brown, 2000; Martins, 2004). These changes were not found here, corroborating the findings of other studies (Fajer-Ávila et al., 2003; Sitjà-Bobadilla et al., 2006; Pahor-Filho et al., 2012; Paixão et al., 2013).

In the *in vitro* tests with *D. cycloancistrium*, from the gills of *A.gigas,* there was 100% efficacy with 660 and 880 mg L^{-1} formalin after 1 h of exposure and 90% efficacy with 330 mg L^{-1} formalin after 2 h of exposure. The mean effective concentration of *D. cycloancistrium* was 115.3 mg L^{-1} formalin after 1 h of exposure and 54.2 mg L^{-1} after 2 h

Table 1
Physical and chemical parameters of water after 1 h of exposure of *Arapaima gigas* to different concentrations of formalin.

Parameters	Concentrations of formalin (mg L^{-1})				
	0	220	330	440	550
OD (mg L^{-1})	7.5 ± 0.04[a]	7.4 ± 0.05[a]	7.4 ± 0.1[a]	7.5 ± 0.03[a]	7.4 ± 0.1[a]
Temperature (°C)	25.9 ± 0.3[a]	25.9 ± 0.2[a]	25.0 ± 0.3[a]	25.9 ± 0.06[a]	25.2 ± 0.06[a]
pH	6.4 ± 0.04[a]	6.4 ± 0.1[a]	6.7 ± 0.1[a]	6.4 ± 0.05[a]	6.5 ± 0.08[a]
CE (μS/cm)	31.2 ± 1.8[a]	32.8 ± 2.7[a]	33.4 ± 1.8[a]	32.7 ± 0.4[a]	33.2 ± 1.0[a]
AT (mg L^{-1})	0.04 ± 0.8[a]	0.04 ± 0.01[a]	0.05 ± 0.04[a]	0.06 ± 0.02[a]	0.06 ± 0.03[a]
Nitrite (mg L^{-1})	0.02 ± 0.03[a]	0.03 ± 0.004[a]	0.05 ± 0.01[a]	0.07 ± 0.005[a]	0.07 ± 0.01[a]
Alc ($CaCO_3$ mg L^{-1})	6.0 ± 0[a]	5.6 ± 1.3[a]	6.4 ± 0.4[a]	5.9 ± 0.6[a]	6.6 ± 0.7[a]
DT ($CaCO_3$ mg L^{-1})	2.5 ± 0[a]	2.9 ± 0.3[a]	2.9 ± 0.5[a]	3.7 ± 0.5[a]	3.9 ± 0.52[a]
CO_2 (mg L^{-1})	10.7 ± 1.4[a]	9.2 ± 0.5[a]	9.8 ± 1.4[a]	10.2 ± 1.4[a]	9.4 ± 2.5[a]
Ca^{++} (mEq L^{-1})	0.8 ± 0[a]	0.9 ± 0.2[a]	0.9 ± 0.04[a]	0.9 ± 0.02[a]	0.9 ± 0.01[a]
Mg^{++} (mEq L^{-1})	0.08 ± 0[a]	0.08 ± 0.01[a]	0.07 ± 0.0[a]	0.07 ± 0[a]	0.07 ± 0.01[a]
Na^+ (mEq L^{-1})	1.8 ± 0.002[a]	1.9 ± 1.1[a]	1.9 ± 0.2[a]	1.9 ± 0.01[a]	1.9 ± 0.02[a]
K^+ (mEq L^{-1})	1.6 ± 2.2[a]	1.6 ± 0.1[a]	1.6 ± 0.08[a]	1.7 ± 0.1[a]	1.7 ± 0.02[a]

Values express means ± standard deviation. Different letters in the same line indicate significant differences by Tukey test ($p < 0.05$). OD = Dissolved oxygen; CE = Electrical conductivity; AT = Total ammonia; Alc = Total alkalinity; DT = Total hardness; CO_2 = Carbon dioxide.

Table 2
Physical and chemical parameters of aquaria water after 12 h of exposure of *Arapaima gigas* to different concentrations of formalin.

