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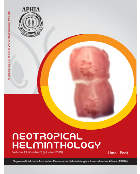

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

MORTALITY OF *ARAPAIMA GIGAS* (SCHINZ, 1822) (ARAPAIMIDAE) CAUSED BY *DAWESTREMA CYCLOANCISTRIUM* PRICE & NOWLING, 1967 (MONOGENOIDEA) FROM FISH-PONDS IN THE PERUVIAN AMAZON AND THE USE OF SALT FOR ITS TREATMENT

MORTALIDAD DE *ARAPAIMA GIGAS* (SCHINZ, 1822) (ARAPAIMIDAE) CAUSADA POR *DAWESTREMA CYCLOANCISTRIUM* PRICE & NOWLING, 1967 (MONOGENOIDEA) PROCEDENTES DE ESTANQUES DE PECES EN LA AMAZONÍA PERUANA Y EL USO DE SAL PARA SU TRATAMIENTO

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ABSTRACT

Salt is commonly recommended as an inexpensive treatment against monogenoids in freshwater fish culture; however, few studies have scientifically evaluated its efficacy. In the present study we tested the efficacy of salt treatments against *Dawestrema cycloancistrium* Price & Nowling, 1967 infestation on farmed "pirarucu" (*Arapaima gigas* (Schinz, 1822)). Fish-farmers from the city of Iquitos, in Peru started to notice mortalities on the *A. gigas* cultivated in their ponds. In order to determine to cause of the mortalities, 26 samples from 13 fish-farmers were sent to the "Laboratorio de Parasitologia y Sanidad Acuícola" of the "Instituto de Investigaciones de la Amazonía Peruana", Iquitos-Peru. Gills analyzed revealed high infestation levels by *D. cycloanistrium*. To treat infested fish, salt in three concentrations were tested: T1 = 15 g·L^{-1}; T2 = 25 g·L^{-1}; and T3 = 30 g·L^{-1}. Treatment 3 (30 g·L^{-1}) was most effective against the infestation of *D. cycloancistrium*. The number of parasites detached and counted 24 h after the application of treatments was higher than 30 min after, showing that parasites continue detaching from the gill of their hosts, even 24 h after the application of salt. Other concentrations of salt and different exposure times need to be tested, in order to find the most effective and safest treatment for *A. gigas* infested by *D. cycloancistrium*.

Keywords: fish-farm – Iquitos – Monogenoid – Parasite – sodium chloride

RESUMEN

La sal se recomienda comúnmente como un tratamiento económico contra los monogenoideos en peces de agua dulce; sin embargo, pocos estudios han evaluado científicamente su eficacia. En el presente estudio probamos la efectividad del tratamiento con sal contra la infestación de *Dawestrema cycloancistrium* Price & Nowling, 1967 en paiches (*Arapaima gigas* (Schinz, 1822)) provenientes de cultivo. Piscicultores de la ciudad de Iquitos, Perú, comenzaron a notar la mortalidad en especímenes de *A. gigas* cultivados en sus estanques. Para determinar la causa de la mortalidad, se enviaron 26 muestras de 13 piscicultores al Laboratorio de Parasitología y Sanidad Acuícola del Instituto de Investigaciones de la Amazonía Peruana, Iquitos-Perú. Las branquias analizadas mostraron altos niveles de infestación por *D. cycloanistrium*. Para el tratamiento de peces infestados, se probó la sal en tres concentraciones: T1 = 15 gL^{-1}; T2 = 25 gL^{-1}; y T3 = 30 gL^{-1}. El tratamiento 3 (30 gL^{-1}) demostró ser el más efectivo contra la infestación por *D. cycloancistrium*. El número de parásitos liberados y contados 24 horas después de la aplicación de los tratamientos fue superior a los 30 minutos posteriores a la aplicación del tratamiento, lo que demuestra que los parásitos continúan separándose de los arcos branquiales de los huéspedes hasta 24 horas después de la aplicación de la sal. Es necesario evaluar otras concentraciones de sal y diferentes tiempos de exposición para encontrar el tratamiento más efectivo y seguro para *A. gigas* infestadas por *D. cycloancistrium*.

