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Sodium chloride against *Dawestrema cycloancistrium* in juvenile *Arapaima gigas*

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Abstract
The aim of the present study was to assess the use of sodium chloride (NaCl) against monogenoid trematodes infestations in pirarucu (*Arapaima gigas*). Two assays were conducted with pirarucu juveniles, the first comprising an in vitro exposure to 8, 9, 10, and 11 g·L^{-1} NaCl and the second, in vivo exposure test to NaCl at the same concentrations. The best in vitro results were observed for 1-h exposures at 9, 10, and 11 g·L^{-1} NaCl, resulting in 60% and 100% parasite mortality, respectively. In vivo exposures to 8 and 10 g·L^{-1} NaCl were 36 and 22% efficient, respectively, following 2 h of exposure, with no mortality. Some dose-dependent changes were observed in exposed fish, such as decreased hemoglobin and MCHC values at the highest NaCl concentration compared to the other NaCl concentrations and the control group. Most frequent gill tissue alterations observed were capillary dilatation and mucus secretions compared to the control group at the highest NaCl concentrations (10 and 11 g·L^{-1}). Decreased mean ($p<0.05$) water pH values and increased mean electrical conductivity were noted compared to the control group. In conclusion, our findings suggest that NaCl exhibits low toxicity toward pirarucu without physiological alterations and anthelmintic activity to the monogenoid directly influenced by exposure time and concentration. Moreover, 100% of the monogenoids present in the gills of the fish were identified as *Dawestrema cycloancistrium*.

Keywords Monogenoids · Histological analysis · Parasitic diseases · Gills · Pirarucu · Biochemical

Introduction

Dactylogirose is a disease caused by parasites belonging to the Monogenoidea class that mainly affects commercial freshwater fish, such as *Piaractus mesopotamicus* (Leão et al. 2017), *Cyprinus carpio* (Zoral et al. 2017), *Colossoma macropomum* (Rocha et al. 2018), and *Arapaima gigas* (pirarucu) (Maciel and Alves 2018; Queiroz et al. 2020).

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Monogenoid infestations can cause from mild to severe lesions in the host skin, including skin pigmentation losses and small erosions to large ulcerations with significant swellings and secondary infections. In addition, monogenoids can also alter gill structures, impairing gas exchanges and osmoregulatory processes (Jerônimo et al. 2014; Costa et al. 2017).

The occurrence of monogenoids in pirarucu is a limiting factor for its rearing in production systems, with the predominance of two species, *Dawestrema cycloancistrium* and *Dawestrema cycloancistrioides*, affecting pirarucu juveniles from the first days of life (Araújo et al. 2009; Mathews et al. 2014; Serrano-Martínez et al. 2015; Andrade-Porto et al. 2017; Queiroz et al. 2020). Monogenoids in pirarucu can cause significant physiology alterations (Queiroz et al. 2020), as well as morphological effects on gill lamellae, such as hyperplasia and edema. When present in initial pirarucu phase, these parasites can contribute to low survival rates and high economic losses (Tavares-Dias and Martins 2017).

Parasitic infestations in fish farming activities can be avoided by adopting Good Management Practices (GMPs) (Miura et al. 2018; Silva et al. 2019) and nutritional adjustments (Lima et al. 2021). Furthermore, the use of prophylaxis and disease treatment products, as well as immunostimulants, is also recommended. Some records concerning therapeutic and prophylactic treatments for pirarucu are available, including the use of chemicals applied in therapeutic baths for monogenoid control, i.e., formaldehyde (Andrade-Porto et al. 2017) and herbal medicines, such as essential *Mentha piperita* (Malheiros et al. 2016) and *Piper aduncum* (Miura et al. 2018) oils and aqueous *P. aduncum* extracts (Queiroz et al. 2020). In addition, sodium chloride (NaCl), a natural product widely employed in aquaculture activities, has been reported as a promising alternative concerning pirarucu growth and survival, due to its various benefits and low costs (Silva et al. 2019; Tavares-Dias 2021).

In this context, this study aimed to evaluate the effectiveness of NaCl in the control of monogenoid infestations in pirarucu and its effects on pirarucu histophysiology and toxicity. The monogenoid species present in pirarucu gills were also identified.