Parameters	Concentrations of formalin (mg L^{-1})				
	0	33	44	55	66
OD (mg L^{-1})	7.5 ± 0.03[a]	7.39 ± 0.08[a]	7.37 ± 0.08[a]	7.4 ± 0.06[a]	7.4 ± 0.1[a]
Temperature (°C)	25.6 ± 0.2[a]	25.8 ± 0.06[a]	25.6 ± 0.3[a]	25.4 ± 0.23[a]	25.5 ± 0.1a
pH	70. ± 0.01[a]	6.9 ± 0.03[a]	6.7 ± 0.5[a]	7.1 ± 0.06[a]	7.1 ± 0.06[a]
CE (μS/cm)	33.5 ± 1.0[a]	35.7 ± 0.2[a]	38.8 ± 1.3[b]	40.8 ± 1.2[b]	41.9 ± 1.8[b]
AT (mg L^{-1})	0.03 ± 0.03[a]	0.04 ± 0.10[a]	0.07 ± 0.01[b]	0.09 ± 0.01[b]	0.1 ± 0.01[b]
Nitrite (mg L^{-1})	0.02 ± 0.01[a]	0.03 ± 0.4[a]	0.05 ± 0.7[a]	0.04 ± 0.5[a]	0.03 ± 0.7[a]
Alc ($CaCO_3$ mg L^{-1})	8.6 ± 0.7[a]	7.9 ± 0.3[a]	8.8 ± 2.4[a]	9.4 ± 1.6[a]	9.9 ± 1.2[a]
DT ($CaCO_3$ mg L^{-1})	3.75 ± 0.4[a]	4.4 ± 0.6[a]	4.7 ± 0.3[a]	3.9 ± 0.6[a]	4.6 ± 0.3[a]
CO_2 (mg L^{-1})	11.2 ± 3.5[a]	10.2 ± 3.4[a]	10.7 ± 2.3[a]	11.8 ± 0.3[a]	11.5 ± 0.9[a]
Ca^{++} (mEq L^{-1})	0.8 ± 0.01[a]	0.8 ± 0.04[a]	0.8 ± 0.06[a]	0.9 ± 0.08[a]	0.9 ± 0.1[a]
Mg^{++} (mEq L^{-1})	0.1 ± 0.01[a]	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.1 ± 0.01[a]
Na^{+} (mEq L^{-1})	2.1 ± 0.13[a]	2.1 ± 0.02[a]	2.3 ± 0.03[a]	2.4 ± 0.04[a]	2.5 ± 0.06[a]
K^{+} (mEq L^{-1})	1.7 ± 0.03[a]	1.7 ± 0.09[a]	1.8 ± 0.06[a]	1.8 ± 0.03[a]	1.9 ± 0.09[a]

Values express means ± standard deviation. Different letters in the same line indicate significant differences by Tukey test ($p < 0.05$). OD = Dissolved oxygen; CE = Electrical conductivity; AT = Total ammonia; Alc = Total alkalinity; DT = Total hardness; CO_2 = Carbon dioxide.

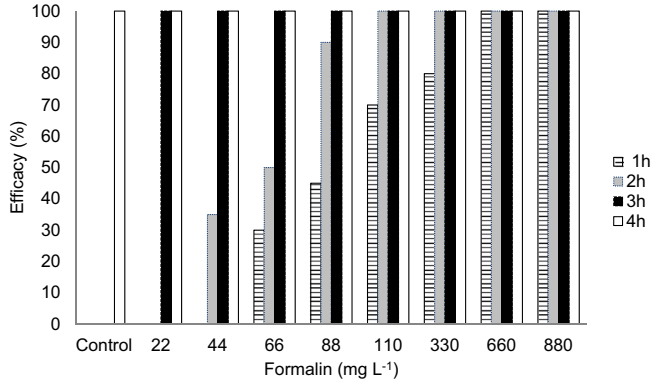

Fig. 1. *In vitro* efficacy, of different concentrations of formalin, for *Dawestrema cycloancistrium* in the gills of *Arapaima gigas*.

Table 3
Prevalence (P) and mean abundance (AM) of *Dawestrema cycloancistrium* in the gills of *Arapaima gigas* after 1 and 12 h baths with different concentrations of formalin.

After bath of 1 h			
Formalin (mg L^{-1})	P (%)	AM	Eficcacy (%)
0	100	270.2 ± 45.1[a]	0
220	100	158.1 ± 33.7[a]	40.2
330	100	103.7 ± 15.9[a]	62.6
440	100	18.7 ± 6.6[b]	93.6
550	100	2.7 ± 1.0[b]	99.3
After bath of 12 h			
0	100	273.2 ± 35.2[a]	0
33	100	186.3 ± 51.6[ab]	34.3
44	100	154.9 ± 39.5[ab]	43.7
55	100	151.2 ± 31.3[ab]	44.5
66	100	121.8 ± 10.8[b]	55.5

Different letters in the same column indicate significant differences by Dunn's test ($p < 0.05$).

of exposure. However, for *Heterobothrium ecuadori,* the mean effective concentration was 225 mg L^{-1} formalin after 30 min of exposure, decreasing to 87 and 47 mg L^{-1} after 60 and 105 min respectively (Fajer-Ávila et al., 2003). These results indicate that formalin was more toxic to *H. ecuadori* than to *D. cycloancistrium* in this assay and beyond that, the sensitivity of this therapeutic product is species-specific. Therefore, formalin should be previously tested *in vitro* for it to be used in therapeutic fish baths (Fajer-Ávila et al., 2003; Sitjà-Bobadilla et al., 2006).

Table 4
Prevalence (P) and mean abundance (AM) of *Dawestrema cycloancistrium* in the gills of *Arapaima gigas* after 96 h of recovery from 1 and 12 h baths with different concentrations of formalin.

