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Parasitic fauna and histopathology of farmed freshwater ornamental fish in Brazil

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ABSTRACT

Ornamental fish farming represents a consolidated market over the world. However, confinement is a factor that favors the occurrence of diseases. This study aimed to report the parasitic fauna of ornamental fish from three facilities, as well as to observe the histological pathogenesis caused by the parasites. Between May 2015 and February 2016, a total of 781 ornamental fishes were used for parasitological and histopathological analysis. Water quality was measured in fishponds from each facility. Ciliate protozoans *Ichthyophthirius multifiliis*; *Trichodina* sp.; the monogeneans *Dactylogyrus extensus*, *D. minutus* and *Diaphorocleidus kabatai*; metacercariae of the digeneans; the cestode *Bothriocephalus acheilognathi*; the nematode *Rhabdochona* sp.; and the branchiuran *Argulus japonicus* were found in the examined fish. The greatest prevalence and mean intensity was observed in the gills of *Gymnocorymbus ternetzi* parasitized by *D. kabatai*, followed by the protozoan parasite *I. multifiliis* on the body surface of *Xiphophorus maculatus*. Histopathological analysis showed epithelial interlamellar hyperplasia of the secondary lamellae, partial fusion of the secondary lamellae, telangiectasia, justalamellar edema and eosinophilic inflammatory infiltrate. The intestine of cestode parasitized fish showed necrosis in the submucosa, intestinal obstruction and lymphoeosinophilic inflammatory infiltrate. It is important to know the parasitic fauna of farmed fish and the pathogenesis caused by the parasites in order to ensure fish production and the health of the hosts.
Statement of relevance: Ornamental fish production as a consolidate activity around the world faces problems of parasite infection leading to fish mortality and economic losses. To ensure farming production, it is important to monitor the status of fish health. Parasitic fauna and histopathological analysis are used as important tools for the diagnosis of tissue lesions.

1. Introduction

Fish farming is rapidly growing and ornamental fish farming is an important economic activity (Santos et al., 2014). According to Lima et al. (2001), Brazil is recognized as the main supplier of ornamental fish species, however most of the production is as a result of capture. These captured ornamental fish are exported, while the internal market is supplied mainly by allocthonous species produced in captivity (Nottingham and Ramos, 2006). In 2007, the amount of ornamental fish imports in Brazil was relatively low, yielding about US$5 million (Monticini, 2010). This low amount can be explained by the enthusiastic entrance into the market of new domestic producers of ornamental fish in recent years. The market entry of fish farmers is stimulated by the rapid growth of fish, well adapted to culture conditions and by the desire to diminish the extractive capture of the native fish species, since many of them are threatened by extinction (Tlusty, 2002; Zuanon et al., 2011).

Intensive fish farming has favored the occurrence and dissemination of parasitic diseases as a result of imbalances in the host/parasite/environment relationship (Jerônimo et al., 2012), which predispose the fishes to disease outbreaks (Portz et al., 2013). The equilibrium in this triad is easily disturbed by increased numbers of parasites, high levels of nitrogen compounds from excessive feeding, high stocking density, poor water quality, inadequate handling and lack of the best management practices (Garcia et al., 2003; Eiras, 2004; Giorgiadis et al., 2001).

The pathogenic action of parasites, especially those that cause lesions on the hosts, has been studied mainly in fish of economic importance (Lom and Dyková, 1992; Martins et al., 2015). Depending on the

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mode of parasite attachment, focal, multifocal and diffuse lesions can be found (Khan, 2012). Previous studies have shown that *Ichthyophthirius multifiliis* Fouquet, 1876 (Mohammadi et al., 2012); trichodinids (Yemmen et al., 2011); monogeneans (Fujimoto et al., 2014); digeneans (Omrani et al., 2010); nematodes (Menezes et al., 2006); cestodes (Dezfuli et al., 2011); and branchiurans (Saha and Bandyopadhyay, 2015) are responsible for tissue damage in ornamental fishes.

