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Original Article

Parasitological diagnosis in ornamental freshwater fish from different fish farmers of five Brazilian states

Diagnóstico parasitológico em peixes ornamentais de diferentes pisciculturas de cinco estados brasileiros

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Abstract

This study aimed to search parasites in 333 ornamental fish from five Brazilian states (Ceará, Minas Gerais, São Paulo, Paraná and Santa Catarina). Fish were sent from eight farms located in the municipalities of Fortaleza, Patrocínio do Muriaé, São Francisco do Glória, Cascavel, Timbó, Iguape, Jacareí and Mairinque. All fish received anesthesia earlier to euthanasia procedures. After the search for parasites, it was verified that 70.6% (235/333) of fishes were infected by at least one type of parasite, being 12 types of parasites identified: monogeneans, digenean metacercariae, cestodes, nematodes, *Lernaea cyprinacea*, trichodinids, *Piscinoodinium pillulare*, *Ichthyophthirius multifiliis*, diplomonad flagellates, *Ichthyobodo* sp., *Chilodonella* sp., and *Tetrahymena* sp. The proportion of infected fishes among the farms is compared through statistical tests, besides, animal handling adopted in each farm is also discussed. The importance of ensuring fish health in order to make the ornamental freshwater fish industry economically viable and reduce losses in production is highlighted.

Keywords: aquatic health, ornamental fish farm, parasitic infection.

Resumo

O objetivo deste estudo foi pesquisar parasitas presentes em 333 peixes ornamentais de cinco estados brasileiros (Ceará, Minas Gerais, São Paulo, Paraná e Santa Catarina). Os peixes eram oriundos de oito pisciculturas localizadas nos municípios de Fortaleza, Patrocínio do Muriaé, São Francisco do Glória, Cascavel, Timbó, Iguape, Jacareí e Mairinque. Todos os peixes foram anestesiados e eutanasiados para a pesquisa de parasitas. Após análise microscópica, foi verificado que 70.6% (235/333) dos peixes estavam infectados por ao menos uma espécie de parasita, sendo identificados 12 tipos: monogenoides, metacercárias, cestoides, nematoides, *Lernaea cyprinacea*, tricodinídeos, *Piscinoodinium pillulare*, *Ichthyophthirius multifiliis*, flagelados diplomonados, *Ichthyobodo* sp., *Chilodonella* sp., e *Tetrahymena* sp. A proporção de peixes infectados entre cada piscicultura é comparada através de testes estatísticos, além disso, o manejo adotado pelas diferentes pisciculturas também é discutido. A importância de assegurar a sanidade de peixes ornamentais, de forma a tornar a atividade economicamente viável e reduzir perdas na produção é enfatizada.

Palavras-chave: sanidade aquícola, piscicultura ornamental, infecção parasitária.

1. Introduction

The ornamental freshwater fish industry has grown substantially over the past decades worldwide (FAO, 2020). In Brazil, this activity has become more common, with fish being the fourth most prevalent pet in Brazilian residences, present in approximately 20.8 million houses (ABINPET, 2022). The Amazon is a critical center for the extraction of ornamental fish from the northern region of Brazil, whose main market is exportation to Europe, Asian and United States (Anjos et al., 2009), while Minas Gerais is the main producer hub, supplying animals to different Brazilian regions (Magalhães and Jacobi, 2013).

The intensification of aquaculture is responsible for the occurrence and the dissemination of parasitological diseases, as the result of an imbalance between host, parasite and environment, which raises diseases outbreaks ratios (Santos et al., 2017). With intensive fish farming,

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stressful situations may occur, causing an imbalance of this triad. This imbalance may be due to excessive amounts of ammonia, nitrite and nitrate, low dissolved oxygen, water eutrophication, lack of control of the water temperature, frequent pH imbalance and inadequate handling (Moraes and Martins, 2004), causing stress and making fish susceptible to infectious and parasitic diseases. Biosecurity, internal and external barriers, disinfection and antisepsis of equipment and aquaria, quarantining of fish prior to entry into the system, among other prophylactics measures, must be taken in order to preserve the physiological conditions and health of the animals to avoid parasite proliferation. The lack of correct hygiene handling practices and failure of proper water treatment equipment installation, cause severe sanitary problems because of inefficient pathogen control (Cardoso et al., 2017, 2018, 2020a; Relvas et al., 2020).