After bath of 1 h			
Formalin (mg L^{-1})	P (%)	AM	Eficcacy (%)
0	100	298.0 ± 10.0[a]	0
220	100	157.5 ± 4.5[a]	47.1
330	100	105.0 ± 17.0[a]	64.7
440	100	19.5 ± 10.5[b]	93.4
550	100	2.5 ± 0.5[b]	99.1
After bath of 12 h			
0	100	278.0 ± 12.0[a]	0
33	100	183.5 ± 5.5[ab]	33.9
44	100	151.0 ± 12.0[ab]	45.7
55	100	149.0 ± 11.0[ab]	46.4
66	100	122.0 ± 7.0[b]	56.1

Different letters in the same column indicate significant differences by Dunn's test ($p < 0.05$).

The different development phases of monogeneans (e.g, oncomiracidium and adults) may or may not be affected by formalin (Yildiz and Pulatsu, 1999; Sitjà-Bobadilla et al., 2006). Larvae and adults of *Sparicotyle chrysophrii* in gills of *Sparus aurata* exposed to 300 mg L^{-1} formalin for 30 min showed 100% efficacy *in vitro* and prevented the hatching of eggs (Sitjà-Bobadilla et al., 2006). In this study, during *in vitro* tests, it was observed that larvae of *D. cycloancistrium* in gills of *A. gigas* were more sensitive to formalin than adults (data not shown).

For *D. cycloancistrium* in the gills of *A. gigas*, the efficacy of the short baths (1 h) was 93.6 and 99.3% when 444 and 550 mg L^{-1} formalin were used, respectively, whereas in the baths of 12 h the highest efficacy was lower. Similarly, in therapeutic baths with 250 mg L^{-1} formalin during 1 h for the control of *Benedenia seriolae* and *Zeuxapta seriolae* in *Seriola lalandi lalandi*, the efficacy was of 80% (Sharp et al., 2004). For *Hemigrammus* sp. exposed to 27.5 mg L^{-1} formalin during 1 h, the efficacy was 77.7% (Paixão et al., 2013). In contrast, for *Haliotrema abaddon* in the gills of *Glaucosoma hebraicum* with 25 mg L^{-1} formalin during 10 and 24 h, there was no antiparasitic efficacy (Stephens et al., 2003).

Arapaima gigas exposed to 22, 44, 66, 88 and 110 mg L^{-1} formalin presented clinical signs and behavioral changes such as erratic swimming, lethargy, crowding on the water surface, loss of hydrodynamic equilibrium, spasms and agonistic confrontation, which were observed only at 88 and 110 mg L^{-1} (Andrade-Porto et al., 2017). No mortality of *A. gigas* was observed whatsoever during the 1 h and 12 h therapeutic

Table 5
Blood parameters of *Arapaima gigas* exposed to different concentrations of formalin during 1 h.

Parameters	Concentrations of formalin (mg L^{-1})				
	0	220	330	440	550
Hemoglobin (mg dL^{-1})	7.0 ± 0.9[a]	6.8 ± 2.3[a]	6.7 ± 0.9[a]	6.7 ± 1.0[a]	6.6 ± 1.7[a]
Hematocrit (%)	27.9 ± 13.3[a]	24.6 ± 7.8[a]	24.5 ± 4.5[a]	24.5 ± 3.3[a]	23.4 ± 5.9[a]
RBC ($\times 10^6$ μL^{-1})	1.90 ± 0.29[a]	1.87 ± 0.55[a]	1.84 ± 0.30[a]	1.73 ± 0.28[a]	1.63 ± 0.42[a]
CHCM (g dL^{-1})	27.2 ± 2.8[a]	27.9 ± 2.7[a]	28.2 ± 2.6[a]	28.7 ± 1.5[a]	28.9 ± 1.9[a]
HCM (pg)	37.7 ± 7.7[a]	37.8 ± 6.7[a]	38.8 ± 6.3[a]	41.3 ± 7.6[a]	41.9 ± 8.8[a]
VCM (f L^{-1})	144.5 ± 26.5[a]	144.6 ± 18.9[a]	145.2 ± 35.0[a]	145.8 ± 29.9[a]	146.0 ± 27.8[a]
Glucose (mg dL^{-1})	45.2 ± 13.7[a]	45.7 ± 17.1[a]	46.6 ± 11.4[a]	46.8 ± 9.5[a]	46.9 ± 18.0[a]
Total protein (g L^{-1})	1.6 ± 0.6[a]	1.6 ± 0.6[a]	1.6 ± 0.2[a]	1.8 ± 0.4[a]	1.7 ± 0.2[a]
Cortisol (ng mL^{-1})	93.9 ± 51.6[a]	93.2 ± 38.7[a]	94.7 ± 29.7[a]	94.4 ± 38.0[a]	95.2 ± 27.7[a]
Cl^- (mmol L^{-1})	51.5 ± 10.1[a]	50.7 ± 15.4[a]	50.2 ± 13.8[a]	49.9 ± 17.6[a]	48.7 ± 15.1[a]
Ca^{++} (mmol L^{-1})	0.5 ± 0.1[a]	0.5 ± 0.1[a]	0.5 ± 0.2[a]	0.5 ± 0.1[a]	0.5 ± 0.2[a]
Mg^{++} (mmol L^{-1})	0.1 ± 0.02[a]	0.1 ± 0.04[a]	0.1 ± 0.05[a]	0.1 ± 0.10[a]	0.1 ± 0.03[a]
Na^+ (mmol L^{-1})	1.2 ± 0.2[a]	1.2 ± 0.4[a]	1.2 ± 0.6[a]	1.2 ± 0.4[a]	1.2 ± 0.4[a]
K^+ (mmol L^{-1})	2.2 ± 0.7[a]	2.0 ± 1.9[a]	1.9 ± 1.4[a]	1.9 ± 0.5[a]	1.9 ± 1.4[a]

