

Parasitic infections in juveniles of *Arapaima gigas* (Schinz, 1822) cultivated in the Peruvian Amazon

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ABSTRACT. The paiche, *Arapaima gigas* represents a socio-economically important species in the Peruvian Amazon, and actually an intensive production for human consumption has emerged during the last years. Therefore, more studies are required in fish farming development, especially concerning populations of parasites that affect fish production yields. Eighty specimens of paiche collected between September and October of 2011 from semi-intensive fish farm in Loreto State, Peru, were examined for their helminthic parasites. Five species were recorded parasitizing *A. gigas*: *Dawestrema cycloancistrium* and *Dawestrema cycloancistrioides* (Monogenea) on gills, *Trichodina* sp. (Protozoa) on the skin, *Caballerotrema arapaimense* (Trematoda) in stomachs and *Philometra senticosa* (Nematoda) in the swim bladder. Highest prevalence was recorded for *D. cycloancistrium* (100.0%), *D. cycloancistrioides* (83.0%) and *Trichodina* sp. (50.0%) and highest values of mean intensity and mean abundance were recorded for *D. cycloancistrium* (260) parasites per individual. The results confirm the necessity of constant monitoring of fish, seeking the diagnosis and timely control of infestations with parasites, in order to eradicate the mortality of the host that leads unviable the fish farming intended for human consumption.

Key words: *Arapaima gigas*, fish parasites, aquaculture, Peruvian Amazon

Introduction

In fish farming, the intensive exploitation allows the handling of high densities of organisms per unit area. Indeed, this type of management frequently leads to breakage the balance between pathogen and host, consequently resulting in the emergence of infectious and parasitic diseases that cause various problems ranging from slow up growth, reduced fertility rates, and occurrence of severe epidemics that resulting in high mortality [1–3].

The *Arapaima gigas* Schinz, 1822 (Osteoglossiformes: Osteoglossidae)known as paiche or pirarucu is a species endemic of the Basin Amazon and is listed in Appendix II-B of the Convention on the International Trade in Endangered Species (CITES) [4]. The *A. gigas* can reach up to three meters in length and 200 kg of total weight [5–7] and is a much appreciated species with great acceptance on the Amazonian market being regarded as a food fish of the highest quality. In the Peruvian Amazon the *A. gigas* is considered a species with great potential for management in controlled environments aiming human nutrition and ornamental purposes. However, to allow the breeding to become entirely feasible, it turns out the necessity to solve the problem of diseases and parasites upsurges affecting this species in controlled environments, a consequence of intensive farming under inadequate management.

The increment of parasitic infections in the aquaculture environment has also been associated with the low quality of water and inadequate management. All these environmental factors have been responsible for high infection with monogenean, copepods and Argulidae in fish cultivated in Peru [8–10]. Therefore, with the gradual increase of intensive and semi-intensive fish farming in the Peruvian Amazon, there is a need for constant monitoring of the fish for the diagnosis and timely control of infestations with parasites. In this sense, the present study aims to evaluate the parasitic infection in *A. gigas* bred in a fish farm of the Peruvian Amazon.

Materials and Methods

Between September and October 2011, which corresponds to relative dry season, 80 individuals of the species *A. gigas* born in captivity were collected in two earthen ponds of 120 m^2 with drag nets, from a semi-intensive fish farm, belonging to the fish aquaculture station Quistococha Research Center (CIQ), of the Institute for Amazonian Research (IIAP), located in the northeast of Loreto State: 3°48'48.9" N and 073°19'18.2" W.

The physicochemical parameters of the water were measured three times daily (at 8 AM, noon and at 4 PM) with daily checks of dissolved oxygen, pH, temperature and conductivity by means of an YSI multiparameter meter (Model MPS 556). Ammonium values, hardness, carbon dioxide and total alkalinity were monitored weekly in the morning at 8 AM, using a complete package for analysis of freshwater (LaMotte AQ-2).

Fish were fed twice daily with extruded diet containing 25% crude protein and 2.6 Mcal/kg of digestible energy and feeding rate of 5% of the biomass of the pond. The sampled fish presented length of 18.50±0.10 cm and weight of 86.06±0.86 g; these data were collected in individual records. Following, the fish were sacrificed by cerebral puncture and placed in individual containers.

Using a stereoscope the body surface, fins, nostrils, mouth, opercula and gills were examined for possible injuries and excess of mucus production. By means of a scalpel, we also performed scraping of the skin, fins and gills to observe any attached parasites.

