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Pathological assessment of farmed yellowtail tetra *Astyanax altiparanae* infested by *Acusicola* sp. (Ergasilidae)

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ABSTRACT

This study registers for the first time the copepod *Acusicola* sp. infesting the gills of farmed yellowtail tetra *Astyanax altiparanae*, as well as new information on gross pathology and gill alterations. Five specimens of yellowtail tetra showing slow swimming, respiratory difficulty, scaleness and darkened skin were examined. Fish were analysed *in situ* for ectoparasites diagnosis. Fragments of the gill arches were removed and processed according to usual histopathology. The gills showed focal and multifocal paleness and whitish areas besides the congestion and hemorrhage signs on the gill filaments. Several white spots attached to the gill filament apex with slight movement were identified as the ergasilid crustacean. Histopathological analysis revealed the gill and blood vessels compression. It was also observed proliferative alterations close to gill filament apex, hyperplasia, total fusion and subepithelial oedema of the secondary lamellae, proliferation of the mucus cells, and inflammatory infiltrate by eosinophilic granular cells surrounding the parasite attachment region. The best management practices and the implementation of diagnostic program are also discussed.

1. Introduction

Diseases caused by crustaceans may compromise the host health including of wild and farmed freshwater and marine fishes worldwide (Johnson et al., 2004). Copepods are the most diversified ectoparasites among the crustaceans being responsible for deleterious effects on their hosts (Johnson et al., 2004; Boxshall, 2005), besides the monogenean helminthes (Boxshall, 2005). Caligid crustaceans are responsible for diseases in marine farmed fishes (Fast, 2014), while Branchiura cause impact on the freshwater fish production (Møller, 2009).

Ergasilid are found mainly on the gills of fish (Lima et al., 2013), although atypical sites of attachment have been reported as for example *Ergasilus sieboldi* Von Nordmann, 1832 on the external surface of the operculum and in the base of pectoral fins of pikeperch *Stizostedion lucioperca* (Molnár and Székely, 1997). Among the different genera, peculiar characteristics for attachment influence directly their pathology on the hosts (Thatcher and Boeger, 1983; Thatcher, 1998). Representatives of the genus *Brasergasilus* Thatcher and Boeger, 1983 generally use their antennae to penetrate the gill filaments being more pathogenic to the host, while in the genus *Ergasilus* Von Nordmann, 1832, may not perforate the filaments (Thatcher and Boeger, 1983; Thatcher, 1998). On the other hand, the genus *Acusicola* Creissey (1970) is well adapted to host attachment by a strategy named "hand over hand", in which do not occur the penetration of the gill filament, however their antennae involve and compress the gill filament producing a tourniquet effect (Thatcher and Boeger, 1983; Motta Amado and Rocha Falavigna, 1996; Thatcher, 1998).

According to Lima et al. (2013), seven species belonging to the genus *Acusicola* have been described in freshwater fishes in Brazil: *A. brasiliensis* Amado and Rocha, 1996; *A. lycengraudilis* Thatcher and Boeger, 1985; *A. paracunula* Amado and Rocha, 1996; *A. pellonidis* Thatcher and Boeger, 1983 *A. rotunda* Amado and Rocha,