Values express means ± standard deviation. Different letters in the same line indicate significant differences by Tukey test. RBC = Total red blood cells, CHCM = Mean corpuscular hemoglobin concentration, HCM = Mean corpuscular hemoglobin, VCM = Mean corpuscular volume, Cl = Chloride, Ca^{++} = Calcium; Mg^{++} = Magnesium; Na^+ = Sodium; K^{+-} = Potassium.

Table 6
Blood parameters of *Arapaima gigas* exposed to different concentrations of formalin during 12 h.

Parameters	Concentrations of formalin (mg L^{-1})				
	0	33	44	55	66
Hb (mg dL^{-1})	6.7 ± 1.7[a]	5.1 ± 2.0[a]	5.6 ± 0.9[a]	6.3 ± 1.0[a]	6.4 ± 1.9[a]
Ht (%)	31.8 ± 5.1[a]	22.1 ± 6.8[b]	23.8 ± 3.5[b]	25.3 ± 2.5[b]	28.9 ± 3.8[ab]
RBC ($\times 10^6$ μL^{-1})	1.420 ± 0.44[a]	1.37 ± 0.42[a]	1.38 ± 0.75[a]	1.38 ± 0.29[a]	1.39 ± 0.45[a]
CHCM (g dL^{-1})	24.8 ± 5.4[a]	24.9 ± 4.3[a]	25.3 ± 3.8[a]	25.9 ± 3.7[a]	26.3 ± 6.6[a]
HCM (pg)	41.4 ± 11.6[a]	41.5 ± 11.5[a]	42.4 ± 16.5[a]	43.2 ± 12.4[a]	43.7 ± 10.6[a]
VCM (fL)	178.1 ± 37.6[a]	175.0 ± 33.7[a]	175.6 ± 68.8[a]	176.2 ± 45.9[a]	176.9 ± 78.3[a]
Glucose (mg dL^{-1})	37.2 ± 6.4[a]	39.7 ± 6.9[a]	41.4 ± 9.7[a]	51.6 ± 10.5[b]	53.3 ± 7.8[b]
Total protein (g L^{-1})	1.3 ± 0.1[a]	1.3 ± 0.4[a]	1.4 ± 0.2[a]	1.4 ± 0.4[a]	1.4 ± 2.8[a]
Cortisol (ng mL^{-1})	63.9 ± 26.5[a]	66.9 ± 34.5[a]	72.6 ± 34.7[b]	114.0 ± 0.5[c]	120.8 ± 7.4[d]
Cl^- (mmol L^{-1})	55.5 ± 18.2[a]	51.1 ± 9.9[a]	49.9 ± 9.4[a]	40.1 ± 12.9[b]	30.1 ± 11.3[c]
Ca^{++} (mmol L^{-1})	0.6 ± 0.02[a]	0.5 ± 0.05[b]	0.4 ± 0.04[c]	0.3 ± 0.03[c]	0.3 ± 0.03[c]
Mg^{++} (mmol L^{-1})	0.09 ± 0.02[a]	0.09 ± 0.01[a]	0.10 ± 0.01[a]	0.09 ± 0.01[a]	0.10 ± 0.02[a]
Na^+ (mmol L^{-1})	1.4 ± 0.20[a]	1.4 ± 0.2[a]	1.4 ± 0.1[a]	1.4 ± 0.07[a]	1.4 ± 0.16[a]
K^+ (mmol L^{-1})	1.4 ± 0.10[a]	1.3 ± 0.2[a]	1.3 ± 0.1[a]	1.3 ± 0.2[a]	1.3 ± 1.3[a]

Values express means ± standard deviation. Different letters in the same line indicate significant differences by Tukey test. Hb = hemoglobin; Ht = hematocrit; RBC = Total number of erythrocytes; CHCM = Mean corpuscular hemoglobin concentration; HCM = Mean corpuscular hemoglobin; VCM = Mean corpuscular volume; Cl = Chloride; Ca^{++} = Calcium; Mg^{++} = Magnesium; Na^+ = sodium; K^{+-} = Potassium. Means ± standard deviation.

Table 7
Formalin residues in the muscle of *Arapaima gigas* after 1 and 12 h baths following 96 h of recovery from these baths.