For examination of the gills, the samples were separated and placed in glass containers with a 1:4 formalin solution. After one hour, the gills were stirred and then removed from the container. Helminths were allowed to settle and were subsequently collected with the aid of a small probe and a dissecting microscope (Nikon SM-30). For parasitological examination of their stomachs and intestines, the organs were removed and placed in Petri plates containing distilled water and then examined. The counting of protozoa from the skin scrapings of each fish was performed using McMaster chamber under light microscopy and estimated by evaluating the number of parasites/field. For study of sclerotized structures, parasites were fixed in a solution of ammonium picrate glycerine (GAP) and mounted in Canada balsam according to Malmberg [11] and Ergens [12]. Some specimens were mounted unstained in Gray and Wess' medium. To visualize internal structures, parasites were fixed in hot formaldehyde solution (4%) and stained with Gomori's trichrome.

The identification of the parasites was based on the methodology of Thatcher [13], Kritsky et al. [14] and Moravec [15]. The parasitic indexes calculated for assessement of the level of infestation of parasites were; prevalence, mean intensity and mean abundance [16,17].

Results

The values of the physicochemical parameters of the water in the culture ponds were: dissolved oxygen (5.64±0.8 mg L^{-1}), pH (4.83±0.10), temperature (27.23±1.50°C) and conductivity (106.1±14.0 µs cm^{-1}). Ammonium values (0.20±0.10 mg L^{-1}), hardness (21.40±1.80 mg L^{-1}), carbon dioxide (3.2±0.9 mg L^{-1}) and total alkalinity (16.14±0.80 mg L^{-1}).

The necropsy of juvenile of *A. gigas*, evidenced the infestation by monogeneans of *Dawestrema cycloancistrium* Price et Nowlin, 1967 and *Dawestrema cycloancistrioides* Kritsky, Boeger et Thatcher, 1985 (Monogenea: Dactylogyridae) on the gill filaments of the fish. The monogenean species with the highest prevalence was *D. cycloancistrium* parasitizing all sampled fish (80), with the mean intensity equal to the mean

Table 1. Values of prevalence (P), mean intensity (MI) and mean abundance (MA) of parasites collected from juveniles of *Arapaima gigas* cultivated in the Peruvian Amazon

Parasites	P	MI	MA	Site of infection
Dawestrema cycloancistrium	100.0%	260	260	Gills
Dawestrema cycloancistrioides	83.0%	6.9	5.7	Gills
Trichodina sp.	50.0 %	159.7	95.8	Skin
Philometra senticosa	30.0%	19.5	4.9	Swim bladder
Caballerotrema arapaimense	18.0%	5.6	4.5	Stomach

abundance (260). *D. cycloancistrioides* showed the lowest abundance (Table 1).

Endoparasites *Philometra senticosa* Bayles, 1927 (Nematoda: Philometridae) and *Caballerotrema arapaimense* Thatcher, 1980 (Trematoda: Echinostomatidae) were found in the swim bladder and stomachs, respectively. In the skin, the greatest occurrences were observed for *Trichodina* sp. (Protozoa: Ciliophora) (Table 1).

Discussion

The increment of parasitic infections in breading environments has been associated with low quality of water and inadequate management [18,19]. According to Buschmann [20] and Mariano et al. [21], intensive and semi-intensive fish farming generates a large accumulation of organic matter on the pond bottom produced from the excreta, dead matter and uneaten food. This organic matter produces hypoxia and anoxia that creates an unbalance in the homeostasis of fish, eventually leading to the increase of the oxidative stress of biomolecules, promoting various physiological and biochemical alterations and causing cell impairment and death [22,23]. In the present study, in the ponds of cultivated *A. gigas* the physical and chemical parameters of the water were within the expected range of values for tropical species. However, high prevalence and intensity of ectoparasites were observed. We suspect that the high parasitism by ectoparasites was due to the imbalance in the homeostasis of fish. *A. gigas* shows the habit of breathing oxygen directly from the atmosphere which makes these animals coming to the water surface constantly. This need to come to the water surface brings some disadvantages for a juvenile *A. gigas*, because fish are more exposed to attack by winged predators (birds and bats), fish are exposed to water supersaturated oxygen (> 200 mg L^{-1}), high pH in the afternoon (> 9.0), and high concentrations of toxic ammonia (> 0.5 mg/L) in excessively fertilized ponds. The fish may also be exposed to hyperthermia or superheated water, common in the superficial portion of the water in ponds, sometimes exceeding 35°C [24] leading them more susceptible to infestation by parasites.