Material and methods

Fish acclimatization

A total of 180 juvenile pirarucu (8.1 ± 0.6 g and 10.1 ± 0.1 cm), *Arapaima gigas*, were randomly distributed in 500 L polyethylene tanks under continuous flow and constant aeration conditions. The fish were initially acclimatized for 15 days and fed until apparent satiety six times a day with commercial extruded feed for carnivorous fish. The study was carried out according to Brazilian animal welfare standards and International Organization for Standardization standards (2006) and was approved by an Ethics Committee (Protocol No 013/2015).

Water quality parameters were determined throughout the experimental period, remaining within the pirarucu comfort range during the acclimatization period ($OD = 6.9$ mg/L: $T = 25.82$ ºC; $pH = 6.43$; *Electrical Conductivity* $= 66.85.57$ µS/cm). All animals underwent parasitological screening prior to the NaCl assays, with a mean infestation rate (*MI*) of 55 ± 1.0.

In vitro NaCl effectiveness

The antiparasitic in vitro effect of NaCl on monogenoids employed concentrations determined in previous trials based on toxic concentrations for pirarucu (Araújo et al. 2009). Five treatments were conducted, namely, 0 (controls, no NaCl exposure), 8, 9, 10, and 11 g·L^{-1} NaCl, in duplicate.

Parasite sampling was performed using 10 gill arches naturally infested with monogenoids ($N=5$). The gill arches were placed individually in Petri dishes, randomly distributed among the different NaCl concentrations, and observed under a Zeiss® Stemi 2000-C stereomicroscope (Carl Zeiss, Oberkochen, Germany). Parasites were quantified by selecting a microscope field containing 20 monogenoids for each gill arch and assessing parasite mortality rates every 15 min, with the results expressed per hour (Andrade-Porto et al. 2017). Monogenoids were considered dead when immobile, even following external stimuli. The test ended when 100% of the parasites in the control group died and the results were expressed as the percentage (%) of dead parasites/h, within a maximum of 4 h.

Sodium chloride toxicity ($LC_{50\text{-}96\,h}$), effectiveness, and water quality

The lethal NaCl concentration ($LC_{50\text{-}96\,h}$) was previously determined for the in vivo efficacy analysis. A random design was applied employing seven NaCl concentrations (8, 9, 10, 11, 13, and 15 g·L^{-1}) compared to controls (no NaCl), in triplicate. All experiments were carried out in 80 L glass aquaria containing five fish in each of the 21 experimental units. Fish were randomly distributed and allocated in aquaria with no water exchange and continuous aeration under a 12-h photoperiod and acclimated for 24 h prior to the experiments. Mortality rates were recorded at 24, 48, 72, and 96 h, and behavioral changes were evaluated throughout the entire experiment. The $LC_{50\text{-}96\,h}$ values were calculated according to the trimmed Spearman-Karber method (Hamilton et al. 1977). Zero toxicity was estimated qualitatively according to Zucker (1985) (Table 1).

The bioassays were performed in a static system, under constant aeration and a 12-h photoperiod dark to assess the antiparasitic efficacy of NaCl in pirarucu. A total of 75 fish were fasted for 24 h, distributed in 80-L glass aquaria ($N=5$), and exposed to 8, 9, 10, and 11 g·L^{-1} NaCl and a control condition without NaCl. Each experiment lasted 2 h and was conducted in triplicate. At the end of the experiments, three fish were sampled from each aquarium to assess NaCl treatment effectiveness ($N=9$). Immediately after the NaCl baths (2 h), the fish gills were removed, and individual arches were analyzed under a stereomicroscope. Sodium chloride effectiveness was calculated as ((Parasite means in the control – Parasite means in the treatment) × 100/ Parasite means in the control group) (Martins et al. 2001). The remaining animals were observed for an additional 48 HPT (hours post treatment) to assess survival under the same environmental conditions as

Table 1 Parasitic indices of *Arapaima gigas* parasitized by *Dawestrema cycloancistrium* after 2 h of NaCl treatment compared to the controls

Indices	Control	Concentrations (g·L^{-1})			
		8	9	10	11
Prevalence (%)	100	100	100	88.9	100
Average intensity	44.5	28.6	48.5	54.5	35.4
Average abundance	44.5	28.6	48.5	54.5	35.4
Intensity	24 to 64	8 to 56	18 to 90	0 to 104	6 to 92

the acclimatization period. The parasite indices were calculated according to Bush et al. (1997).