Palabras claves: Cloruro de Sodio – Iquitos – Monogenoideo – Parásito – Estanque de peces

INTRODUCTION

Not all species marketed in the Peruvian Amazon come from fishing in natural environments; some of them come from aquaculture production that tries to compensate the decrease of natural resources, exploited in an unsustainable manner. This activity in the Peruvian Amazon has been experiencing a constant growth of more than 15% per year for a decade. Within the main cultivated species stands out the arapaima *Arapaima gigas* (Schinz, 1822) (Arapaimidae), known in Peru as "paiche" (García-Dávila *et al.,* 2018).

The arapaima has a high economic potential in fish farming, both for the production of meat with a view to national and international markets, and for the production of fingerlings for exportation to different countries of the world (García-Dávila *et al.,* 2018).

With the intensification of breeding systems, there is a need for greater knowledge about the appropriate management to provide improvement in fish health conditions, especially in the early stages of production, larviculture and fish farming. At these stages, when fish are continually exposed to adverse conditions and their immune system still does not respond properly, they become more susceptible to parasites and diseases (Tavares-Dias & Martins*,* 2017).

The lack of information, mainly on the production and management of arapaima, has markedly impeded the development of fish farming (Imbiriba, 1991). Within the obstacles found for the production of arapaima, parasitic diseases play an important role in the quantity and quality of fish production (Gaines *et al.,* 2012). Monogenoideans are ectoparasites with present a direct life cycle and can rapidly multiply and disperse in fish ponds, reaching high intensities. These parasites are responsibles of major losses in fish culture (Flores-Crespo & Flores, 2003). In the Peruvian Amazon there are reports concerning to high infestation by species of Monogenoidea, with cases of mortalities and economic losses in fish-farmers.

In fish-farming, the salt (sodium chloride) is used as a mediator of stress when fish are manipulated (Lim & Webter, 2001) and for treating parasitic diseases. It is less harmful to fish hosts compared to other anti-parasitic treatments, such as formalin or malachite green and its low cost and availability make it the recommended treatment against a variety of fish diseases. Nevertheless, despite its practical use there have been few studies to test the effective concentration against monogenoideans.

Taking into consideration the importance of *A. gigas* for aquaculture, the risk of infestations by monogenoideans and the possibility of using salt for its treatment, the present study evaluated the parasitism of *Dawestrema cycloancistrium* Price & Nowling, 1967 on this fish species, by assessing the impact imposed by the parasite on its host by calculating parasitological indices and by testing the use of salt in different concentrations against this parasite.

MATERIAL AND METHODS

Study area and Fish

Twenty-six *A. gigas* (21.5 ± 6.5 cm average length; 70.7 ± 15.8 g average weight) were collected from thirteen fish ponds located along the Iquitos-Nauta highway in March 2019 (Fig. 1). All specimens of *A. gigas* collected belong to one producer, who distributed all fishes to fish-farmers in February 2019. One week after receiving the fishes, all fish-farmers started to notice mortalities on some specimens of *A. gigas.* Mortalities didn't stop, so, fish-farmers decided to send samples for parasitological analyses to the laboratory of "Parasitología y Sanidad Acuícola" of the "Instituto de Investigaciones de la Amazonía Peruana" (IIAP) in Iquitos, Peru. Two specimens of *A. gigas* were analyzed from each fish pond.

Collection, identification of parasites and parasitological indices

In order to determine the species of parasites in *A. gigas* and to evaluate the parasitic load, the skin, gills and internal organs were analyzed. Samples of the skin were taken by using a spatula and observed under microscope. Gill archers were removed and observed under microscope. To analyze internal organs, a longitudinal cut from the anus to the operculum opening was made. Organs were placed individually in Petri dishes and analyzed under stereoscope.

Gill archers were removed and placed in vials containing heated water (68 °C). Each vial was shaken vigorously and 96% ethanol was added (final concentration approximately 75-80%). The content of each vial was examined using a dissecting microscope. Helminths were removed from the gills or sediment using dissection needles. Some specimens were stained with Gomori's trichrome (Humason, 1979) and mounted in Dammar's gum, to determine internal morphology, while others were mounted in Hoyer's mounting medium (Humason, 1979), for the study of sclerotized structures. Illustrations were prepared with the aid of a microprojector. Sclerotized structures of all parasites were photographed with a digital camera (Axio Cam ERc 5S) connected to a microscope (ZEISS Primo Star). The identification of the species was based on the taxonomic keys of Kritsky *et al.* (1985). The ecological terms in parasitology followed those provided by Bush *et al.* (1997). In order to compare the results of each treatment, an ANOVA – Tukey Test was conducted by using the software BioEstat 5.3.