The *A. gigas* has a diverse fauna of parasites, including species of myxozoa, monogeneans, nematodes, cestodes, digeneans, crustaceans, acanthocephalan and pentastomids. However, dealing mostly with taxonomic descriptions were conducted on specimens collected in rivers of the Amazon [1,14,25,26].The *A. gigas* has piscivorous habits, but their food items vary according to their development, starting with the ingestion of zooplankton, shellfish, and finally fish represent the most important item of their diet [27]. In the Peruvian Amazon, for breading *A. gigas*, the fish are initially fed with phytoplankton and zooplankton (fingerlings stage), followed by small crustaceans, molluscs, forage fish and extruded food (< 18 cm length/juvenile stage) and finally extruded food (juvenile stage – 18 cm length and adult stage). In the present study, three ectoparasites and two endoparasites were identified in *A. gigas*. The presence of several groups of parasites in farmed *A. gigas* may be related to the presence of various nutritional items. The animals that sustain the diet, may act as intermediate hosts of trematodes, cestodes, nematodes and acanthocephalans. Indeed, it is also known that the diet and feeding habits of the fish are considered important for determining composition of their parasite fauna, and may have a strong influence on the population of parasites transmitted trophically [28–31].

Parasites that have a direct life cycle, such as monogeneans and protozoan, are more frequently found in lentic environments as this type of environment favors the transmission of these parasites [31]. In regions of tropical and semi-tropical climate, the life cycle of ectoparasites can be completed in less than one day and they can proliferate explosively [32]. The climate in the region of this study is tropical humid with annual temperature averaging in 28.3°C with relative humidity of 85%, which favors acceleration of the life cycles. In the earthen ponds where fish were collected, the water circulation is almost negligible or nonexistent and these ponds have high density of fish. This drawbacks favor the contact with ectoparasites [32,33], and may explain the fact that fishes have shown elevated infestation with monogenean and protozoa.

Among the various groups of helminthes which parasitize freshwater fishes, monogeneans are represented by many species and cause considerable economic losses in fish farming in different regions of the world [32,34]. In *A. gigas* three species of monogeneans assigned to *Dawestrema* genus (i. e., *D. cycloancistrium*, *D. cycloancistrioides* and *D. punctatum*) have been reported from natural environments and two from fish farm (i. e., *D. cycloancistrium* and *D. cycloancistrioides*)

[1,14,35,36], evidencing a high specificity of *Dawestrema* species parasitizing *A. gigas*. However, this specificity may be related to the fact that many of monogeneans which parasitize fish are host-specific, because of co-evolution with their hosts [37].

The Monogenoidea *D. cycloancistrium* and *D. cycloancistrioides* were previously described by Araujo et al. [35] and Mathews et al. [38] parasitizing the gills of *A. gigas* fingerlings collected from fish farms of the state of Amazonas, Northern of Brazil and state of Loreto, Northern of Peru, respectively. Furthermore, Bonar et al. [36] identified specimens of *D. cycloancistrium* in gills of *A. gigas* juveniles from an aquarium in the United States, confirming the occurrence of this parasite in cultivated *A. gigas*.

In the present study, *D. cycloancistrium* showed high parasitism along with prevalence rates of 100%, and mean intensity and mean abundance of 260. Our results agree with Araujo et al. [35], who reported similar prevalence, with mean intensity and mean abundance of 280 for *A. gigas* collected from fish farming. In addition, Bonar et al. [36] reported a prevalence of 100% without informing, however, other parasitic indexes. Notwithstanding, Mathews et al. [38] have described a prevalence of 100% in *A.gigas* fingerlings collected from fish farms in Northeast of Peru.

The presence of a monogenean species in a given host may be influenced by numerous factors. It is known that the monogeneans have various forms of fixation which are related to mechanical and chemical factors that stimulate the permanence of the parasites on their hosts [39,40]. According to Buchmann [41], the mucous cells of the hosts contain proteins, polypeptides and carbohydrates which are responsible for the recognition of the host and facilitate the encounter between the parasite and the host. However, there may be no competition between these species of monogeneans on the gills of *A. gigas* in general. Indeed, there is no competition in the distribution of congeneric monogeneans [42]. This fact could explain a high prevalence of two monogenean *Dawestrema* species on gills of *A. gigas*.

Species from the Philometridae and Echinostomatidae families can use several paratenic or transport hosts until they reach their definitive host and complete their development. Therefore, fish can be either definitive or intermediate host for these parasites. The prevalence and intensity of these endoparasites were lower than the ones described for this same species in natural environment [25,26]. However, the low prevalence and intensity of endoparasites in *A. gigas* found in the present study may be related by absence of arthropods and molluscs in the cultured ponds. It is known that nematodes and digeneans generally use arthropods and molluscs as intermediate hosts, infecting various animals that sustain proteins in their diet [43,44]. Furthermore, Poulin [45] stated that in fish, the richness of endoparasites is proportional to the amount of animal food in the diet of the host.

The results in the present study indicated a high prevalence and abundance of monogenean *D. cycloancistrium*, *D. cycloancistrioides* and protozoa *Trichodina* sp. The results confirm the necessity of constant monitoring of fish, seeking the diagnosis and timely control of infestations by parasites, in order to eradicate mortality of the host that leads unviable the fish farming intended for human consumption.

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