Monogenoid species were identified according to Kritsky et al. (1985). The parasites were mounted on slides and coverslips under a Zeiss® Stemi 2000-C stereomicroscope, employing a Gray and Wass solution (Thatcher 2006). Identifications were carried out under an optical Zeiss Axio Lab A1 microscope coupled to a digital Zeiss AxioCam ERc5s camera.

Water quality monitoring was carried out throughout the entire experimental period. Dissolved oxygen (DO), temperature (°C), and electrical conductivity (μS) were determined using a digital YSI 85/10 oximeter, while pH values were determined employing a YSI 60–10 digital pH meter.

Hematology and histopathology examination

Pirarucu blood samples were obtained following the prophylactic NaCl baths by caudal vein puncture ($N = 9$/aquarium) using 3-mL syringes rinsed with 10% EDTA and stored at 4 °C. The following analyses were performed: hematocrit (Htc%), employing the microhematocrit method, erythrocyte counts (RBC erythrocytes·μL^{-1}), using a hemocytometer in a formalin citrate solution and hemoglobin concentrations ([Hb] g·dL^{-1}), employing the cyanomethemoglobin method through a commercial kit. Hematimetric indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin concentrations (MCHC), and mean corpuscular hemoglobin (MCH), were calculated from the Htc, RBC, and [Hb] values, according to Wintrobe (1934).

Plasma was obtained by whole blood centrifugation using a refrigerated Eppendorf 5430 R centrifuge and was used for glucose (mg·dL^{-1}) determinations by the glucose oxidase enzymatic-colorimetric method, while total proteins (PPT–g·dL^{-1}) were determined by the biuret method, both employing commercial kits, and determined using a UV/Visible BIOPLUS 2000 spectrophotometer. Cortisol was determined by the Elisa method using commercial kits (Direct ELISA. The EIAsy Way—Cortisol DIAG. BIOCHEM Canada) and readings were performed on a DLS Active EIA DSL-10–2000 ACTIVE microplate spectrophotometer calibrated to 450 nm.

Concerning the histopathological analysis, gill arches ($N = 9$/per aquarium) were fixed in 10% neutral buffered formalin and processed by usual histology techniques (Brancroft and Gamble 2002). Samples were dehydrated cleared in xylol and embedded in paraffin blocks, and 4-μm cross-sections were obtained and stained with hematoxylin–eosin and observed, interpreted, and photographed under a Zeiss Axio Lab A1 microscope coupled to a digital Zeiss AxioCam ERc5s camera. Twenty random fields on the histological slides were chosen for qualitative descriptive statistical classifications.

Descriptive and qualitative analyses were performed for histopathological change categorization employing the mean alteration value (MAV), determined according to Schwaiger et al. (1997), and the histological alteration index (HAI), determined according to Poleksić and Mitrović-Tutundžić (1994).

Statistical analyses

The data were subjected to a 5% alpha normality test (Kolmogorov–Smirnov, Anderson–Darling, Shapiro–Wilk, and Watson). A normal distribution was observed, so an analysis of variance (one-way ANOVA) was performed, and means were compared by either the Tukey or Dunn tests ($p < 0.05$). All statistics were performed using the R software.

Results

Prophylactic in vitro and in vivo NaCl effects

The results of the in vitro test employing different NaCl concentrations demonstrated parasite mortality after 1 h of exposure. The 9, 10, and 11 g·L⁻NaCl concentrations were the most effective after 1 h of exposure, totaling 60% and 100% parasite mortality rates, respectively (Fig. 1A). Concerning the control group, 30% and 100% parasite mortality rates were observed following 2 and 4 h of exposure (Fig. 1A).

