



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



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### ARTICLE INFO

#### Article history:

Received 5 December 2014

Received in revised form 18 August 2015

Accepted 18 August 2015

Available online 29 August 2015

#### Keywords:

Fish

Copepoda

Histopathology

Pathogenesis

Gills

### 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.

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### 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).

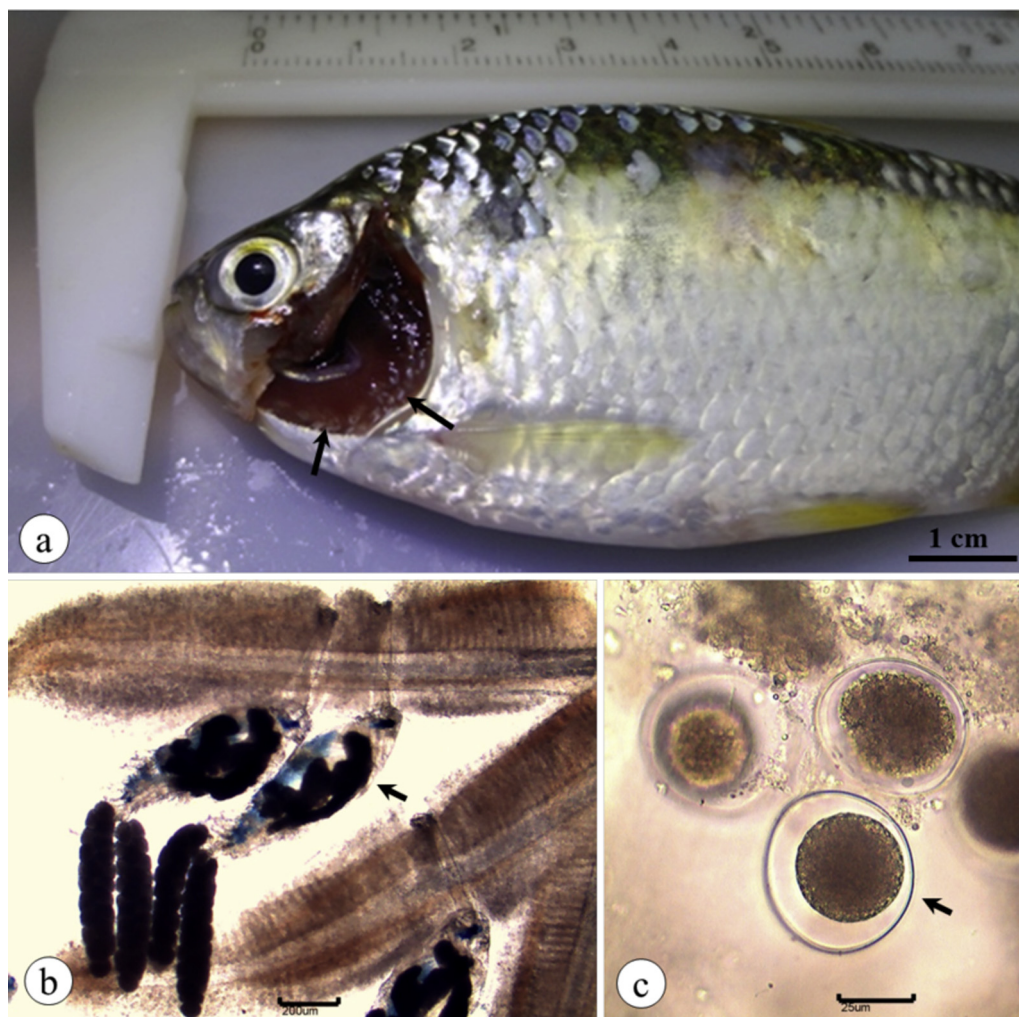
Ergasilid are found mainly on the gills of fish (Lima et al., 2013), although atypical sites of attachment have been reported as for example *Ergasilus sieboldi* Von Nordmann, 1832 on the external surface of the operculum and in the base of pectoral fins

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 *Braser-gasilus* 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. pel-lonidis* 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. tucunarensis* 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* ( $10.0 \pm 2.0$  g) were obtained from a fish farm located in Porto Ferreira city ( $21^{\circ}51'14''$ S,  $47^{\circ}28'44''$ O), São Paulo State, Southeast Brazil, that showed chronic fish mortality reaching 100%. The fish were kept in ponds 800 m<sup>2</sup> 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 ( $75 \text{ mg L}^{-1}$ ), 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,

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