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MASSIVE INFESTATION BY *GUSSEVIA UNDULATA* (PLATYHELMINTHES: MONOGENEA: DACTYLOGYRIDAE) IN FINGERLINGS OF *CICHLA MONOCULUS* CULTURED IN THE PERUVIAN AMAZON

INFESTACIÓN MASIVA POR *GUSSEVIA UNDULADA* (PLATYHELMINTHES: MONOGENEA: DACTYLOGYRIDAE) EN ALEVINOS DE *CICHLA MONOCULUS* CULTIVADO EN LA AMAZONÍA PERUANA

Patrick Mathews Delgado^{1*}, John Paul Mathews Delgado² & Rosa Ismiño Orbe³

 ¹University of Strasbourg, Institute Charles Sadron, Department of Biomedical Sciences, 67034, Strasbourg, France. patrickmathews83@gmail.com; Phone:+33 (0) 388414219; Fax:+33 (0) 388414099.
 ²National Institute of Amazonian Research, Department of Ecology, 69060-001 Manaus, Amazonas, Brazil.
 ³Research Institute of the Peruvian Amazon, Department of Aquatic Ecosystems, 265556 Iquitos, Loreto, Peru.

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Abstract

Cichla monoculus (Spix & Agassiz, 1831) is a species with great potential for breeding in controlled environments in the Peruvian Amazon. This fact that has led to semi-intensive and intensive production intended for human consumption. However, more studies are required regarding the population of parasites that are affecting breeding and development of the species. This study identified a high infestation of a monogenean species, *Gussevia undulata* (Kritsky, Thatcher, Boeger, 1986), in semi-intensively fish farming of *C. monoculus*. The prevalence was 100%, with mortality of all fish. The mean intensity and mean abundance of the parasite was 168.5 of parasites per individual. This is the first report of high infestation by *G. undulata* in *C. monoculus* cultured from the Peruvian Amazon.

Keywords: Cichla monoculus - fish farming - Gussevia undulata - infection - monogenea - parasite.

Resumen

Cichla monoculus (Spix & Agassiz, 1831) es una especie con gran potencial para su crianza en ambientes controlados en la Amazonía Peruana, hecho que ha llevado a su producción semi-intensiva e intensiva destinada al consumo humano. Sin embargo, se necesitan más estudios sobre la población de parásitos que afectan la producción y desarrollo de la especie. Este estudio identificó una alta infestación de monogeneos de la especie *Gussevia undulata* (Kritsky, Thatcher, Boeger, 1986) en un cultivo de *C. monoculus*. La prevalencia fue del 100%, con una mortalidad de todos los peces. La intensidad media y la abundancia media del parásito fue 168,5 parásitos por pez. Este es el primer informe de una alta infestación por *G. undulata* en *C. monoculus* cultivado en la Amazonía peruana.

Palabras clave: Cichla monoculus - Gussevia undulata - infección - monogeneo - parásito - piscicultura.

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 break 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, until the appearance of severe epidemics resulting in high mortality (Thatcher, 1991; Pavanelli *et al.*, 1998; Scholz, 1999; Cable *et al.*, 2002; Lemos *et al.*, 2007; Araujo *et al.*, 2009; Del Rio-Zaragosa *et al.*, 2010).

Cichlids have wide geographical distribution. Currently there are 1533 known species, with 320 reported for South America (Kullander, 1988). These species inhabit a wide variety of aquatic ecosystems. Moreover, the fish represent high economic importance, given that they are marketed for human nutrition with a promissing potential for intensive and extensive aquaculture (Kullander & Ferreira, 2006; Araujo *et al.*, 2009).

Cichla monoculus (Spix & Agassiz, 1831) can reach up to 70 cm in length and 9 kg of total weight (Chellappa et al., 2003; Keith et al., 2000) and is a much appreciated species with great acceptance on the Amazonian market being regarded as a food fish of the highest quality. Due to its zootechnical characteristics, the C. monoculus 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 upsurge affecting this species in controlled environments, as a consequence of intensive farming under inadequate management (Varella & Malta, 1995).

Among the various groups of helminthes which parasite fishes from freshwater, the monogeneans, represented by many species, cause substantial economic losses in fish farms around the world (Jones, 2001; Marcogliese *et al.*, 2001). An important characteristic is that the fish parasitized by monogeneans, show necrosis and bleeding wounds in the integument and gills, serving as a gateway to different pathogenic protozoa and bacteria of very difficult controlling (FloresCrespo & Flores, 1993; Harris *et al.*, 1998; Del Rio-Zaragoza *et al.*, 2010). It represents, thus, a serious concern, since monogeneans affect a huge variety of Cichlids in tropical and semitropical regions where parasites are favored by ecological conditions (Martins *et al.*, 2002; Flores-Crespo & Flores, 2003).

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 by monogeneans. In this sense, the present study aims to evaluate the monogenean infestation in *C. monoculus* bred in a fish farm in the Peruvian Amazon.