After bath of 1 h		
Formalin (mg L^{-1})	Exposure (μg/g)	Recovery (μg/g)
0	0.6 ± 0.2[Aa]	0.6 ± 0.1[Aa]
220	1.4 ± 0.7[Aab]	0.6 ± 0.01[Ba]
330	1.6 ± 0.9[Aab]	0.7 ± 0.02[Ba]
440	2.3 ± 1.2[Ab]	0.7 ± 0.01[Ba]
550	2.0 ± 1.3[Ab]	0.9 ± 0.01[Ba]
After bath of 12 h		
0	0.5 ± 0.06[Aa]	0.5 ± 0.04[Aab]
33	0.7 ± 0.1[Aab]	0.5 ± 0.02[aB]
44	0.8 ± 0.1[Abc]	0.5 ± 0.00[abB]
55	0.9 ± 0.1[Acd]	0.6 ± 0.02[abB]
66	1.0 ± 0.2[Ad]	0.7 ± 0.02[bB]

Values express means ± standard deviation. Different letters in the same collunm indicate significant difference by Dunn's test ($p < 0.05$). Different letters in the same line indicate significant difference by Dunn test ($p < 0.05$).

Table 8
Fomalin residues in aquaria water after 1 and 12 h baths of *Arapaima gigas*.

After bath of 1 h	
Concentrations (mg L^{-1})	Residues (mg L^{-1})
220	88.5 ± 9.6[a]
330	103.9 ± 3.6[a]
440	126.3 ± 3.4[a]
550	200.4 ± 16.2[a]
After bath of 12 h	
33	8.4 ± 0.3[a]
44	10.9 ± 0.3[a]
55	14.7 ± 0.1[a]
66	17.1 ± 0.4[a]

Values express means ± standard deviation. Different letters in the same column indicate significant differences by Dunn's test ($p < 0.05$).

baths with formalin, since the lethal concentration for this fish was previously determined. In contrast, Paixão et al. (2013) found 100% and 66.6% mortality for *Hemigrammus* sp. exposed to 275 and 110 mg L^{-1} formalin during 1 h, respectively. However, these authors used concentrations close to the lethal concentration for *Hemigrammus* sp.

Studies have suggested that formalin used in fish for the control of

ectoparasites in long-term baths is less effective than in short baths, as it is rapidly consumed within the water column (Treves-Brown, 2000; Stephens et al., 2003); similar to what occurred in the present study. Thus, the formalin residue in the aquaria water of *A. gigas*, after the short (1 h) and long (12*h*) baths ranged from 25 to 40%, in relation to the concentration added at the beginning of the experiment. Twenty-four-hour baths with 27.5 mg L^{-1} formalin for *Pterophyllum scalare*, in six alternate-day applications, showed 71% efficacy in controlling monogeneans (Fujimoto et al., 2006). In contrast, for *G. hebraicum*, prolonged 27.5 mg L^{-1} formalin baths during 24 h were not effective in controlling monogeneans (Stephens et al., 2003). Nevertheless, as formaldehyde is a human carcinogen (Noordiana et al., 2011; Lai et al., 2016) and a toxic product, herbal medicines such as *Mentha piperita* have been assayed in order to control monogeneans in *A. gigas* (Malheiros et al., 2016). Therefore, formalin should be handled carefully during antiparasitic treatments in fish farming (Andrade-Porto et al., 2017).

One of the effects of formalin is to cause tissue changes in fish gills, which can lead to osmoregulatory disorders (Treves-Brown, 2000; Jung et al., 2003; Andrade-Porto et al., 2017). In general, formalin may affect the gills, acting on the ionic regulation that induces a rapid decrease in plasma electrolyte levels due to the inhibition of Na^+ influx and, consequently, the inhibition of the Na^+/K^+-ATPase enzyme in the gills (Treves-Brown, 2000; Jung et al., 2003). The inhibition of the Na^+ e Cl^- branchial influx, together with the increase in Na^+ efflux, leads to a decrease in these plasma ions. In this study with *A. gigas*, 12 h baths with 55 and 66 mg L^{-1} formalin decreased chloride levels, and in fish exposed to 44, 55 and 66 mg L^{-1} there was also a decrease in plasma calcium levels.

For *A. gigas,* 1 h baths with different concentrations of formalin did not cause any alteration in blood parameters. However, there was a reduction in hematocrit in fish exposed to 33, 44 e 55 mg L^{-1} formalin. For *O. niloticus,* low concentrations of formalin (1.56, 3.1, 6.25, 12.5 and 25.9 mg L^{-1} caused a reduction in the number of erythrocytes, indicating an anemic process (Omoregie et al., 1994).

Stress-induced changes are defined as primary, secondary and tertiary responses. Primary responses are mediated by the neuroendocrine system, by means of rapid release of the catecholamine hormones (adrenaline and noradrenaline) that are released and synthesized by chromaffin cells and cortisol by interrenal cells. These trigger secondary responses involving various physiological and biochemical effects associated with stress, which include metabolic, hematological, hydromineral, and structural changes. Tertiary responses affect the animals' organism, compromising their growth, their resistance to diseases and their reproductive success (Wedemeyer, 1997; Wendelaar Bonga, 1997; Jorgensen and Jørgensen and Buchmann, 2007). Glucose and plasma cortisol are common indicators of stress in fish (Omoregie et al., 1994; Jung et al., 2003; Araújo et al., 2004; Jorgensen and Jørgensen and Buchmann, 2007).

