Outbreak of *Notozothecium bethae* (Monogenea: Dactylogyridae) in *Myleus schomburgkii* (Actinopterygii: Characiformes) cultured in the Peruvian Amazon

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Abstract The presence of *Notozothecium bethae* was investigated in 76 black band myleus (*Myleus schomburgkii* [Actinopterygii: Characiformes]) born and kept in captivity in two semi-intensive breeding cages in the northern region of Peru. Among the 76 cultivated specimens of *M. schomburgkii* examined in the present study, 100 % had monogenean on the gill and the parasites were identified as *Notozothecium bethae*. During the survey no bacteria was isolated, and no protozoan or other metazoan parasites were found. The prevalence of *N. bethae* remained constant throughout the present study. Mean intensity of *N. bethae* in the months of April and May was exceptionally high in both facilities. The massive infection of *N. bethae* on the gills of *M. schomburgkii* was associated with mortality. This is the first report of *N. bethae* in *M. schomburgkii* born in captivity and cultured in the Amazon region. The mortality due the presence of this parasite emphasise the need for disease control strategies of cultured *M. schomburgkii*.

Keywords Monogenean · Fish parasites · *Myleus schomburgkii* · Aquaculture · Peruvian Amazon

Introduction

The freshwater fish *Myleus schomburgkii* Jardine, 1841, is a rheophilic omnivorous species from the Family Serrasalminae, popularly known in Peru as “banda negra” (English name = black band myleus) and widespread in the middle and lower Amazon River basin, Nanay River, upper Orinoco River basin in South America (Jégu 2003). This fish can grow up to 42 cm and reach 600 g, and it is considered as a potential species for the diversification of aquaculture production in the Peruvian Amazon (Garcia et al. 2012).

Among the various groups of helminthes which parasitize freshwater fishes, monogeneans are represented by many species and cause expressive economic losses in fish farming around the world (Marcogliese et al. 2001; Venmathi Maran et al. 2014). Although there is a general agreement that these parasites are not harmful in natural conditions, in intensively cultured fish they may reach high intensities and produce injuries on gills such as laceration of the filaments, epithelium destruction, gills borders become thicker and opercula remain open with necrotic areas, generating anastomosis which obstruct gas exchange in capillaries, thus diminishing the respiratory function that in severe cases produces death by asphyxia (Flores-crespo and Flores 2003). In the Amazon region several species of monogeneans have been reported parasitizing cultured fish of economic importance for human nutrition and ornamental purposes (Mathews et al. 2012, 2013a, b, 2014a, b; Soberon et al. 2014); however, little is known about parasites that affect *M. schomburgkii* in fish farms.

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Therefore, with gradual increase of intensive and semi-intensive fish farming in the Peruvian Amazon, there is a need of constant monitoring of the fish for diagnosis and timely control of infestations by monogeneans. In this sense, the present study aims to evaluate the monogenean infestation in *M. schomburgkii* bred in a fish farm in the Peruvian Amazon. This study reports for the first time, the infestation by *Notozothecium bethae* on the gills of *M. schomburgkii* in fish farm, and describes pathological signs and mortalities caused by the outbreak of this parasitic disease.

Materials and methods

From January to May 2013, a total of 76 specimens of *M. schomburgkii* were examined for parasitological and microbiological analysis. The fishes were collected with drag nets from two semi-intensive breeding cages. The cages were placed in two earthen ponds of 120 m²: Location A and Location B, from where 40 and 36 specimens were collected respectively. At Location A, the water that fills the tanks originates from artesian wells, the exchange of the water is nonexistent and the water circulation is almost negligible or nonexistent. At Location B, the water used to fill the tanks was obtained by a nearby natural lake, and exchange of water, where the culturing cages of *M. schomburgkii* are located, is randomly performed or during long periods is not performed (once at every 6 months). Both facilities are located in the state of Loreto, northern region of Peru, between latitudes 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 4 PM) with daily checks of dissolved oxygen, pH, temperature and conductivity by means of a YSI multiparameter meter (Model MPS 556). Ammonium values, hardness, carbon dioxide and total alkalinity were monitored weekly and in the morning (8 AM), using a complete package for analysis of freshwater (LaMotte AQ-2). Fish were fed thrice daily (at 8 AM., noon, and 4 PM.) with extruded diet containing 25 % crude protein and 2.6 Mcal/kg of digestible energy.

For parasitological analysis, body surface, mouth, gills, body cavity and all organs were examined under a stereomicroscope for metazoan parasites. Fresh scrapings of the internal organs were observed using a light microscope (Nikon SM-30), from 100× to 1000× magnification, for myxozoans and protozoan parasites. Prevalence and mean intensity were calculated according to Bush et al. (1997). Microbiological examination was carried out by classical methods as described by Viale et al. (2006). To study the monogeneans, permanent slides were prepared with total parasites assembly according to Thatcher (1991). For the

study of sclerotized structures, parasites were fixed in a solution of ammonium picrate glycerine (GAP) and mounted in Canada balsam. 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 Kritsky et al. (1996).

The association of presence of *N. bethae* and place of sample collection, age (young or adult), and sex (male or female) was analyzed by Chi square and Fisher's tests, and $P \leq 0.05$ values were considered significant.

Results

Among the 76 cultivated specimens of *M. schomburgkii* examined, 100 % ($n = 76$) had monogenean on the gill (Fig. 1a). After microscopic examination the parasites were identified as *Notozothecium bethae* according to the criteria given by Kritsky et al. (1996) (Fig. 1b–d). During the survey no bacteria was isolated, and no protozoan or other metazoan parasites were found.