MATERIAL AND METHODS

Between September and October 2011, which corresponds to the relative dry season, 50 individuals of the species *C. monoculus* were collected with drag nets, from a semi-intensive fish farm, located in the northeast of Loreto (Peru), between latitudes 3° 48' 48.9" N and 073° 19' 18.2" W, with average annual temperature of 26.3 °C and relative humidity of 85% at 328 masl.

The physicochemical parameters of the water were measured three times daily (at 8 AM, noon and 4 PM) with daily checks of dissolved oxygen (5.64 \pm 0.8 mg L⁻¹), pH (4.83 \pm 0.10), temperature (27.23 \pm 1.50 °C) and conductivity (106.1 \pm 14.0 µs cm⁻¹) by means of a YSI multiparameter meter (Model MPS 556). Ammonium total (0.20 \pm 0.10 mg L⁻¹), hardness (21.40 \pm 1.80 mg L⁻¹), carbon dioxide (3.2 \pm 0.9 mg L⁻¹) and total alkalinity (16.14 \pm 0.80 mg L⁻¹) were monitored weekly and in the morning (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⁻¹ of digestible energy and feeding rate of 5% of the biomass of the pond.

The sampled fish presented length of 5.60 ± 0.10 cm and weight of 6.60 ± 0.86 kg. Having identified the parasite infestation, the fish were transferred to concrete tanks covered with tiles to undergo long-term baths containing 0.5 to 2.0 mg·L⁻¹ of

potassium permanganate and 1% formalin during one hour. In the absence of improvement, we sacrificed and burned all the fish from the respective pond. Systematically, the fish have been previously weighed (in g), measured (in cm, total length) and numbered; these data were collected in individual records. Following, the fish were sacrificed by cerebral puncture and placed in individual containers. The research was authorized by the Instituto de Investigaciones de la Amazonia Peruana-IIAP and necropsy of fish was conducted within the ethical standards.

Using a stereoscope we examined the body surface, fins, nostrils, mouth, opercula and gills, looking 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 possible attached parasites.

For examination of the gills, the samples were separated and placed in glass containers with a 1:4,000 formalin solution. After one h, the gills were stirred in the liquid and then removed from the container. Helminths were allowed to settle on the bottom and were subsequently collected with the aid of a small probe and a dissecting microscope (Nikon SM-30). The identification of the parasites was based on the methodology of Kritsky et al. (1989) and Thatcher (2006). The specimens were deposited in the collection of invertebrates from Ecosystem Aquatic Program, Research Institute of the Peruvian Amazon and thereafter some specimens shall be deposited in the collection of Helminths of Instituto Nacional de Pesquisas da Amazônia (INPA).

To study the monogeneans, permanent slides were prepared with total parasites assembly according to the method HYP (Hundred Year Permanence). For the study of sclerotized structures, parasites were fixed in a solution of ammonium picrate glycerine (GAP) and mounted in Canada balsam according to Malmberg (1957). Some specimens were mounted unstained in Gray and Wess' medium. To visualize internal structures, parasites were fixed in hot formaldehyde solution (4%) for staining with Gomori's trichrome. The parasitic indexes calculated for assessing the level of infestation of parasites in the fish were prevalence, mean intensity and mean abundance (Margolis *et al.*, 1982; Bush *et al.*, 1997).

RESULTS

The necropsy of fingerlings from *C. monoculus* bred in controlled environments in the Peruvian Amazon evidenced the infestation by the monogenean *Gussevia undulata* (Kritsky, Thatcher and, Boeger, 1986) in the gill filaments of the fish.

Indeed, the totality of the examined fish showed a high parasitic infestation by *G. undulata*. The mean intensity was equal to the mean abundance, provided that the number of parasitized fish was the same as those examined (Table 1).

 Table 1. Parasitic indexes of Gussevia undulata in fingerlings of Cichla monoculus cultured in the Peruvian Amazon.

Parasitic indexes	Gussevia undulata
Prevalence (%)	100
Abundance (count)	9864
Mean abundance (count)	168.5
Mean intensity (count)	168.5

DISCUSSION

Even though in the ponds of cultivated *C. monoculus* the physical and chemical parameters of the water were within the expected range of values for tropical species, massive infestation of monogenean was found. However, the increment of parasitic infections in artificial environments has been associated with low quality of water and inadequate management (Thoney & Hargis, 1991).

Several studies report the parasitism of neotropical cichlids by monogeneans belonging to the genus *Gussevia* Kohn and Paperna 1964 (Kritsky *et al.*, 1989; Vidal-Martinez *et al.*, 2001; Yamada *et al.*, 2010; Mendoza-Franco *et al.*, 2010). For South America, thirteen species of *Gussevia* have been described for eight species of cichlids (Kritsky *et al.*, 1986, 1989), evidencing a high specificity of the genus *Gussevia* in parasitizing cichlids.