In vivo tests with A. gigas exposed to of salt

The experimental design was a completely randomized block with three treatments and three replicates with one fish each. In vivo tests consisted of therapeutic baths of 30 minutes with three concentrations of salt: T1 = 15 $g{\cdot}L^{-1}$; T2 = 25 $g{\cdot}L^{-1}$; T3 = 30 $g{\cdot}L^{-1}$. Therapeutic baths were performed in 50 L glass aquarium, with a static water system and constant aeration. After 30 min of treatment, fishes were placed in other glass aquariums with clean water.

To evaluate the effectiveness of salt in each treatment, parasites released into the water were counted. For this, the water from each aquarium was filtered in qualitative Whatman filter papers n° 1, in order to visualize possible monogenoids 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. In order to continue testing the effectiveness of salt twenty-four h after the application of each treatment, the water of each experimental unit was filtered again and analyzed. This process was repeated during two days.

Physical and chemical parameters

Samples of water from fish-ponds were taken to the lab in order to measure the values of Nitrite and Ammonium (ppm). Those parameters were measured by using a LAMOTE testing kit. Additionally, information concerning to temperature ($T^{O}C$), dissolved oxygen (ppm) and pH were measured daily by fish-farmers. That information was provided to evaluate the influence of water quality on the infestation of monogenoids.

Ethic aspects
Statement on ethical approval from an ethics committee and license for working with fish species were followed according to the following resolutions: Resolution No132-2014-GRL-DIREPRO; Resolution No21-2016 GRL-DIREPRO; and PTH-068-16-PEC-SANIPES.

RESULTS

Identification of the parasite species and parasitological indices
The monogenoidean *Dawestrema cycloancistrium* Price & Nowling, 1967 was identified parasitizing the gill filaments of specimens of *A. gigas,* with a prevalence of 100%, 6320 parasites recorded with an intensity of 79-880, and mean abundance of 243.1 ± 164.1 (Table 1). The mean number of parasites recorded was 486 ± 287.

The main characteristics of the species are: copulatory complex includes a tubular cirrus with expanded base that coils 6 times, accessory piece terminally enclosing cirrus shaft. Vagina tubular, ventrolateral, sclerotized, proximally coiled, with distal petal-shaped sclerotization protruding from aperture. Egg elongate ovate, with proximal filament; exceptionally long. Ventral anchor robust, with elongate straight point, heavy base, ornate deep root, superficial root with conspicuous saddle-like fold; dorsal anchor with curved point and shaft, fold of superficial root weakly developed. Ventral bar plate-like, with anterior medial projection arising near posterior margin; Dorsal bar with globose ends, heavy ridge along posterior margin (Figure 2).

Table 1. Parasitological indices of *Dawestrema cycloancistrium* Price & Nowling, 1967 in infested *Arapaima gigas* (Schinz, 1822). AF = analyzed fish; PF = parasitic fish; P% = prevalence; I = intensity; mI = mean intensity; mA = mean abundance

Fish Farms (FF)	AF	PF	P%	I	mI	mA
FF 1	2	2	100	299 (140-159)	149.50	149.50
FF2	2	2	100	231 (79-159)	115.50	115.50
FF3	2	2	100	251 (96-155)	125.50	125.50
FF4	2	2	100	626 (294-332)	313.00	313.00
FF5	2	2	100	473(231-242)	236.50	236.50
FF6	2	2	100	583(269-314)	291.50	291.50
FF7	2	2	100	1188(308-880)	594.00	594.00
FF8	2	2	100	229(98-131)	114.50	114.50
FF9	2	2	100	682(321-361)	341.00	341.00
FF10	2	2	100	699(333-366)	349.50	349.50
FF11	2	2	100	242(84-158)	121.00	121.00
FF12	2	2	100	199(97-102)	99.50	99.50
FF13	2	2	100	618 (260-358)	309.00	309.00

Efficacy of salt against the infestation of D. cycloancistrium

Treatment 3 (T3 = 30 g·L^{-1}) showed the best results after counting the detached parasites 30 min and 24 h after the application of each treatment. The number of parasites counted 24 h after was higher than 30 min after. Additionally, in day 2, the number of parasites counted was considerably reduced (table 2). The mean number of parasites detached recorded after two days of treatment was: T1 = 16; T2 = 52 and T3 = 167. Tukey test results showed: T1 and T2 = $p > 0.05$; T1 and T3 = $p < 0.01$ and T2 and T3 = $p < 0.05$.