Monitoring of parasitism and histological analysis of the organs in farmed fish can ensure the early diagnosis of pathogens prior to their dissemination. Histological examination of fish organs is an important tool for rapid diagnosis (Takashima and Hibiya, 1995; Genten et al., 2009). For example, gill alterations such as hypertrophy, edema, necrosis, epithelial desquamation, hyperplasia, fusion of the secondary lamellae and telangiectasia are reported in parasitized fish (Roberts, 2001; Campos et al., 2011). Intestinal helminths can provoke an inflammatory reaction at their attachment site. Depending on the intensity of the parasitic infestation, they can also provoke intestinal hemorrhages, inflammation and loss of gastrointestinal function (Molnár, 2005; Alvarez-Leon, 2007; Dezfuli et al., 2007, 2011; Alvarez-Pellitero et al., 2008).

The aim of this study was to assess the parasitic fauna in farmed ornamental fishes from three facilities in Southern Brazil, as well as to evaluate the pathogenesis caused by the parasites through histological analysis.

2. Material and methods

2.1. Fish collection

Between May 2015 and February 2016, a total of 781 ornamental fishes were collected each three months from three facilities located in the State of Santa Catarina, Brazil: fish farm A (FA) (26° 22′ 12″ S 48° 43′ 20″ W), fish farm B (FB) (27° 29′ 39″ S 48° 39′ 20″ W) and fish farm C (FC) (26° 49′ 24″ S 49° 16′ 18″ W), characterized in Table 1.

The number of sampled fish (n) and biometry are as follows:

Fish farm A: blood swordtail (*Xiphophorus helleri* Heckel 1848) (4.1 ± 0.8 g, 6.9 ± 0.5 cm, n = 30); wagtail platy (*Xiphophorus maculatus* Gunther 1866) (1.6 ± 1.1 g, 4.5 ± 0.7 cm, n = 30); common platy (*Xiphophorus maculatus*) (0.8 ± 0.6 g, 3.1 ± 2.0 cm, n = 15).

Table 1
Characteristics of the fish farms used in this study.

Characteristics	FA	FB	FC
Fish farm size	0.0018 ha	22 ha	0.28 ha
Culture system	Semi intensive	Semi intensive	Intensive
Pond size	0.0004 ha	0.03 ha	0.02 ha
Water source	Rain water	Velho River	Fortuna River
Water exchange rate	5–15%	5%	No exchange
Fish source	Own production	Own production	Own production
Stocking density	No control	No control	1 fish/m^3
Feeding frequency	2 times a day	Once a day	2 times a day
Larval diet (powder)	36% CP	55% CP	46% CP
Breeding diet (pellet)	2–3% (biomass)	3% (biomass)	3% (biomass)
Aeration	No	No	Yes
Control of water quality	Yes	No	No
Fertilization	Yes[a]	Yes[a]	Yes[a]
Water renewal	Yes	Yes	Yes
Mortalities	No	20%	No
TR (cm)	25 ± 8.5	18 ± 10	23.2 ± 26.3
AM ($mg \cdot L^{-1}$)	0.4 ± 0.3	0.1 ± 0.2	0.1 ± 0.1
pH	6.5 ± 1.2	6.0 ± 3.2	7.2 ± 0.9
DO ($mg \cdot L^{-1}$)	6.0 ± 3.4	5.3 ± 1.6	6.8 ± 2.0
TE (°C)	21 ± 1.5	22.4 ± 2.6	23.6 ± 3.7
SAL (‰)	0.06 ± 0.0	0.02 ± 0.0	0.02 ± 0.0

FA: Araquari. FB: Biguaçu. FC: Timbó. CP: crude protein. Mean values ± standard deviation of water quality in the fish farms studied from Southern Brazil. TR: transparency, AM: ammonia, DO: dissolved oxygen, TE: temperature, SAL: salinity.
[a] Fertilization only when needed.