In the Central and Peruvian Amazon several species of monogeneans of the genus Gussevia have been reported parasitizing cichlid of economic importance for human nutrition and ornamental purposes. Among the species of monogeneans, Gussevia asota and G. astronoti have been described in parasitizing freshwater Astronotus ocellatus, G. spiralocirra (Pterophyllum scalare), G. elephus and G. obtusa (*Uaru amphiancanthoides*), *G. alioides*, *G. dispar* and G. disparoides (Heros severus and *Cichlasoma amazonarum*), *G. longihaptor* and *G.* undulata (Cichla monoculus), G. tucunarense, G. longihaptor, G. disparoides, G. arilla, G. dispar and G. undulata parasitizing Cichla ocellaris (Kritsky et al., 1989; Mendoza-Franco et al., 2010; Azevedo et al., 2010). Despite, any parasitism by the monogenean G. undulata in C. monoculus has been reported. In our study we report for the first time the parasitism by the G. undulata in fingerlings of C. monoculus bred in controlled environments in the Peruvian Amazon.

In the study described herein, the fingerlings of *C. monoculus* presented high levels of parasitism by the monogenean *G. undulata*. Parasites that have a direct life cycle, such as monogeneans, are more frequently found in lentic environments. Moreover, this type of environment favors the transmission of these parasites (Flores-Crespo *et al.*, 2003; Azevedo *et al.*, 2007), which justifies the fact that the fish had elevated parasite infection, since the same are confined to their culture in earthen ponds where water circulation is almost negligible or nonexistent.

In natural aquatic environments with low levels of dissolved oxygen, fish undergo stress and end up susceptible to intense parasitic infestation (Pavanelli et al., 2004; Hogue & Swig, 2007). In confined environments where fish are raised this situation is aggravated as a result of the capture, transport, inadequate nutrition, low amount of dissolved oxygen, temperature and pH with large variations and poor installations (Malta et al., 2001; Hogue & Swig, 2007). Under these conditions, monogeneans can cause large bias and even death, as these parasites induce the gills to produce an excessive amount of mucus, which difficult breathing and consequently prejudice the fish growth. According to Buschmann (2001) and Mariano et al. (2010), intensive fish farming generates a large accumulation of organic matter on the pond bottom produced from the excreta, dead matter and the fraction of uneaten food. This organic matter produces hypoxia and anoxia that creates an unbalance in the homeostasis of the fish, eventually leading to the increase of the oxidative stress of biomolecules, promoting thus various physiological and biochemical alterations, causing cell impairment and death (Sherry, 2003; Van der Oost et al., 2003). Therefore, these adverse effects of poor water quality reduce the self resistance of the fish, which turns out as a favorable condition to the parasite proliferation. This fact may justify the high parasitic infestation by G. undulata in C. monoculus from fish farming.

The results described herein are in accordance with Kritsky *et al.* (1989) and Yamada *et al.* (2011), who found the monogenean *G. undulata* parasitizing gills of two species of cichlid, *C. ocellaris* and *C. kelberi* respectively, being common the setting of this kind of monogenoide parasite in this organ (Kritsky *et al.*, 1989). Indeed, several studies report the parasitism of *G. disparoides* in gills of *H. severus, C. amazonarum* and *C. ocellaris* and all these fish species are currently being raised in confined environments in the Peruvian Amazon.

Furthermore, in a study with *C. kelberi*, captured from the wild, Yamada *et al.* (2011) found 18.42% of prevalence and mean intensity of one and four

monogeneans of *G. tucunarense*. However, the results differ from our study where we found a prevalence of 100% and mean intensity of 50 and 100 parasites of *G. tucunarense*. A possible reason for the low levels of infestation reported by Yamada *et al.* (2011) may be fact that the fish originate from nature. Nevertheless, Kritsky *et al.* (1989) found 100% prevalence of *G. undulata* in *C. ocellaris*, although without informing other parasitic indexes.

Parasites of the genus *Gussevia* are considered specific for cichlids and therefore may show low susceptibility when present in favorable breeding conditions. This is the first report of *G. undulata* parasitizing *C. monoculus* in fish farming in the Peruvian Amazon. The high parasitism turns out as an important reason according to which the fish stopped taking food, occasionally leading to mortality.

The results of this study and studies addressing various aspects of parasite in other species bred in the same region (Iannacone & Luque, 1991; Mathews *et al.*, 2007; Dinis *et al.*, 2007; Mathews *et al.*, 2011) confirm the necessity of constant monitoring of fish, seeking the diagnosis and timely control of infestations by monogeneans, in order to eradicate, once installed in cropping systems, the use of highly toxic products that cause mortality of the host or leave unviable the fish farming intended for human consumption. The present study reports thus, a serious pathological problem that resulted in the mortality of most fish and alerts to the need for preventing actions.

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Correspondence to author/ Autor para correspondencia: Patrick Mathews Delgado

University of Strasbourg, Institute Charles Sadron, Department of Biomedical Sciences, 67034, Strasbourg, France. Phone:+33 (0) 388414219; Fax:+33 (0) 388414099.

E-mail/ correo electrónico: patrickmathews83@gmail.com