No fish mortality was recorded for 96 for the control group and in fish exposed to 8, 9, and 10 $g{\cdot}L^{-1}$ NaCl. Fish mortality rates at the end of the experiment were of 20% at 11 $g{\cdot}L^{-1}$ and 100% at 13 and 15 $g{\cdot}L^{-1}$. Clinical signs in exposed fish were observed at 11, 13, and 15 $g{\cdot}L^{-1}$ NaCl exposure, cataracts and hemorrhagic tails, while fin erosion was observed at 13 $g{\cdot}L^{-1}$ NaCl exposure. Increased body volume, general edema, and scale sloughing were noted at 15 $g{\cdot}L^{-1}$ NaCl exposure. No behavioral changes were observed at any of the employed NaCl concentrations. The calculated NaCl $LC_{50\text{-}96\ h}$ for pirarucu juveniles was of 11.64 $g{\cdot}L^{-1}$ at a 95% confidence interval, ranging from 11.34 to 11.97 $g{\cdot}L^{-1}$. The linear regression representing the 96-h NaCl concentration and response curve for pirarucu juveniles is displayed in Fig. 2.

Sodium chloride concentrations of 8 and 10 $g{\cdot}L^{-1}$ were the most efficient, at 36 and 22%, respectively, after 2 h of exposure (Fig. 1B). Despite this, parasite loads ranging from 257 to 490 parasites were observed in all treatments (unpublished data). No fish mortality occurred during the entire bioassay period (48 HPT) at all NaCl concentrations. A 100% *D. cycloancistrium* prevalence in gill arches was observed after 2 h of NaCl exposure, except for samples exposed to 10 $g{\cdot}L^{-1}$ NaCl (88.9%), with no observed difference concerning the determined parasitological indices ($p > 0.05$; Table 1).

Physical–chemical water parameters were altered after NaCl exposure, with reduced average pH values in all treatments compared to the controls ($p < 0.05$; Table 2). Increasing water electrical conductivity values were noted with increasing NaCl concentrations in both the treatments and the control group ($p < 0.05$; Table 2).

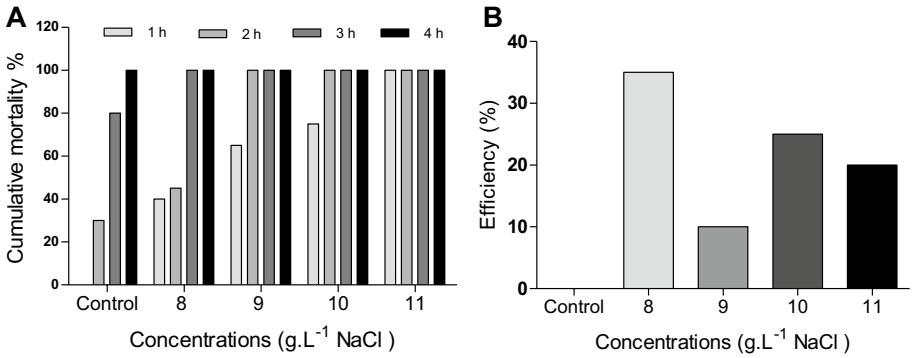

Fig. 1 Sodium chloride in vitro and in vivo effectiveness against monogenoids. Cumulative mortality of monogenoids exposed to different NaCl concentrations in in vitro tests (**A**); effectiveness percentage (%) of NaCl against monogenoids in *Arapaima gigas*, following 2 h of exposure (**B**)

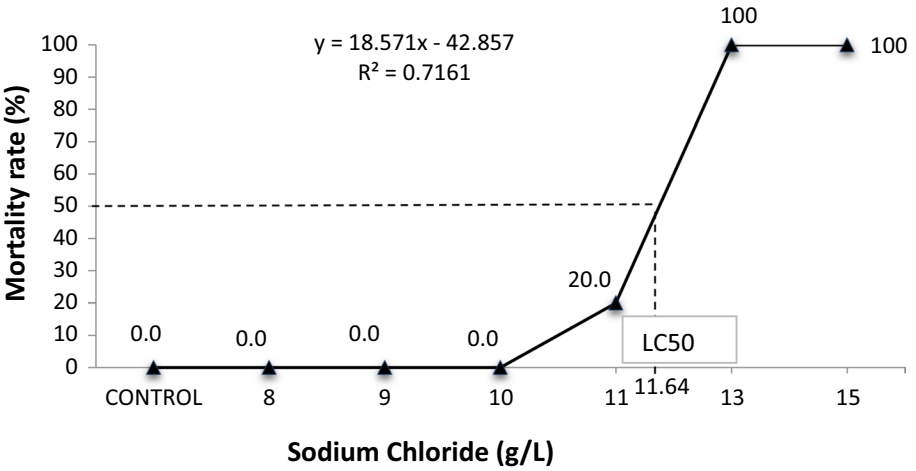

Fig. 2 Relationship between sodium chloride concentrations and *Arapaima gigas* juvenile mortality rates in 96 h