The monitoring of fish health status must be one of the most important activities in farms, wholesalers, stores, and in importation and exportation holding facilities. Transport of live animals contributes to parasite dissemination not only in ornamental fish, but also in native fish and farmed fish (Tavares-Dias et al., 2009), endangering native populations and posing a threat to the aquaculture industry. The introduction of a new pathogen may have devastating effects on the health and economic viability of aquatic animal populations if they do not evolve appropriate defense mechanisms against pathogens (Cardoso and Balian, 2018). The most common diseases affecting cultured fish are caused by exotic pathogens, which are inadvertently introduced into a region via infected fish from another geographic area. The implementation of methods to prevent or manage the transmission of infectious diseases is important in preventing the escape of pathogens from a farm, so that it does not affect wild populations or adjacent farms (Whittington and Chong, 2007; Becker et al., 2014; Trujillo-González et al., 2018).

Parasitic infections represent an important challenge for commercial suppliers of ornamental fish, and only an effective biosecurity program will maintain healthy animals, reducing the risk of acquiring disease in a facility. In this context, knowing the parasite groups that can affect ornamental fish is essential for aquaculture development. However, in Brazil, there is still a lack of data and robust research being conducted. Additionally, research is not being conducted by official laboratories, making the present research important in order to initiate an epidemiological mapping of sanitary conditions of fish grown in national territory. Therefore, the aim of this study was to identify the parasite groups of ornamental freshwater fish from some fish producing regions that had different animal handling characteristics.

2. Materials and Methods

2.1. Animals studied

Animal handling and experimental designs were approved by the School of Veterinary Medicine and Animal Science Institutional Animal Care and Use Committee (Ethics Committee – CEUA nº. 8380210119). Between November 2018 and March 2019, 48 species of ornamental freshwater fish were studied from 10 different families, totaling 333 specimens (Table 1). Fish were sent from five states, located in three different Brazilian regions (south, southwest and northeast: Cascavel (Paraná – PR) (n = 37), Fortaleza (Ceará – CE) (n = 38), Iguape (São Paulo – SP) (n = 21), Jacareí (São Paulo – SP) (n = 31), Patrocínio do Muriaé (Minas Gerais - MG) (n = 50), São Francisco do Glória (MG) (n = 57) and Timbó (Santa Catarina – SC) (n = 55).

Animals were transported alive in plastic bags with oxygen to a wholesale shop in the city of São Paulo, where the parasitological analysis were performed. For this study, animals were randomly collected from the plastic bags at the moment of arrival, and fish species were identified and euthanized.

2.2. Water quality and evaluation of biosecurity practices in fish farms

During technical visits at the farms, the water quality and biosecurity practices were verified through the presence or absence of methods for disease and parasite prevention (filters, biofilters, and UV radiation devices), staff routines towards fish care were observed, along with feeding practices, fallowing and quarantine routines, equipment to control water quality (mechanical aerators), and water quality testing routines (e.g., measurement of pH, ammonia, and dissolved oxygen). In addition, contact with wild animals, such as birds and crustaceans, was evaluated.

2.3. Clinical evaluation, collection and parasite analysis

Prior to necropsy procedures, fish were clinically examined and classified as either 'alert' or 'prostrated'. After that, one of the following clinical signs were identified and recorded: difficulty to breathe properly, skin wounds or ulcers, irregular, clamped or damaged fins, excessive mucus, and white spots.

For parasite analysis, skin scraping was performed first, followed by a sedation procedure with Eugenol (100 mg L^{-1}) diluted in a 4 L container (Roubach et al., 2005). Animals were sedated after being in contact with the solution for approximately 5 min or less, depending on the species, followed by spinal cord section euthanasia (Noga, 2010). Following euthanasia, gill and viscera wet mounts were performed through optic microscopic evaluation on 4 x, 10 x, 20 x and 40 x magnification. The parasites were identified based on their structure, morphology and movement pattern (Woo, 2006; Noga, 2010; Martins et al., 2015; Cardoso et al., 2018). For a better examination of tissues by wet mount, samples were mounted between a glass slide and coverslip with a drop of saline solution (Martins et al., 2015).

2.4. Statistical analysis

We evaluated whether there was a difference in the proportion of infected animals between each municipality. A Chi-square test was performed to compare the proportion of infected animals in different municipalities and the alpha value established to reject the null hypothesis was

Table 1. Fishes analyzed from different ornamental fish farms in Brazil. A: Fortaleza (CE); B: Patrocínio do Muriaé (MG); C: São Francisco do Glória (MG); D: Cascavel (PR); E: Timbó (SC); F: Iguape (SP); G: Jacareí (SP); H: Mairinque (SP).