Physical and chemical parameters

Fish-farmers registered a mean temperature of 28 ± 2 ^{O}C, dissolved oxygen of 3.5 ± 1.5 mg·L^{-1}, pH of 6.5 ± 0.6. Water samples revealed high values of Nitrite (> 0.05 ppm) and ammonium (> 0.1 ppm).

Table 2. Number of parasites counted 30 minutes and 24 hours after the application of each treatment. T = treatment; R = replica; T1 = 15 g·L^{-1}; T2 = 25 g·L^{-1}; T3 = 30 g·L^{-1}

	Day 1		Day 2		
Treatments	Number of parasites counted 30 min after	Number of parasites counted 24 h after	Number of parasites counted 30 min after	Number of parasites counted 24 h after	Sum of Parasites
T1 R1	2	4	1	1	8
T1 R2	4	15	1	1	21
T1 R3	3	7	6	3	19
T2 R1	18	83	6	2	109
T2 R2	9	24	2	0	35
T2 R3	1	7	3	1	12
T3 R1	65	152	0	0	217
T3 R2	28	105	2	0	135
T3 R3	6	138	7	0	151

Figure 1. Lateral view of specimen of *Arapaima gigas* (Schinz, 1822). Scale bar = 10 cm.

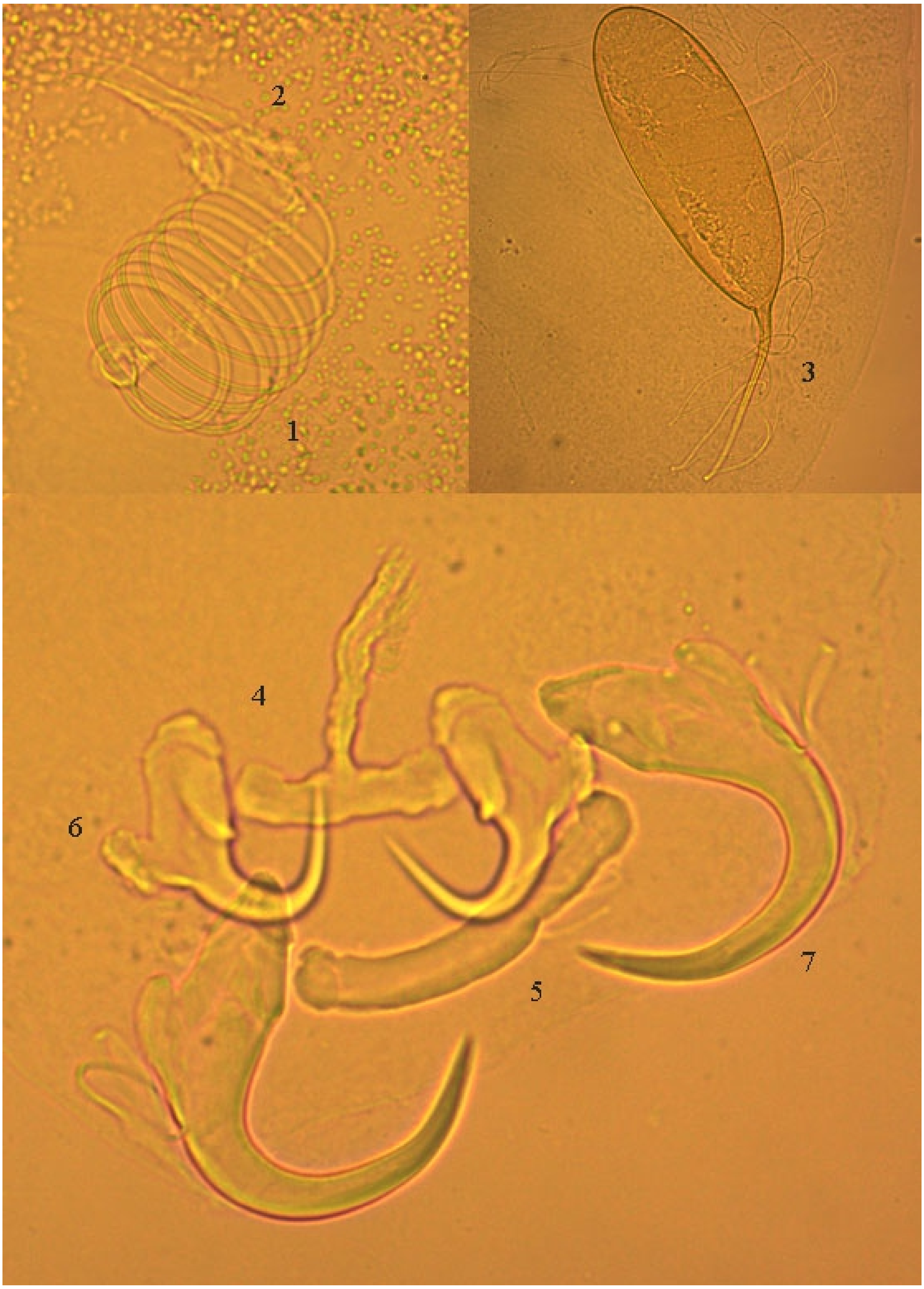

Figure 2. Sclerotised structures of *Dawestrema cycloancistrium* Price & Nowling, 196. 1. Male copulatory organ (MCO); 2. Accessory piece; 3. Egg; 4. Ventral bar; 5. Dorsal bar; 6. Ventral anchor; 7. Dorsal anchor. Scale bar: 1 – 2 = 100µm; 3 = 100µm; 4 – 7 = 30µm.

DISCUSSION

In Peru, there are reports of high levels of infestation by *D. cycloancistrium* affecting specimens of *A. gigas* from fish-farming (Iannacone & Luque, 1991; Mathews *et al.,* 2013; Mathews *et al.,* 2014; Serrano-Martínez *et al.,* 2015). In the present study, this monogenoid was identified parasitizing the gills of *A. gigas* from different fish-farms located in Iquitos, Peru.

Fish cultivated in fish-farms are exposed to poor water quality, crowding, inadequate manipulation and many other stressors which may negatively affect their immune system and consequently their resistance to parasitological diseases (Sado *et al.,* 2010, Jha *et al*., 2007). In the present study, high values of Nitrite and Ammonium were registered in the samples collected from the ponds. We assume that poor water quality influenced negatively in the health of the *A. gigas,* becoming the fish more susceptible to infestation by monogenoids.

Lentic environments favor the transmission of monogenoids (Flores-Crespo & Flores, 2003). In regions with tropical weather, the life cycle of monogenoids can be completed in h. In this way, parasites proliferate rapidly, increasing the transmission from one individual to another. According to the owners of the sampled fish-farms, temperatures registered daily oscillate between 27 to 29 ^{O}C, favoring the speed of life cycle of *D. cycloancistrium*, justifying the high parasitological indices recorded.