Physiological and histological pirarucu parameters following NaCl exposure

Decreased hemoglobin concentrations and MCHC values were observed in fish exposed to 11 g·L^{-1} in relation to the control and the other NaCl concentrations after 2 h ($p < 0.05$; Table 3). Other hematological parameters (blood glucose and cortisol) were not altered following NaCl exposure ($p > 0.05$; Table 3).

Histological gill alterations in pirarucu exposed to NaCl were classified as stages I to III (Table 4). All treatments, including the control, exhibited histological gill alterations comprising all three stages. The most frequent alterations were lamellar epithelium hyperplasia, vascular congestion, mucous cell (MC) hyperactivation, partial lamellae fusion, hemorrhage, marginal canal dilation, and epithelial detachment (Fig. 3A).

At the highest NaCl concentrations (10 and 11 g·L^{-1}), a higher frequency of marginal canal dilation was noted, as well as the appearance of capillary dilatation and mucus secretions. Mean MAV ranged from 0.28 to 0.71 and were more frequent

Table 2 Physical–chemical water variables after 2 h of *Arapaima gigas* exposure to different NaCl concentrations

Variable	Control	Concentrations (mg·L^{-1})			
		8	9	10	11
O_2 (mg·L^{-1})	6.90 ± 0.51	6.72 ± 0.09	6.74 ± 0.0 2	6.63 ± 0.04	6.62 ± 0.05
T °C	27.7 ± 0.26	27.7 ± 0.17	27.57 ± 0.66	27.60 ± 0.10	27.67 ± 0.35
pH	7.61 ± 0.42^{a}	6.41 ± 0.12^{b}	6.79 ± 0.12^{b}	6.46 ± 0.23^{b}	6.71 ± 0.09^{b}
EC (µS·cm^{-1})	31.8 ± 0.26^{a}	18.522 ± 48^{b}	19.580 ± 606^{c}	22.400 ± 332^{d}	23.826 ± 128^{e}

O_2 dissolved oxygen, *T* temperature, *EC* electrical conductivity. Values are expressed as means ± standard deviations

Table 3 Blood parameters in *Arapaima gigas* exposed to different NaCl concentrations

Parameter	Control	Concentrations ($g \cdot L^{-1}$)			
		8	9	10	11
Hb ($g \cdot dL^{-1}$)	10.61 ± 1.19^{a}	10.92 ± 0.91^{a}	10.48 ± 1.01^{a}	10.46 ± 0.78^{a}	9.19 ± 0.88^{b}
Ht (%)	27.83 ± 4.55	29.22 ± 2.90	26.43 ± 2.98	26.33 ± 2.59	31.04 ± 2.57
RBC ($\times 10^{6} \cdot \mu L^{-1}$)	1.13 ± 0.33	1.36 ± 0.23	1.40 ± 0.23	1.20 ± 0.22	1.20 ± 0.18
MCV (fL)	237.9 ± 4.46	213.07 ± 6.88	208.13 ± 36.96	225.77 ± 24.54	264.44 ± 24.12
MCH (pg)	91.08 ± 9.54	79.95 ± 9.37	74.86 ± 15.85	87.10 ± 10.88	75.90 ± 12.57
MCHC ($g \cdot dL^{-1}$)	38.46 ± 2.86^{a}	37.87 ± 1.08^{a}	36.59 ± 1.81^{a}	38.75 ± 3.35^{a}	30.53 ± 3.21^{b}
Glucose ($mg \cdot dL^{-1}$)	62.88 ± 25.17	99.39 ± 27.65	70.43 ± 22.40	49.88 ± 10.12	77.92 ± 18.68
Cortisol ($ng \cdot mL^{-1}$)	50.49 ± 16.08	67.90 ± 7.49	78.13 ± 16.77	60.11 ± 15.47	68.06 ± 20.73

Values (means ± SD) with different letters compare treatments within columns ($p < 0.05$)