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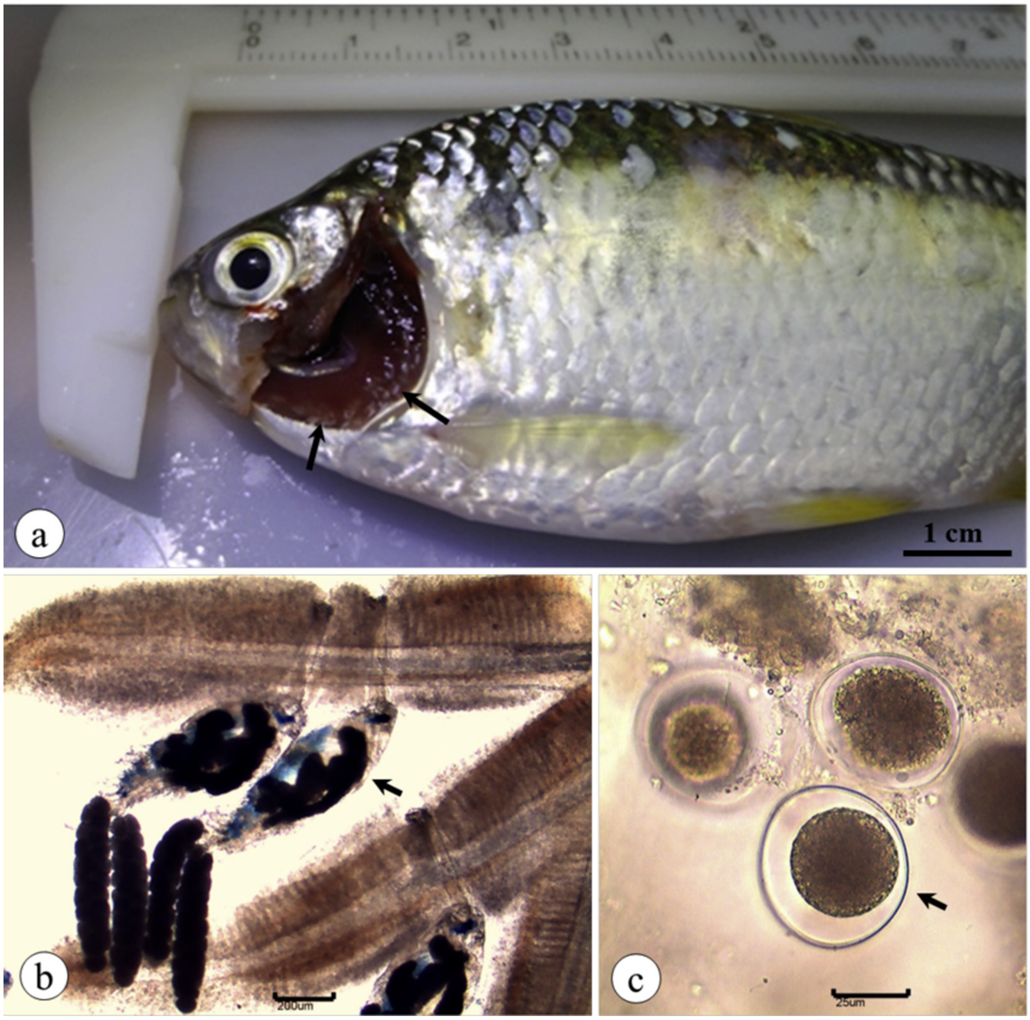

Fig. 1. *Astyanax altiparanae* highly parasitized by *Acusicola* sp., showing white spots of parasites in the gills (arrows) (a), detail of attached female embracing the gill filament (b), and eggs released by the parasite in the respiratory organ in microscopical analysis (c).

1996; *A. spinulosa* Amado and Rocha, 1996; A. *tucunarense* Thatcher, 1984. In yellowtail tetra *Astyanax altiparanae* from natural environment *Acusicola* sp. was registered by Lizama et al. (2008) with 1% prevalence.

In the last years, lambari (*A. altiparanae*) has received especial attention in several regions of Brazil due to its low cost of production and favorable zootechnical characteristics (Lopes et al., 2014). This fish is appreciated as food for human consumption and as bait fish for sport fishing of carnivorous fish (Sabbag et al., 2011). According to the data of IBGE (2015), its production reached 255 tons in 2013 but few works have been done on the health of cultured fish.

Sanitary problems has inevitably emerged with the high stocking density in ponds, and this study registers for the first time the copepod *Acusicola* sp. on the gills of yellowtail tetra from fish ponds and provides new information on the parasitic pathogenesis during a mortality outbreak.