Pavanelli & Takemoto (2008) recommended the use of salt in concentrations between 1 to 3% during 60 min for treating ectoparasites infestations. According to Vargas *et al.* (2003), the treatment using 3% of salt for 10 min is efficient against the parasitism by species of *Gyrodactylus* von Nordmann 1832 but is less efficient against species of *Dactylogyrus* Diesing, 1850. Kubitza (2000) suggested a higher concentration of salt (3.5 to 5% for five to 10 minutes) and Cone (1995) recommended using 4.5 to 5% of salt for 2.5 minutes. The results of those authors showed that the use of one product can be effective for some species but ineffective for others. In this way, it is important to test the effectiveness of one product for a defined species because not all species can react in the same way against any treatment.

Exposing freshwater organisms to saline conditions disrupts their osmoregulation, resulting in water loss and dehydration. Ectoparasites or free-living parasitic stages are more severely affected by such disruption in osmoregulation compared to their fish host (Shephard, 1994). Salt baths using high concentrations during a short exposition time act aggressively against parasites and are more effective than long-term baths (Bakke *et al.,* 2007). In the present study the treatment using the highest concentration of salt showed the best results. The absence of motility and the detachment of the parasites during the application of the treatment and 24 h later confirm the efficiency of salt in high concentrations against the infestation of *D. cycloancistrium*.

Salt is a safer treatment option in aquaculture compared to other broad anti-parasitic treatments such as formalin or malachite green, despite reports of increased mortalities amongst fish (Buchmann, 1997). In the current study, no mortalities occurred during the application of treatments but we consider necessary to test other concentrations of salt and different exposition times, in order to find the most effective and safer treatment for *A. gigas* infested by *D. cycloancistrium*.

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