Fish farm B: blood swordtail (*Xiphophorus helleri*) (1.8 ± 1.2 g, 5.2 ± 1.5 cm, n = 57); black swordtail (*Xiphophorus helleri*) (2.3 ± 0.8 g, 6.0 ± 0.8 cm, n = 15); wagtail platy (*Xiphophorus maculatus*) (0.6 ± 0.1 g, 3.3 ± 0.2 cm, n = 15); hawaii platy (*Xiphophorus maculatus*) (1.3 ± 0.4 g, 4.4 ± 0.5 cm, n = 30); blue platy (*Xiphophorus maculatus*) (1.4 ± 0.8 g, 4.3 ± 0.7 cm, n = 60); mickey mouse platy (*Xiphophorus maculatus*) (1.3 ± 0.4 g, 4.3 ± 0.4 cm, n = 45); black tetra (*Gymnocorymbus ternetzi* Boulenger, 1895) (4.0 ± 1.7 g, 6.0 ± 0.5 cm, n = 15); pink tetra (*Gymnocorymbus ternetzi*) (4.3 ± 1.0 g, 6.0 ± 0.7 cm, n = 60); zebrafish (*Danio rerio* Hamilton, 1822) (0.5 ± 0.2 g, 3.4 ± 0.6 cm, n = 60); jewel tetra (*Hyphessobrycon eques* Steindachner, 1882) (1.2 ± 0.4 g, 4.3 ± 0.4 cm, n = 60); goldfinned barb (*Puntius sachsii* Ahl, 1923) (2.6 ± 2 g. 6.0 ± 0.9 cm, n = 60).

Fish farm C: Koi carp (*Cyprinus carpio* Koi Linnaeus, 1758) (4.4 ± 1.2 g, 6.8 ± 1.9 cm, n = 229).

The ornamental fishes were collected with net and kept alive in plastic bags to be transported to the laboratory for parasitological and histopathological analysis.

In each fish collection, the water quality parameters were measured: transparency with Secchi disc, ammonia and pH measured with commercial kit ammonia freshwater Hanna (HI 38049, São Paulo, Brazil), dissolved oxygen, water temperature and salinity measured with a multiparameter Hanna (HI 9828, São Paulo, Brazil). Concomitantly to the collections was asked to the producers which management practices usually were adopted for better understanding the data.

2.2. Parasitological analysis

The fish were anesthetized in eugenol (75 $mg \cdot L^{-1}$) and euthanized by cerebral concussion. These procedures were previously approved by the Ethics Committee on Animal Use from the Federal University of Santa Catarina (CEUA/UFSC PP00928).

Macroscopic observation of the body surface and organs was performed to verify any lesions and/or alterations caused by pathogens. Parasitological analysis was performed according to Jerônimo et al. (2013). Scrapings from the body surface and fresh samples of the internal organs were mounted on glass slides with a drop of saline solution 0.65% for microscopic observation. The eyes were placed in Petri dishes, dissected and observed under a stereomicroscope, while the gill arches were placed in flasks containing hot water at 55 °C, agitated and fixed in 70% ethanol for later parasite quantification. Parasites were counted according to Jerônimo et al. (2016) and parasitological indices (prevalence and mean intensity) were calculated as recommended by Bush et al. (1997).

The trichodinid protozoans were impregnated with silver nitrate using the method of Klein (1958) and identified according to Pádua et al. (2012), Valladão et al. (2013) and Dove and O'Donoghue (2005). Monogeneans were mounted in Hoyer's medium between a slide and coverslip for observation of sclerotized structures and the copulatory complex (Eiras et al., 2006), for the purpose of identification according to Dzika et al. (2009) and Sujan and Shameem (2015). Cestodes were stained with carmine according to Eiras et al. (2006) and identified according to Brandt et al. (1981) and Scholz (1997). Nematodes were clarified with Amann's lactophenol, mounted in Canada balsam and identified according to Moravec (1998, 2001). Branchiurid crustaceans were clarified with lactic acid and identified according to Cressey (1978), Mousavi et al. (2011), Rushton-Mellor (1994) and Soes et al. (2010).

2.3. Histopathological analysis

Fragments of the gills and intestine of 260 fishes with the highest mean intensities of parasitism were fixed in 10% buffered formalin solution to observe the tissue alterations caused by the parasites. The organs were dehydrated in serial solutions of alcohol, cleared in xylol, embedded in paraffin at 60 °C for posterior cross sections of 5 μm thickness and