Family	Specimen	Type of fish	Common Name	n	Counties	Weight(g)	Length(cm)
Adrianichthyidae	*Oryzias Woworae*	Exotic	Daisy's ricefish	**2**	A	0.35±0.07	3.25±0.07
Callichthyidae	*Corydora paleatus*	Native	Peppered corydoras	**5**	F; G	3.24±1.02	6.08±0.78
Characidae	*Paracheirodon innesi*	Native	Neon tetra	**4**	A	0.40±0.18	2.63±0.10
	Gymnocorymbus ternetzi	Exotic	Black tetra	**12**	A; B; G	1.51±1.51	4.63±0.96
	Hyphessobrycon eques	Native	Jewel tetra	**2**	C	0.20±0.00	2.60±0.00
Cichlidae	*Aequidens rivulatus*	Exotic	Green terror	**1**	C	3.20±0.00	5.60±0.00
	Apistograma cacutuoides	Native	Cockatoo cichlid	**4**	D	2.10±0.54	5.58±0.38
	Astronotus ocellatus	Native	Oscar	**7**	A; D	5.86±4.47	6.61±1.71
	Cichlasoma salvini	Exotic	Yellow belly cichlid	**2**	D	2.90±0.42	5.65±0.35
	Hemichromis bimaculatus	Exotic	Jewelfish	**2**	C	2.70±0.14	5.55±0.07
	Labidochromis caeruleus	Exotic	Blue streak hap	**6**	A	0.92±0.40	4.13±0.45
	Pseudotropheus demasoni	Exotic	Pombo rocks	**2**	A	1.55±0.07	4.85±0.21
	Pterophyllum scalare	Native	Freshwater angelfish	**52**	B; H	1.97±1.70	4.81±1.32
	Rocio octofasciata	Exotic	Jack Dempsey	**3**	A	1.00±0.00	3.80±0.10
	Sympgysodon aequifasciatus	Native	Blue discus	**2**	D	1.80±0.42	4.30±0.42
	Thorichthys meeki	Exotic	Firemouth cichlid	**2**	A	1.95±0.35	4.75±0.21
Cobitidae	*Misgurnus anguillicaudatus*	Exotic	Pond loach	**1**	F	5.80±0.00	10.40±0.00
Cyprinidae	*Carassius auratus*	Exotic	Goldfish	**100**	B; D; E; G	6,29±3.61	7.49±1.40
	Cyprinus carpio	Exotic	Common carp	**8**	C	2.09±0.91	5.49±0.80
	Epalzeorynchos bicolor	Exotic	Redtail sharkminnow	**1**	A	2.00±0.00	6.20±0.00
	Puntius sachsii	Exotic	Goldfinned barb	**4**	C	1.10±0.18	4.13±0.15
	Puntius tetrazona	Exotic	Sumatra barb	**2**	B	2.55±0.07	5.35±0.07
Loricariidae	*Pterygoplichthys parnaibae*	Native	*Pterygoplichthys parnaibae*	**2**	A	1.90±0.85	6.50±0.71
Melanotaeniidae	*Melanotaenia praecox*	Exotic	Dwarf rainbowfish	**6**	D	2.47±1.08	5.15±0.56
Osphronemidae	*Betta splendens*	Exotic	Siamese fighting fish	**25**	C	1.29±0.22	4.61±0.34
	Trichogaster lalius	Exotic	Dwarf gourami	**9**	B; C; D	2.30±0.81	4.77±0.58
	Trichogaster leerii	Exotic	Pearl gourami	**2**	D	2.30±0.42	5.80±0.28
	Trichogaster trichopterus	Exotic	Three spot gourami	**2**	B	4.35±0.21	7.25±0.35
Poeciliidae	*Poecilia reticulata*	Exotic	Guppy	**20**	B; C	0.26±0.13	3.24±0.44
	Poecilia sphenops	Exotic	Molly	**4**	A; C	1.05±0.35	4.15±0.37
	Xiphophorus hellerii	Exotic	Green swordtail	**8**	B; C	3.03±2.80	5.50±1.15
	Xiphophorus maculatus	Exotic	Southern platyfish	**31**	A; B; C; F	0.89±0.36	3.75±0.42
			Total	**333**			

0.05 ($p<0.05$). After the comparison of proportions, if $p<0.05$, pairwise comparisons were calculated through multiple comparisons with *Bonferroni*'s method to adjust the *p*-value. All statistical analyses were computed using R Core Team (2020) software.