2. Material and methods

Diseased yellowtail tetra *A. altiparanae* (10.0 ± 2.0 g) were obtained from a fish farm located in Porto Ferreira city (21°51′14″s, 47°28′44″O), São Paulo State, Southeast Brazil, that showed chronic fish mortality reaching 100%. The fish were kept in ponds 800 m^2 with constant flow of water, fed commercial diet 32% crude protein and showed unspecific clinical signs, slow swimming and respiratory difficulty. The water temperature was 21.0 °C and dissolved oxygen 4.8 mg/L measured at the time of sample collection. Fish were examined *in situ* in order to verify the gross pathology followed by skin, fins and gill scrapings for parasitic diagnosis observed in a light microscope. The parasites were fixed in 10% formalin solution and processed according to Motta Amado and Rocha Falavigna (1996).

Five specimens of yellowtail tetra were euthanized by deepening of anesthesia using clove oil (75 mg L^{-1}), and fixed in 10% buffered formalin solution to confirm the fish species. In addition, fragments of the gill arches were carefully removed and processed according to usual histopathological techniques, embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin-eosin. The slides were analyzed and photomicrographs were obtained using an Olympus BX60 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) with coupled image analyzer (Image Pro Plus version 6.1 for Windows—Copyright© 1993–2006 Media Cybernetics, Inc.).

3. Results

In diseased fish discrete alterations on the skin and fins were observed such as scaleness and darkened skin in some specimens. Nevertheless, the gill organ showed focal and multifocal paleness,

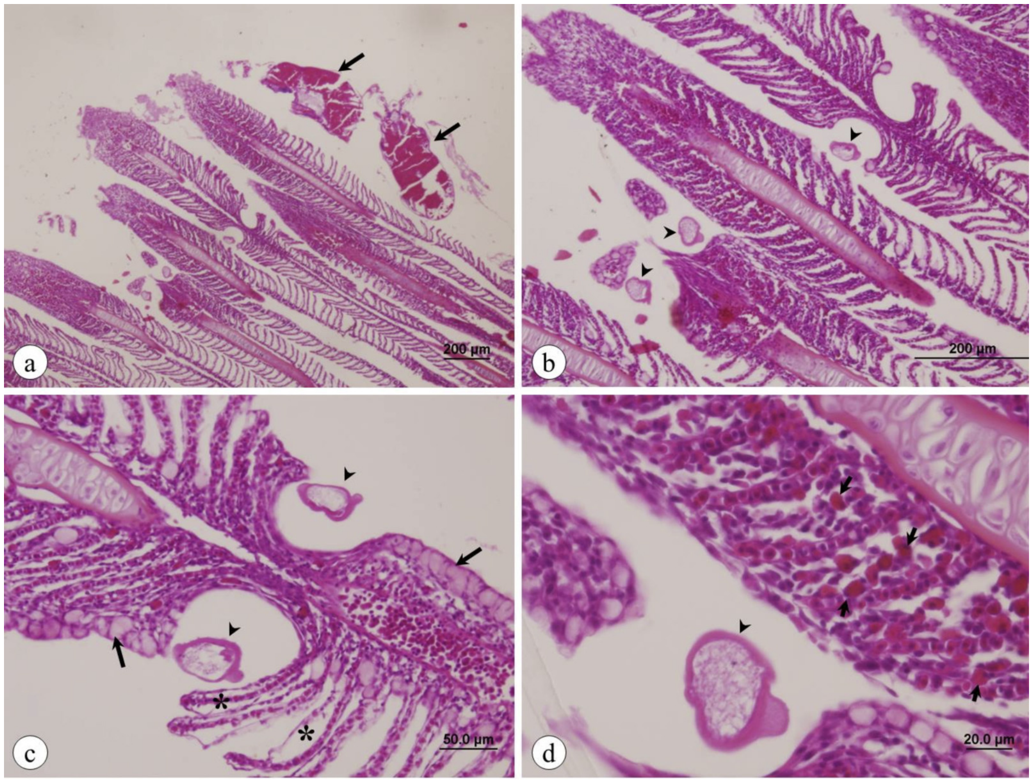

Fig. 2. Histological sections of the gill filaments of *Astyanax altiparanae* parasitized by *Acusicola* sp. The copepod presence (a — arrows), with detail of the antennae embracing the gill filament (b — arrowhead). Note the epithelial proliferation and fusion of the secondary lamellae (b). Detail of attachment area compressed by the parasite antennae (c — arrowhead), proliferation of mucus cells (c — continuous arrow) and subepithelial oedema of the secondary lamellae (c — asterisk). Inflammatory infiltrate composed by eosinophils (d — continuous arrows) adjacent to the antennae attachment area (d — arrowhead). Staining with hematoxylin-eosin.