In *A. gigas*, in 12 h baths with 55 and 66 mg. L^{-1} formalin, glucose levels increased and chloride levels decreased. Similarly, cortisol levels increased in fish exposed to 44, 55 and 66 mg L^{-1} formalin whereas calcium levels decreased. Plasma glucose levels also increased in carps (Kakuta et al., 1991) and formalin-exposed tilapias (Omoregie et al., 1994), as well as in *Colossoma macropomum* exposed to 100, 150, 200 and 250 mg L^{-1} formalin, during different exposure times (Araújo et al., 2004). In contrast, Jung et al. (2003) studied the effects of formalin 100, 212 and 300 mg L^{-1} on *Paralichthys olivaceusis* for 3 h, and they did not observe changes in plasma glucose levels.

In *Oncorhynchus mykiss* infected with *Ichthyophthirius multifiliis* and exposed to 120 mg L^{-1} formalin for 1 h, increased plasma cortisol levels were reported. These were associated with both infection and exposure to formalin (Jørgensen and Jørgensen and Buchmann, 2007). However, during the 12 h formalin baths for *A. gigas* in this study, in addition to the presence of infection by monogeneans, the electrical conductivity and ammonia levels in aquaria water increased. Such changes may also have contributed to the increase in cortisol levels in fish.

Although fish from the controls of this study were not exposed to formalin, 0.52 μg/g (1 h baths) and 0.60 μg/g formalin (2 h baths), formalin was detected in them. These levels were lower in comparison to the ones found in fish exposed to formalin. In Nile tilapia, formalin residue in the muscle of control fish was 1.34 μg/g, similar to the one found in fish exposed to 125 mg L^{-1} formalin (Xu and Rogers, 1995). Formaldehyde concentration in the muscle of *Paralichthys olivaceus* and *Sebastes schlegelii* exposed to different concentrations of formalin was also similar to the one in control fish (Jung et al., 2001). Besides being a natural product in the metabolism of animals and plants, formaldehyde is essential for the biosynthesis of amino acids, thus, naturally occurring in many kinds of food (Owen et al., 1990). It can also be produced in fish tissues during the period in which muscles are stored for analyses (Xu and Rogers, 1995; Noordiana et al., 2011). High levels of formalin do not accumulate in the fish tissues due to subsequent conversion of formaldehyde into other chemical compounds such as through the oxidation of lipids because of microorganism activities (Noordiana et al., 2011). However, the concentrations of formalin found in fish used for our study were not influenced by postmortem changes because formalin residues in the muscle were assayed immediately after fish were killed.

In Brazil, the National Plan for the Control of Residues in Products of Animal Origin (PNCR) by the Ministry of Agriculture, Livestock and Supply (MAPA) has not yet included the determination of formaldehyde residues in fish. According to the United States Environmental Protection Agency (EPA), acceptable daily intake of formaldehyde is 0.2 μg/g body weight per day (Noordiana et al., 2011). Xu and Rogers (1995) cite that endogenous levels of 3.0 to 12.8 μg/g formaldehyde have been found in fish. Noordiana et al. (2011) reported levels of formaldehyde residues varying from 0.38 to 15.7 μg/g per weight of fresh fish commercialized in wet markets. The formalin residue in the *A. gigas* muscle, in this study, after 96 h of recovery was low, presenting no risk of intoxication for consumers in the concentrations tested. Xu and Rogers (1993) demonstrated that in *Morone saxatilis* exposed to 25 and 250 mg L^{-1} formalin for 1 h and 24 h respectively, formalin was no longer detected in the fish muscle after the 4th day of exposure. In Nile tilapia, after baths of 96 h, 1.30 and 1.57 μg/g formalin were reported for fish exposed to 125 and 250 mg L^{-1} formalin respectively, indicating that the concentrations and persistence of formalin in the water affected the absorption of formalin on the part of the fish (Xu and Rogers, 1995).

5. Conclusions

The results, *in vitro and in vivo*, showed that the antiparasitic efficacy of formalin against *D. cycloancistrium* was dose-dependent. Short baths (1 h) with concentrations of 440 and 550 mg L^{-1} formalin are recommended for the control of *D. cycloancistrium* in the gills of *A. gigas* without compromising fish homeostasis. However, caution is advised regarding the use of such concentrations directly into farming tanks, since there may be synergistic and antagonistic effects with variables in different environments.

Acknowledgements

The authors are grateful to FAPEAM for financial support for this study. Dr. M. Tavares-Dias was supported by research fellowships (#375930/2015-9) from the National Council for Research and Technological Development (CNPq, Brazil). We also give thanks to the Thematic Laboratory of Electronical Microscopy (LTME) of the National Institute for Research in the Amazon for the analyses performed.