3. Results

Most of the fish examined in this study were parasitized (Table 2), totalizing 70.6% (235/333) of infected specimens. It was found that 47.4% (158/333) of parasites were on gills, 43.2% (144/333) on scales and 9.3% (31/333) were located in the intestine. The metazoans identified in this study were monogeneans (Figure 1A), digenean metacercariae (Figures 1B, 1C and 1D), cestodes (Figure 1E), nematodes (Figures 1F and 1G) and *Lernaea cyprinacea* [anchor worm] (Figure 1H); while protozoans were trichodinids (Figure 2A), *Piscinoodinium pillulare* (Figure 2B), *Ichthyophthirius multifiliis* (ich) (Figure 2C), diplomonad flagellates (Figure 2D), *Ichthyobodo* sp., *Chilodonella* sp. (Figure 2E), and *Tetrahymena* sp. (Figure 2F).

During the clinical analysis it was found that 92.2% (307/333) of animals were alert upon examination, 7.8% (26/333) were prostrated, 4.8% (16/333) showed some difficult breathing, 2.7% (9/333) had skin wounds or damaged fins, 0.9% (3/333) had excessive mucus, and none of them presented white spots.

In the farms from Fortaleza and Mairinque, fish were raised in fiberglass tanks; the facilities had internal and external barriers, preventing fish from having contact with other animals; there was frequent monitoring and adjustment of pH levels, ammonia, nitrite and nitrate; before introducing new individuals to the system, fish stayed in quarantine; and there was a vet technician responsible for sanitary aspects and animal health in both municipalities. In Cascavel, Jacareí, Patrocínio do Muriaé and Timbó, fish were pound-raised and had contact with outside animals because there were no internal or exterior barriers; there was also no control of water temperature; farms had a poor sanitation system, with frequent low dissolved oxygen and high levels of occurrence of water eutrophication; equipment or aquaria did not have any type of disinfection or antisepsis; there was an inefficient pathogen control. In Iguape and São Francisco do Glória, fish were also pond-raised and had contact with outside animals; the farms did not have any control over water temperature; nor did they implement frequent monitoring and control of pH, ammonia, nitrite and nitrate.

In the northwest region, it was found that 23.7% (9/38) of fish from Fortaleza were parasitized, of which 10.5% (4/38) were infected by *Chilodonella* sp., 5.3% (2/38) by trichodinids, and 2.6% (1/38) by monogeneans, *Tetrahymena* sp. and *I. multifiliis.*

In the southern region, 100% (55/55) of fish from Timbó were parasitized, of which 100% (55/55) were infected by trichodinids, 80% (44/55) by monogeneans and 5.5% (3/55) by digenean metacercariae. In Cascavel, 75.7% (28/37) of fish were parasitized by seven different types of parasites: 32.4% (12/37) by monogeneans and *Chilodonella* sp., 21.6% (8/37) by *P. pillulare*, 18.9% (7/37) by *Tetrahymena* sp., 13.5% (5/37) by *I. multifiliis*, 10.8% (4/37) by trichodinids and 2.7% (1/37) by cestodes.

In the southwest region, 88% (44/50) of fish from Patrocínio do Muriaé were parasitized and eight different types of parasites were found: 46% (23/50) were infected by monogeneans, 44% (22/50) by *Chilodonella* sp., 14% (7/50) by digenean metacercariae, 4% (2/50) by diplomonad