as well as whitish focal areas suggesting necrosis besides the congestion and haemorrhage signs in the gill filaments. Several white spots with slight movement were observed on the gill apex being confirmed the presence of ergasilid crustacean in the microscopical analysis identified as *Acusicola* sp. (Fig. 1a), parasites embracing the gill filaments (Fig. 1b) and several parasite eggs adhered to the gill epithelium (Fig. 1c).

Histopathological analysis confirmed the presence of the parasite squeezing the gill filaments with their antennae in a tourniquet effect with no organ perforation (Fig. 2 a–c), but compressing the blood vessels. Moreover, proliferative alterations in the apex of the gill filaments (Fig. 2a), epithelial hyperplasia with total fusion of the secondary lamellae (Fig. 2b) were associated with proliferation of mucus cells surrounding the parasite attachment site. Besides, the subepithelial oedema of the secondary lamellae (Fig. 2c) was also observed. Inflammatory infiltrate composed by eosinophilic granular cell was found in all examined animals surrounding the parasite attachment area (Fig. 2d). Consequently, haemorrhagic areas, epithelial degeneration, necrosis and ruptured apex of the gill filament were also observed in severely injured filaments.

4. Discussion

Yellowtail tetra infested by *Acusicola* sp. showed serious lesions in the gill filaments, such as epithelial and mucus cells proliferation, congestion, haemorrhage and necrosis. These histopathological changes compromise the hydromineral balance and gas exchange, reducing the availability of oxygen, and in massive infections leading to death. Thatcher (1998) observed similar lesions in *Cichla monoculus* infected by *A. tucunarense* Thatcher 1984. Furthermore, subepithelial oedema with inflammatory infiltrate composed mainly by eosinophilic granular cells was also observed. These granulocytes present in the inflammatory infiltrate during the organ response have been described in the gills of bream *Abramis brama* infested by *Ergasilus sieboldi* Von Nordmann, 1832 (Dezfuli et al., 2003), Tanganyika killifish *Lamprichthys tanganicanus* infested by *Ergasilus sarsi* Caparti, 1944 (Kilian and Avenant-Oldewage, 2013) associated with rodlet cells and mast cells during the host response against crustacean parasites (Dezfuli et al., 2003, 2011; Kilian and Avenant-Oldewage, 2013). Moreover, eosinophilic granular cells have been described during the inflammatory process as a result of parasitic infections such as stomach infection by *Goezia spinulosa* Diesing, 1839 (Nematoda) in pirarucu *Arapaima gigas* (Menezes et al., 2011) and in chronic gill parasitism by *Paratrichodina africana* Kazubski and El-Tantawt, 1986 (Ciliophora) in Nile tilapia *Oreochromis niloticus* (Valladão et al., 2013). Pathological alterations observed in this study were similar to that reported in *Ergasilus lizae* Kroyer, 1863 infestation in gilthead seabream *Sparus aurata* (Lui et al., 2013).

The impact of disease caused by ergasilid crustaceans is related not only to fish health status but also to economic and social impacts in case of cultured fish (Dezfuli et al., 2011), due to reduced respiratory capacity compromising the growth leading to high mortality rates. Knowing the host-parasite relationship and the consequences caused by the parasite on its host, could allow us to detect whether the environment is imbalanced. This is especially true in analyzing fish pond ecosystems when the fish are constantly exposed to high stocking densities, food intake and to dynamic environment. According to the topic, the best management practices implemented in the facilities allied to regular diagnostic program could avoid the parasite outbreak and economic losses due to highly pathogenic parasites, as *Acusicola* and others copepods.

Conflict of interest

The authors declare no conflicts of interest.

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