References

Andrade-Porto, S.M., Ramos, C.A., Tavares-Dias, M., Roque, R., Affonso, E.G., Barcellos, J.F.M., Souza, H.E.M., Araújo, C.S.O., Malta, J.C.O., 2017. Histopathological evaluation of formalin toxicity in Arapaima gigas (Arapaimidae), the giant fish from Amazon. Pesqui. Vet. Bras (In press).

Araújo, L.D., Chagas, E.C., Gomes, L.C., Brandão, F.R., 2004. Efeito de banhos terapêuticos com formalina sobre indicadores de estresse em tambaqui. Pesq. Agrop. Brasileira 39, 217–221.

Araújo, C.S., Gomes, A.L., Tavares-Dias, M., Andrade, S.M.S., Belém-Costa, A., Borges, J.T., Queiroz, M.N., Barbosa, M., 2009a. Parasitic infections in pirarucu fry, *Arapaima gigas* Schinz, 1822 (Arapaimatidae) kept in a semi-intensives fish farm central Amazon. Brazil. Vet. Arhiv. 79, 99–507.

Araújo, C.S., Tavares-Dias, M., Gomes, A.L., Andrade, S.M.S., Lemos, J.R.G., Oliveira, A.T., Cruz, V.R., Affonso, E.G., 2009b. Infecções parasitárias e parâmetros sanguíneos em *Arapaima gigas* Schinz, 1822 (Arapaimidae) cultivados no Estado do Amazonas, Brasil. In: Tavares-Dias, M. (Ed.), Manejo e sanidade de peixes em cultivo. Embrapa Amapá, Macapá, pp. 389–424.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitalogy meets ecology on its own terms: Margolis et al. Revisited. J. Parasitol. 83, 575–583.

Fajer-Ávila, E., Farra, I.A., Aguilar-Zarate, G., Contreras-Arce, R., Zaldivar-Ramirez, J., Betancourt-Lozano, M., 2003. Toxicity of formalin to bullseye puffer fish (*Shoeroides annulatus* Jenyna 1843) and its effectiveness to control ectoparasites. Aquaculture 223, 41–50.

Fujimoto, R.Y., Vendruscolo, L., Schalch, S.H.C., Moraes, F.R., 2006. Avaliação de três diferentes métodos para o controle de monogenéticos e *Capillaria* sp. (Nematoda: Capillariidae) parasitos de acará-bandeira *Pterophyllum scalare* Liechtenstein, 1823. Bol. Inst. Pesca 32 (2), 183–190.

Hamilton, M.A., Russo, R.C., Thurton, R.V., 1977. Trimed Spearman-Karber method for estimating median lethal concentration in toxicity biossays. Environ. Sci. Technol. 11 (7), 714–719.

Jørgensen, T.R., Buchmann, K., 2007. Stress response in rainbow trout during infection with *Ichthyophthirius multifiliis* and formalin bath treatment. Acta Ichthyol. Piscat. 37 (1), 25–28.

Jung, S.H., Kim, J.W., Jeon, I.G., Lee, W.Y., 2001. Formaldehyde residues in formalin-treated olive flounder. *Paralichthys olíFaceus*, black rockfish *Sebastes schlegeli* and seawater. Aquaculture 194, 253–262.

Jung, S.H., Sim, D.S., Park, M., Jo, Q., Kim, Y., 2003. Effects of formalin on haematological and blood chemistry in olive flounder, *Paralichthys olivaceus* (Temminck et Schlegel). Aquac. Res. 34 (14), 1269–1275.

Kakuta, I., Namba, K., Uematsu, K., Murachi, S., 1991. Physiological response of the fish, *Cyprinus carpio*, to formalin exposure.1. Effects of formalin on urine flow, heart rate, respiration. Comp. Biochem. Physiol. 100C, 405–411.

Lai, Y., Yu, R., Hartwell, H.J., Moeller, B.C., Bodnar, W.M., Swenberg, J.A., 2016. Measurement of endogenous versus exogenous formaldehyde–induced DNA–protein crosslinks in animal tissues by stable isotope labeling and ultrasensitive mass spectrometry. Cancer Res. http://dx.doi.org/10.1158/0008-5472.CAN-15-2527.

Malheiros, D.F., Maciel, P.O., Videira, M.N., Tavares-Dias, M., 2016. Toxicity of the essential oil of *Mentha piperita* in *Arapaima gigas* (pirarucu) and antiparasitic effects on Dawestrema spp. (Monogenea). Aquaculture 455, 81–86.

Marinho, R.G.B., Tavares-Dias, M., Dias-Grigório, M.K.R., Neves, L.R., Yoshioka, E.T.O., Boijink, C.L., Takemoto, R.M., 2013. Helminthes and protozoan of farmed pirarucu (*Arapaima gigas*) in eastern Amazon and host-parasite relationship. Arq. Brasil. Med. Vet. Zoot. 65 (4), 1192–1202.