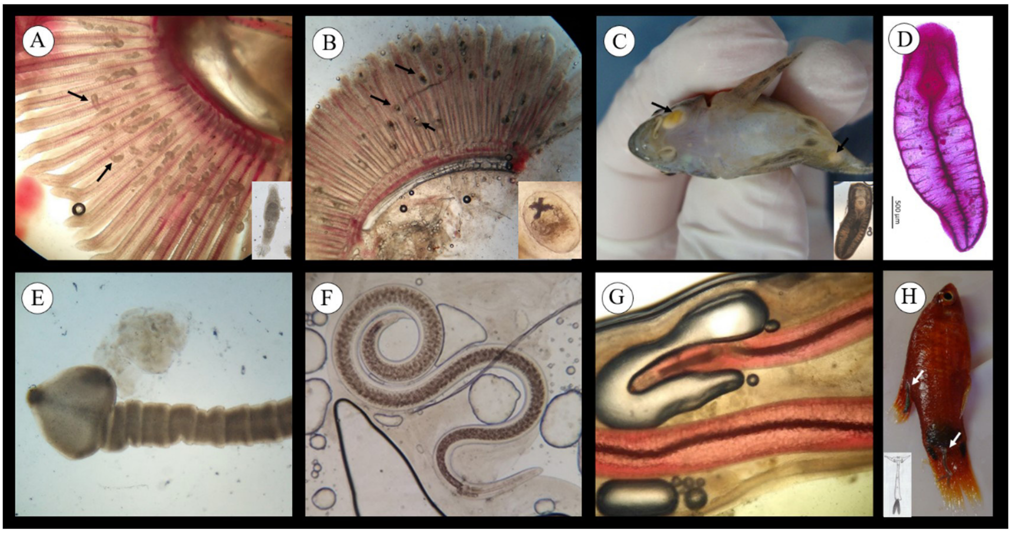

Figure 1. Optical microscopic magnification and naked eye of metazoan parasites: monogeneans in gills of *Astronotus ocellatus* (A) 10x, digenean metacercariae: in gills of *Xiphophorus maculatus* (B) 10x, yellow disease skin similar to the genus *Clinostomum* in *Corydora paleatus* (C, D), cestodes similar to the species *Schyzocotyle acheilognathi* in intestine of *Cichlasoma salvini* (E) 20x, nematodes: in intestine of *Pteroplylum scalare* (F) 20 x, and *Corydora paleatus* (G) 20x and *Lernaea cyprinacea* in skin of *Xiphophorus maculatus* (H). Schematic drawing of 1 H: Noga (2010).

Table 2. Prevalence of parasites recovered from different ornamental fish farms in Brazil. A: Fortaleza (CE); B: Patrocínio do Muriaé (MG); C: São Francisco do Glória (MG); D: Cascavel (PR); E: Timbó (SC); F: Iguape (SP); G: Jacareí (SP); H: Mairinque (SP).

Parasite	A	B	C	D	E	F	G	H
Monogeneans	2.6% (1/38)	46.0% (23/50)	22.8% (13/57)	32.4% (12/37)	80.0% (44/55)	-	87.1% (27/31)	-
Trichodinids	5.3% (2/38)	2.0% (1/50)	14.0% (8/57)	10.8% (4/37)	100.0% (55/55)	-	6.5% (2/31)	-
Chilodonella sp.	10.5% (4/38)	44.0% (22/50)	3.5% (2/57)	32.4% (12/37)	-	-	6.5% (2/31)	-
Metacercariae	-	14.0% (7/50)	5.3% (3/57)	-	5.5% (3/55)	57.14% (12/21)	48.4% (15/31)	-
Ichthyophthirius multifiliis	2.6% (1/38)	2.0% (1/50)	8.8% (5/57)	13.5% (5/37)	-	-	25.8% (8/31)	-
Diplomonad flagellates	-	4.0% (2/50)	-	-	-	-	-	40.9% (18/44)
Piscinoodinium pillulare.	-	-	10.5% (6/57)	21.6% (8/37)	-	-	9.7% (3/31)	-
Nematodes	2.6% (1/38)	2.0% (1/50)	1.8% (1/57)	-	-	14.28% (3/21)	6.5% (2/31)	15.9% (7/44)
Tetrahymena sp.	2.6% (1/38)	-	1.8% (1/57)	18.9% (7/37)	-	-	-	-
Ichthyobodo sp.	-	-	10.5% (6/57)	-	-	-	3.2% (1/31)	-
Cestodes	-	-	8.8% (5/57)	2.7% (1/37)	-	-	-	-
Lernaea cyprinacea	-	4.0% (2/50)	-	-	-	-	-	-
FISHES EVALUATED	**38**	**50**	**57**	**37**	**55**	**21**	**31**	**44**
INFECTED FISHES	**9**	**44**	**33**	**28**	**55**	**14**	**30**	**22**
INFECTED ANIMALS (%)	**23.7%**	**88.0%**	**57.9%**	**75.7%**	**100.0%**	**66.7%**	**96.8%**	**50.0%**