Hb, hemoglobin; *Hct*, hematocrit; *RBC*, red blood cell count; *MCV*, mean corpuscular volume; *MCH*, mean corpuscular hemoglobin; *MCHC*, mean corpuscular hemoglobin concentration

Table 4 Frequency of histopathological gill changes in *Arapaima gigas* exposed to NaCl during 2-h baths

Control			Concentrations ($g \cdot L^{-1}$)			
Alterations	Stage	Control	8	9	10	11
Lamellar epithelium hypertrophy	I	0	0	0	0+	+
Lamellar epithelial hyperplasia	I	+	++	+	+	++
Proliferation of CRMs	I	0	0	0	0	0
Vascular congestion	I	+	+	+	+	++
Pillar cell system constriction	I	0	0	0	0	0
CM proliferation	I	+	+	+	+	+
Partial lamellae fusion	I	0	0+	0+	+	0+
Aneurysm	I	0	0	0	0+	+
Epithelial rupture (hemorrhage)	I	0+	0+	0+	0+	0+
Marginal canal dilation	II	0+	0+	+	+++	++
Epithelial detachment	II	0+	+	0+	+	+
Capillary constriction	II	0	0	0	0+	0
Mucus secretion	II	0	0	0	0	+
Necrosis	III	0	0	0	0	0

0, absent; *0+*, rarely present; +, less common; ++, frequent; +++, very frequent

following 10 $g \cdot L^{-1}$ NaCl exposure, statistically different ($p < 0.05$) compared to the control group (Fig. 3B). All mean NaCl concentrations were higher compared to the control group. The determined HAI values ranged from 0.05 and 0.24, with no statistical differences observed between the treatment and the control group (Fig. 3C; $p > 0.05$). MAV increases were observed at all NaCl concentrations, notably for the 8 $g \cdot L^{-1}$ NaCl exposure, although with no statistical difference (Fig. 3C).