The prevalence of *N. bethae* remained constant throughout the present study (100 %). Mean intensity (MI) of *N. bethae* in the months of April and May were exceptionally high in both facilities (Fig. 2); however, MI was higher in the Location A than in fishes from Location B (MI = 230 and 150 in Location A and Location B, respectively) (Fig. 2). In the fishes from Location A and Location B, at necropsy, the gills were pallid with hypersecretion of mucus and opercula remained open. In both facilities, the age and sex of fish did not affect the occurrence of *N. bethae*. In the present study, a total mortality of 15 fish (37.5 %), was verified for Location A and a total of 9 (25 %) fish had died in Location B.

In the present study, the values of the physicochemical parameters of the water from the culture ponds were dissolved oxygen ($3.2\text{--}5.4 \text{ mg L}^{-1}$), pH (5.44 ± 0.20), temperature ($28.23 \pm 0.9 \text{ }^{\circ}\text{C}$), conductivity ($106.1 \pm 14.0 \text{ } \mu\text{S cm}^{-1}$), ammonium values ($0.08 \pm 0.01 \text{ mg L}^{-1}$), hardness ($21.40 \pm 1.80 \text{ mg L}^{-1}$), carbon dioxide ($3.2 \pm 0.9 \text{ mg L}^{-1}$), and total alkalinity ($16.14 \pm 0.80 \text{ mg L}^{-1}$).

Discussion

Monogenean infestations are frequent worldwide in cultured fish (Flores-Crespo and Flores 2003) and this group is among the helminthes most commonly found in fish with high economic importance for fish farming in the amazon region of Peru (Mathews et al. 2012, 2013a, b, 2014a, b; Soberon et al. 2014). However, this study reports, for the

Fig. 1 *Notozothecium bethae* infecting *Myleus schomburgkii*. **a** Gills of *M. schomburgkii* infected with *N. bethae*. **b** *N. bethae* total. **c–d** Sclerotized structures of *N. bethae* 1 Ventral bar, 2 Dorsal bar, 3

Hooks, 4 Ventral anchor, 5 Dorsal anchor, 6 Copulatory complex. Scale bar 10 µm

Fig. 2 Mean intensity of *Notozothecium bethae* infecting *Myleus schomburgkii* from January to May 2013 in the two breeding cages (Location A and B). Bars Standard Deviation

first time, the presence of *N. bethae* in *M. schomburgkii* born in captivity and cultured in the Amazon region. Furthermore, the massive infection of *N. bethae* on the gills of *M. schomburgkii* was associated with mortality.

Notozothecium bethae was previously described parasitizing the gills of *M. schomburgkii*, *M. pacu*, *M. rhomboidalis* and *Mylesinus paucisquamatus*, collected from natural environments in Brazil and French Guiana (Kritsky et al. 1996, 1998), confirming the occurrence of this species of parasite in *M. schomburgkii* and other congeneric species. However, the presence of *N. bethae* in fish of the genus *Myleus* and *Mylesinus* may be related to the high phylogenetic proximity between them (Strona and Lafferty 2013; Braga et al. 2014).

The monogenean found in this study has a direct cycle and can be easily spread, especially in intensive culture. In the Peruvian Amazon, cage density of amazon fish is suggested at around 40 fish/m³ (Alcántara et al. 2003), which may explain the permanent high levels in the prevalence of *N. bethae*, which exhibited prevalence values above 95 %. Likewise, environmental factors must be considered. In regions with tropical climate, the life cycle of monogenean can be completed in less than 1 day and the monogenean proliferate rapidly (Flores-Crespo and Flores 2003). The climate in the region of this study is tropical humid with annual average temperature of 28.3 °C and relative humidity of 85 %, favoring the speed of life cycle (Flores-Crespo and Flores 2003).

Water quality parameters monitored during the study did not present any significant difference among the two facilities. The values observed were considered adequate for tropical cultured fish (Gomes et al. 2006; Bicudo et al. 2009). However, dissolved oxygen presented a strong variation during the study, reaching its highest values between January and March (5.4 mg L⁻¹) and the lowest values during the months of April and May (3.2 and 3.4 mg L⁻¹ respectively). Indeed, it is known that unsuitable environmental conditions and abrupt variations of the temperature, pH and oxygen concentration in aquaculture can render fishes more susceptible not only to parasites but also to opportunistic bacteria (Garcia et al. 2007).

In the present study, the largest number of monogeneans were observed between the months that correspond to the end of the rainy season (April) and early dry season (May to June). However, monogeneans were detected during each sampling throughout the study period which is in accordance with previous studies that reported presence of monogeneans throughout all seasons (Rabago-Castro et al. 2011; Jerônimo et al. 2011).

At Location A, the facility that had the highest value of MI in the fishes, the water that fills the tanks originates from artesian wells, the exchange of the water is nonexistent and the water circulation is almost negligible or nonexistent. At Location B, the facility that had the lower mean intensity, the water used to fill the tanks was obtained by a nearby natural lake, and exchange of water where the culturing cages of *M. schomburgkii* are located is randomly performed or during long periods is not performed. Hence, the first recommendation to decrease monogenean infection in the fishes at these facilities should be the institution of a water circulation system. Another recommendation would be continues exchange of the water.

Considering that *M. schomburgkii*, is a relatively new especie to aquaculture in the Peruvian Amazon, the presence of the helminths points out the need of improving disease control strategies of cultured *M. schomburgkii* and

investigating the possible transfer of monogeneans from wild to caged fish to better prevent future disease outbreaks. Furthermore, studies on the seasonality of parasites in wild and cultured fish would be necessary to establish prophylactic strategies to decrease the mortality of cultured fish.

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Conflict of interest The authors declare that they have no competing interests.

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