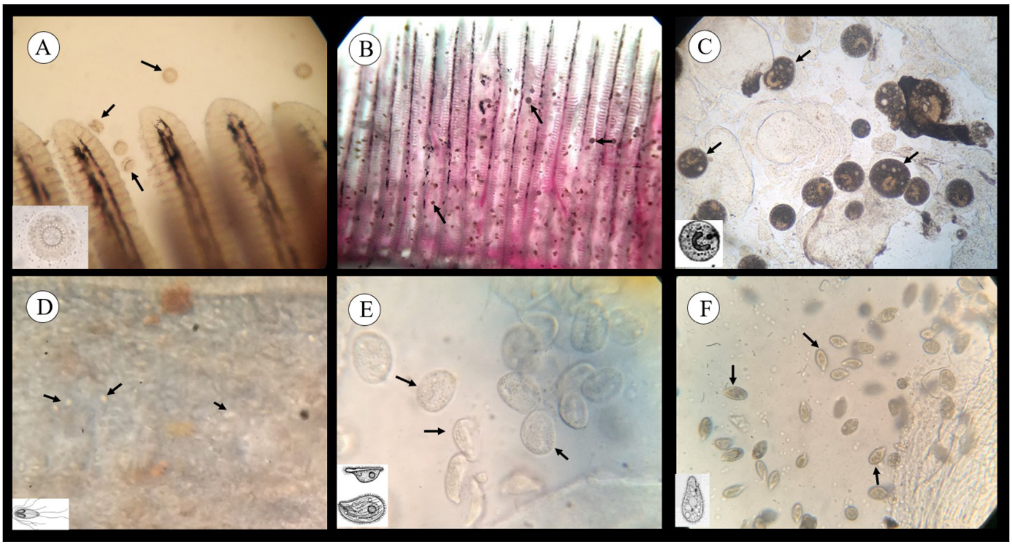

Figure 2. Optical microscopic magnification of protozoans trichodinids in gills of *Labidochromis caeruleus* (A) 10x, *Piscinoodinium* sp. in gill of *Apistograma cacatuoides* (B) 10x, *Ichthyophthirius multifiliis* in skin of *Hyphessobrycon eques* (C) 10x, diplomonad flagellates in *Pterophylum scalare* (D) 40x, *Chilodonella* sp. in skin of *Carassius auratus* (E) 20x, and *Tetrahymena* sp. in skin of *Poecilia reticulata* (F) 20x. Schematic drawing of 2 C,D,E,F: Noga (2010).

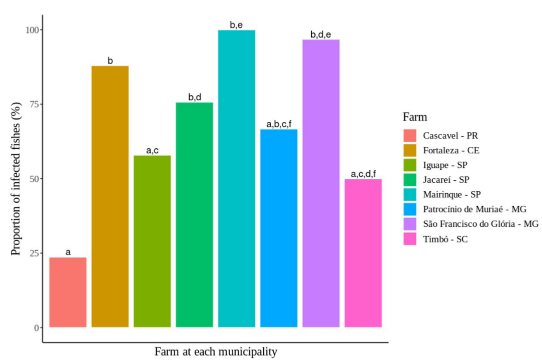

Figure 3. Proportion of infected fish in each municipality analyzed in the study. Note: a,b,c,d,e,f: Equal letters correspond to equal proportions by the pairwise comparisons for proportions with Bonferroni's correction.

flagellates and *L. cyprinacea*, and 2% (1/50) by *I. multifiliis*, trichodinids and nematodes. In São Francisco do Glória, 57.9% (33/57) of fish were parasitized by ten different types of parasites: 22.8% (13/57) by monogeneans, 14% (8/57) by trichodinids, 10.5% (6/57) by *P. pillulare* and *Ichthyobodo* sp., 8.8% (5/57) by *I. multifiliis* and cestodes, 5.3% (3/57) by digenean metacercariae, 3.5% (2/57) by *Chilodonella* sp., and 1.8% (1/57) by tetrahymenids and nematodes. In Iguape, 66.7% (14/21) of fish were parasitized, of which 57.1% (12/21) were infected by digenean metacercariae and 14.3% (3/21) by nematodes. In Jacareí, 96.8% (30/31) of fishes were parasitized by eight different types of parasites: 87.1% (27/31) by monogeneans, 48.4% (15/31) by digenean metacercariae, 25.8% (8/31) by *I. multifiliis*, 9.7% (3/31) by *P. pillulare*, 6.5% (2/31) by trichodinids, *Chilodonella* sp. and nematodes, and 3.2% (1/31) by *Ichthyobodo* sp. Finally, in Mairinque, 50% (22/44) of fish were parasitized, of which 40.9% (18/44) were infected by diplomonad flagellates and 15.9% (7/44) by nematodes.