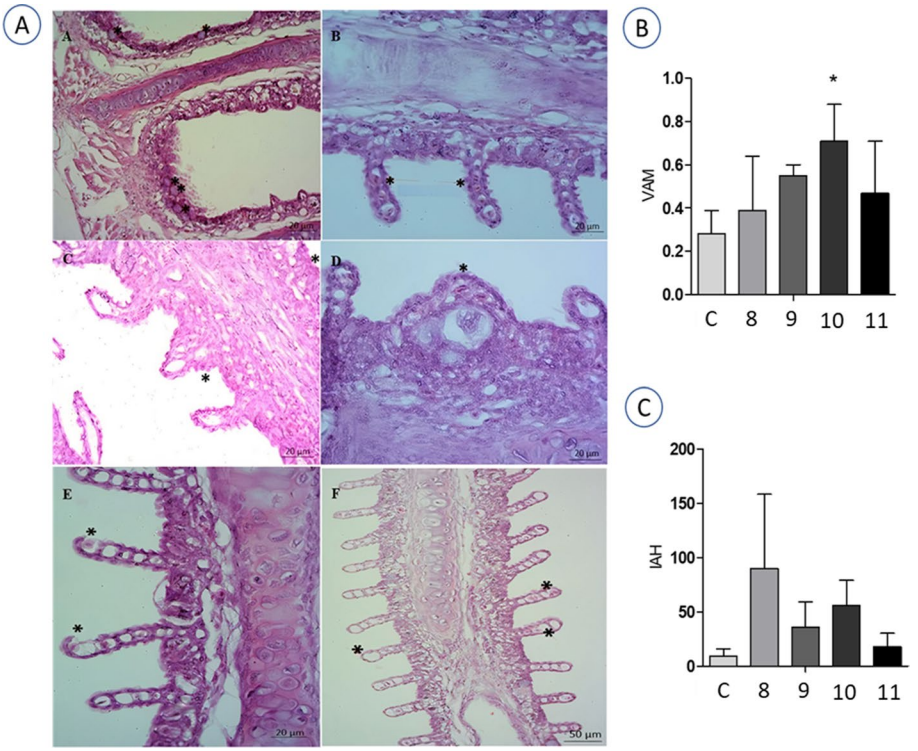

Fig. 3 *Arapaima gigas* gill histopathology following sodium chloride treatment against monogenoids. **A** Gill microphotograph of *A. gigas* exposed to different NaCl concentrations (*/H&E): (**a**) mucosal cells; (**b**) vascular congestion; (**c**) lamellar epithelium hyperplasia; (**d**) lamellar fusion; (**e**) lamellar epithelium rupture; (**f**) marginal canal dilation. **B** Mean alteration value (MAV) of the gills of *A. gigas* submitted to different NaCl concentrations. (**C**) Control. **C** Histological alteration index (HAI) of the gills of *A. gigas* submitted to different NaCl concentrations. (**C**) Control. The vertical columns in panels B and C represent the mean values of each group in the different assessment groups ($n=7$). Vertical bars represent the standard deviations of the means. Columns with (*) in common do not differ from each other at a 5% level by the Tukey tests

Discussion

In the present study, the in vitro assay demonstrated the effectiveness of NaCl against pirarucu monogenoids, allowing for a better understanding of the responses observed in the in vivo efficacy tests. The NaCl effects in the target organism indicate greater safety and reduce the number of fish required for the in vivo test (Park et al. 2014; Zorin et al. 2019; Queiroz et al. 2020). Direct NaCl exposure on pirarucu parasites led to a dose-dependent relationship, with increasing salt concentrations resulting in lower exposure times to reach 100% mortality rates. A 100% effectiveness in the 11 $g{\cdot}L^{-1}$ in vitro assay was observed at 1 HPT, corroborating previous reports for *Poecilia reticulata* (Schelkle et al. 2013) and *Bidyanus bidyanus* (Forwood et al. 2013) displaying the same parasitosis and undergoing similar salt concentration treatments.

The pharmacological effects of NaCl on aquaculture health are associated to osmotic concentration gradient, osmoregulation, and compensatory mechanisms changes, resulting in cellular dehydration and, consequently, parasite death (Kim 2012). Sodium chloride can

be absorbed by monogenoid teguments, which displays a membrane associated to the internal parasite environment and by the parasite excretory system, which contains specialized cells named flame cells comprising collecting ducts and capillaries capable of transporting chloride to the internal parasite environment (Cohen et al. 2004; Woo 2006). In the present study, the dose-dependent effect of NaCl on pirarucu monogenoids reflects the probable parasite mortality cause.

The $LC_{50\text{-}90} = 11.64$ g·L^{-1} calculated for juvenile pirarucu in the present study is higher than those reported for other species at the same growth stage. According to Zucker (1985), NaCl is considered lightly toxic for pirarucu ($> 10 < 100$ ppm), ranking 2 in a 1 to 5 toxicity scale. Our findings, however, suggest that pirarucu are highly tolerant to this compound ($LC_{50\text{-}96\,h} = 11.64$ g·L^{-1}) compared to other freshwater fish.

The low toxicity of NaCl to pirarucu indicated by the calculated $LC_{50\text{-}96\,h}$ allowed for in vivo NaCl effectiveness determinations. At the applied NaCl concentrations, a low efficacy (20.5% — 11 g·L^{-1}) against monogenoids was noted compared to the in vitro test (100% — 11 g·L^{-1}) following the same exposure period.

The effectiveness difference noted in the in vivo and in vitro tests can be attributed to the presence of biological barriers, such as mucus, teguments, and the operculum in the in vivo tests, making it difficult for NaCl to reach the parasites, interfering with product absorption. In addition, abiotic water factors can also decrease NaCl effects and interfere with its effectiveness.

Our findings and other literature reports suggest that NaCl results in differential responses in the host and parasite. Furthermore, its effectiveness is directly related to NaCl concentrations, exposure times, and/or parasite and host species sensitivities. Despite being a cheap alternative for ectoparasite control with no residual effects, NaCl should be used cautiously, respecting its tolerance limits (Tavares-Dias 2021).