Martins, M.L., 2004. Cuidados básicos e alternativas no tratamento de enfermidades de peixes na aquicultura brasileira. In: Ranzani-Paiva, M.J.T., Takemoto, R.M., Lizama, M.A.P. (Eds.), Sanidade de organismos aquáticos. Varela, São Paulo, pp. 357–370.

Martins, M.L., Onaka, E.M., Moraes, F.R., Fujimoto, R.Y., 2001. Mebendazole treatment against *Anacanthorus penilabiatus* (Monogenea, Dactylogyridae) gill parasite of cultivated *Piaractus mesopotamicus* (Osteichthyes, Characidae) in Brazil. Efficacy and hematology. Acta Parasitol. 46, 332–336.

Noordiana, N., Fatimah, A.B., Farhana, Y.C.B., 2011. Formaldehyde content and quality characteristics of selected fish and seafood from wet markets. Int. Food Res. J. 18, 125–136.

Omoregie, E., Eseyin, T.G., Ofojekwu, P.C., 1994. Chronic effects of formalin on erythrocyte counts and plasma glucose of the Nile tilapia, *Oreochromis niloticus*. Asian Fish. Sci. 7, 1–6.

Owen, B.A., Dudney, C.S., Tan, E.L., Easterly, C.E., 1990. Formaldehyde in drinking water: comparative hazard evaluation and an approach to regulation. Regul. Toxicol. Pharmacol. 11, 220–236.

Pahor-Filho, E., Miranda-Filho, K.C., Pereira-Júnior, J., 2012. Parasitology of juvenile mullet (*Mugil liza*) and effect of formaldehyde on parasites and host. Aquaculture 354, 111–116.

Paixão, L.F., Santos, R.F.B., Ramos, F.M., Fujimoto, R.Y., 2013. Efeitos do tratamento com formalina e sulfato de cobre sobre os parâmetros hematológicos e parasitos monogenéticos em juvenis de *Hemigrammus* sp. (Osteichthyes: Characidae). Acta Amaz 43, 211–216.

Rebelatto-Junior, I.A., Lima, A.F., Rodrigues, A.P.O., Maciel, P.O., Kato, H.C.A., Mataveli, M., Rezende, F.P., Varela, E.S., Sousa, A.R.B., Santos, C., Boijink, C.L., Yoshioka, E.T.O., O'sullivan, F.L.A., 2015. Reprodução e engorda do pirarucu: levantamento de processos produtivos e tecnologias. 1a.ed. Embrapa, Brasíliapp. 102.

Sharp, N.J., Diggles, B.K., Poortenaar, C.W., Willis, T.J., 2004. Efficacy of Aqui-S, formalin and praziquantel against the monogeneans, *Benedenia seriolae* and *Zeuxapta seriolae*, infecting yellowtail kingfish *Seriola lalandi lalandi* in New Zealand. Aquaculture 236, 67–83.

Sitjà-Bobadilla, A., Felipe, M.C., Alvarez-Pellitero, P., 2006. *In vivo* and *in vitro* treatments against *Sparicotyle chrysophrii* (Monogenea: Microcotylidae) parasitizing the gills of gilthead sea bream (*Sparus aurata*). Aquaculture 261, 856–864.

Stephens, F.J., Cleary, J.J., Jenkinsb, G., Jonesc, J.B., Raidala, S.R., Thomasa, J.B., 2003. Treatments to control *Haliotrema abaddon* in the west Australian dhufish, *Glaucosoma hebraicum*. Aquaculture 215, 1–10.

Thatcher, V.E., 2006. Amazon Fish Parasites, second ed. Pensoft Publishers, Sofia-Moscow, pp. 508.

Treves-Brown, K.M., 2000. Externally Applied Antimicrobial Agents. In: Applied fish pharmacology. Kluwer Academic Publishers, Netherlands, pp. 161–164.

Wedemeyer, G.A., 1997. Effects of rearing conditions on the health and physiological quality of fish in intensive cultere. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P., Schreck, C.B. (Eds.), Fish Stress and Health in Aquaculture. Experimental Biology Seminar Series. University of Cambridge, Cambridge, USA, pp. 35–71.

Wendelaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.

Xu, D., Rogers, A., 1993. Formaldehyde residue in striped bass muscle. J. Aquat. Anim. Health 5, 306–312.

Xu, D., Rogers, A., 1995. Formaldehyde residue in the muscle of Nile perch. Asian Fish. Sci. 8, 81–88.

Yildiz, H.Y., Pulatsu, S., 1999. Evaluation of the secondary stress response in healthy Nile tilapia (*Oreochromis niloticus* L.) after treatment with a mixture of formalin, malachite green and methylene blue. Aquac. Res. 30, 379–383.

Zagatto, P.A., Bertoletti, E., 2006. Ecotoxicologia aquática: princípios e aplicações. Universidade Federal de São Carlos, São Paulo, pp. 478.

Zar, J.H., 2010. Biostatistical Analysis, fifth ed. Prentice Hall, New Jersey, pp. 944.