The proportion of infected animals between municipalities is shown in Figure 3. The proportion of infections in Fortaleza was not different from that in animals of São Francisco do Glória (p=0.06), Iguape (p=0.09) or Mairinque (p=0.74), but it was different from that in the fish from Patrocínio do Muriaé, Cascavel, Timbó and Jacareí (p<0.01). In Patrocínio do Muriaé, the proportion of infections was different from São Francisco do Glória (p=0.03) and Mairinque (p<0.01), and presented no difference from Cascavel, Iguape, Jacareí (p=1.0) or Timbó (p=0.73). In São Francisco do Glória, the proportion of infections was different from Timbó and Jacareí (p<0.01), and presented no difference from Cascavel, Iguape or Mairinque (p=1.0). In Cascavel, the proportion of infections significantly differed only from Timbó (p=0.01), and it was not significantly different from Iguape (p=1.0), Jacareí (p=0.99) or Mairinque (p=0.91). The proportion of infected animals in Timbó was different from that in Iguape and Mairinque (p<0.01) and presented no difference from Jacareí (p=1.0). The proportion of infections in Iguape was not different from that in Jacareí (p=0.29) or Mairinque (p=1.0). Lastly, in Jacareí, the proportion of infected animals was different from that in Mairinque (p=0.01).

4. Discussion

Our results demonstrate that a great diversity of parasites currently affects ornamental fish from the main producing regions in Brazil. Fortaleza had the lowest proportion of infected fish (23.7% - 9/38) in this study, and thus it was used to compare the proportion of infected animals with the other municipalities.

When compared to Fortaleza, the proportion of infected fish was equal in São Francisco do Glória (57.9% - 33/57), Iguape (66.7% - 14/21) and Mairinque (50% - 22/44). In Fortaleza and Mairinque: both farms employ proper measures for raising their fish, resulting in fewer infected animals in these farms. Although São Francisco do Glória and Iguape raised fish in pound-raised systems, which meant that fish had contact with outside animals and that there was not proper water quality control, the proportion of infected animals was equal to Fortaleza. This result could be explained by the small number of evaluated specimens in Iguape: only 21 analyzed fish belonging to three species (*Corydora paleatus, Xiphophorus maculatus* and *Misgurnus anguillicaudatus*), of which 57.1% (12/21) were infect by digenean metacercariae. In addition, in São Francisco do Glória, even though fish were raised in pond-raised tanks, animal handling was better in this county, which could explain the proportion of infected animals being similar to that of Fortaleza.

The proportion of infected fish in Timbó (100% - 55/55), Jacareí (96.8% - 30/31), Patrocício do Muriaé (88% - 44/50), and Cascavel (75.7% - 28/37) was different from Fortaleza. In these four counties, a greater proportion of infections were observed. Pond-raised fish farms are normally characterized by a lack of proper water quality control or biosecurity measures. These characteristics enable pathogens reproduction and spread, and as a result, more animals were infected in these farms in relation to Fortaleza.

All fish analyzed from Timbó were Goldfish, *Carassius auratus,* from a new producer in the region, and 100% (55/55) of the fish were infected with trichodinids. Trichodinids have been previously described to infect ornamental freshwater fish from different Brazilian regions (Piazza et al., 2006; Tavares-Dias et al., 2009, 2010; Martins et al., 2012; Florindo et al., 2017b; Santos et al., 2017; Cardoso et al., 2018). In natural habitats, these ectoparasites cause no clinical effects and have also been reported in cultured fish (Martins and Ghiraldelli, 2008). However, under highly intensive aquaculture systems with inadequate handling conditions, these ciliates may proliferate extensively and become pathogenic parasites responsible for, not only for serious host inhibited growth, but also losses up to 50% of fish stocks (Madsen et al., 2000; Guiraldelli, 2006). When starting a new farm, it is important to follow biosecurity rules and prophylactic measures to preserve animal health, avoid parasite proliferation, and ensure that animals without health issues are commercialized.

The proportion of infected fish in Timbó did not differ from that in Jacareí (p=1). In Jacareí and Timbó, 87.1% (27/31) and 88% (44/55) of fish were infected with monogeneans, respectively. These flatworms have already been reported in different ornamental freshwater fish from Brazil (Tavares-Dias et al., 2009, 2010; Florindo et al., 2017a; Santos et al., 2017; Cardoso et al., 2018; Hoshino et al., 2018). They have a direct life cycle and can cause respiratory disease when infecting gills, or scale loss may occur if the parasite is attached to the skin. Large numbers of monogeneans on either the skin or gills may result in significant damage and mortality (Cardoso et al., 2017; Francis-Floyd et al., 2019). In this study, only 4.8% (16/333) of the infected fish presented difficulty in breathing properly, and 2.7% (9/333) showed scale irritation or damage during clinical assessment. Herein, it is also important to highlight that scale damage might have been caused during the transport of animals. Despite these findings, these results demonstrate that few fish presented sings of infection when commercialized. Heavy monogenean infestations are usually indicators of poor sanitation and deteriorating water quality (Chen et al., 2020), a scenario found in producers from the counties of Jacareí and Timbó. If left untreated, monogeneans can lead to serious economic loss for fish farmers.