Hematology, biochemistry, and histology are commonly employed to assess fish health conditions and the effect of xenobiotics (Andrade-Porto et al. 2017; Rodrigues et al. 2020; Queiroz et al. 2020; Ventura et al. 2020). Herein, no significant blood changes were observed following NaCl exposure. However, decreased hemoglobin and HCM values were noted following exposure to the highest NaCl concentration (11 g·L^{-1}). Under adverse conditions, the organism attempts to adapt by employing compensatory mechanisms, in this case increasing water cell absorption and decreasing hemoglobin concentrations. These results corroborate reports for *Cyprinus carpio* under prolonged exposure (40 days) to 3.0 g·L^{-1} NaCl, resulting in altered erythrogram values, including decreased hemoglobin and MCHC values and increased mortality rates (Mubarik et al. 2018). Prolonged exposure in *Labeo rohita* to 8.0 g·L^{-1} NaCl increased total erythrocytes and thrombocytes and hematocrit percentages and decreased hemoglobin values (Murmu et al. 2019). In another study, however, a short 15-min NaCl bath (15 g·L^{-1}) did not significantly alter *Peckoltia oligospila* blood parameters (Santos et al. 2020), while *Sander lucioperca* exposed to short baths (30 min) at 10 and 20 g·L^{-1} NaCl exhibited significant increases in hemoglobin concentrations, hematocrit percentages, and number of erythrocytes (Demska-Zakęs et al. 2021). These results suggest that NaCl alters fish oxygen transport capacity in a species-specific and dose-dependent manner. Pirarucu survival was not compromised in the present study, indicating that the fish were able to adapt to the evaluated conditions.

The different NaCl concentrations evaluated herein resulted in altered mean glucose and cortisol values, albeit not statistically different ($p > 0.05$), suggesting that NaCl does not reverse the stress situation induced by the parasitosis in all groups. In addition, higher NaCl concentrations than those tested herein induce hypercotisolemia

and hyperglycemia in other species, such as *Brycon amazonicus* (Urbinati and Carneiro 2006) and *Colossoma macropomum* (Chagas et al. 2012).

Fish gills are responsible for gas exchanges, osmoregulation, acid–base regulation, and the excretion of nitrogenous wastes (Evans et al. 2005). Their structures are sensitive and may present different responses to environmental stressors (Nilsson 2017; Rodrigues et al. 2017; Uğurlu et al. 2019). In the present study, histological changes were observed with increasing NaCl concentrations. The increased HAI values and MAV observed at 8 and 10 $g{\cdot}L^{-1}$ NaCl exposure were mainly associated to marginal gill canal dilation and lamellar epithelium hyperplasia, respectively. These changes may indicate pirarucu sensitivity to NaCl, although they were reversible, returning to normal when the stressor agent was removed from the environment (Sayed et al. 2020).

In the present study, 100% of the monogenoids present in the gills of the fish were identified as *D. cycloancistrium*, probably because this is the most common species in pirarucu, corroborating Andrade-Porto et al. (2017) and Queiroz et al. (2020). Some changes were also observed in the gills of fish belonging to the control group, probably due to the presence of monogenoid parasites. Other cases of reversible lesions caused by monogenoids in fish gills have been reported previously by Jerônimo et al. (2014) and Costa et al. (2017). Thus, despite the observed changes, therapeutic NaCl baths in juvenile pirarucu at the concentrations evaluated herein do not interfere with fish homeostasis.

The results of this work suggest that NaCl exhibits anthelmintic activity against *D. cycloancistrium* in pirarucu influenced by exposure time and concentration. Pirarucu exhibit a high tolerance to NaCl (11 $g{\cdot}L^{-1}$), with 100% survival rate during 2-h exposure, without significant hematological, hormonal, and histopathological alterations. However, the concentrations employed herein exhibit low efficacy ($<36\%$) against *D. cycloancistrium*, indicating that other studies evaluating the influence of long and/or sequenced baths employing higher NaCl concentrations are required in *Arapaima gigas*.

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Author contribution Elizabeth Gusmão Affonso: Conceptualization, funding acquisition, supervision, validation, writing — original draft preparation and review and editing.

Marieta Nascimento de Queiroz: Formal analysis, investigation, methodology, project administration, writing — original draft preparation.

Eduardo Akifumi Ono: Investigation, visualization, writing — review and editing.

Sanny Maria De Andrade Porto: Investigation, resources, writing — review and editing.

Gustavo da Silva Claudiano: Validation, supervision, writing — review and editing.

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Data availability Not applicable.

Declarations

Ethics approval The study was carried out according to Brazilian animal welfare standards and International Organization for Standardization standards (2006) and was approved by an Ethics Committee (Protocol No 013/2015). For human, not applicable.

Competing interests There is no conflict of interest between authors.

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