In Jacareí, 25.8% (8/31) of the fish were infected by *I. multifiliis*. This protozoan is a widespread parasite with no host specificity and has been described in a range of ornamental fish in Brazil (Piazza et al., 2006; Tavares-Dias et al., 2009, 2010; Eiras et al., 2012; Santos et al., 2017; Cardoso et al., 2018; Hoshino et al., 2018). Fishery utensils used in fish farms and stressful situations, such as overcrowding, low dissolved oxygen, and high temperatures, favor the *I. multifiliis* life cycle and disease outbreaks (von Gersdorff Jørgensen, 2016). In this study, none of the fish infected with *I. multifiliis* presented the telltale clinical sing of *I. multifiliis* infestation during clinical assessment, white spots. This demonstrates that infected fish may have the parasite but may not yet have developed common clinical signs of the disease prior to removal from the system. Although *I. multifiliis* showed low prevalence rates in the analyzed fish, sanitary handling must be considered to avoid the introduction of diseased or asymptomatic fish.

In Iguape, fish were pond-raised and had contact with animals outside of the pounds, including birds, which are common hosts for of adult digeneans, from which metacercariae are part of their life cycle. An important finding was yellow spot disease in *Corydora paleatus*. The morphology matched the genus *Clinostomum* that have zoonotic potential, but this was the only parasite that we had the opportunity to attempt molecular identification and there was no amplification of the PCR reaction. A total of 57.1% (12/21) of fishes from Iguape were infected with digenean metacercariae. This digenean life stages commonly found in ornamental fish raised in tanks or lakes with access to other hosts, such as crustaceans, mollusks, birds and mammals (Alves et al., 2001; Piazza et al., 2006; Santos et al., 2017; Cardoso et al., 2018; Hoshino et al., 2018). Except for the counties of Mairinque and Fortaleza, all farms evaluated had pound-raised systems that enabled the direct contact of fish with birds, mammals, mollusks and crustaceans. Trematode cysts can infect gills, skin, muscles and viscera, and are generally innocuous; however, high infestations can be lethal (Dias et al., 2003). When in metacercaria form, these worms cannot be treated; therefore, precautions must be taken to prevent the parasitosis.

In Patrocínio do Muiaré and Cascavel, the proportion of infected animals was not significantly different (p=1).

Of the fish from Patrocínio do Muriaé, 44% (22/50) were infected with *Chilodonella* sp., while, in Cascavel, 21.6% (8/37) and 18.9% (7/37) were infected with *P. pillulare* and *Tetrahymena* sp., respectively. These ectoparasites have been frequently reported in studies from different Brazilian regions (Piazza et al., 2006; Tavares-Dias et al., 2009, 2010; Eiras et al., 2012; Florindo et al., 2017b; Cardoso et al., 2018) and their transmission occurs especially through direct contact between infested and healthy fish. Elevated intensities of these protozoan parasites are common in cultured fish because the parasite disseminates in fish population via the use of routine utensils and water used during fish transport (Martins et al., 2002, 2015; Tavares-Dias et al., 2010). Pathological changes caused by these agents are related to their abrasive action on the host epithelium, with the gill filaments being the most sensitive organ for parasites to attack. Clinical signs are non-specific, indicating respiratory difficulty and skin irritation, resulting in excessive mucus (Martins et al., 2015). In this study, only 0.9% (3/333) of fish had excessive mucus during clinical assessment, demonstrating that few infected fish present signs of infection when commercialized, even though they are infected.

Lernaea cyprinacea was found just in Patrocínio do Muriaé. This parasite is a non-native species that was first cataloged in Brazil in the 1960s, being disseminated from Brazilian regions, like São Paulo, southwest Brazil (Cardoso et al., 2018), Sergipe northeastern Brazil (Assis et al., 2014), and Pará, north Brazil (Barros et al., 2024), corroborating the data obtained from the present study. This invasive parasite can cause severe damage on fish populations and spread rapidly in a farming environment (Assis et al., 2014), so the adoption of quarantine and not disposing of waste water in common sewage are actions that prevent the spread and establishment of the parasite.

All fish that came from Mairinque were angelfish, *Pterophyllum scalare*, which were raised in polyethylene tanks, a material that is easy cleaned and disinfected. Only two types of parasites were found in this farm: 15.9% (7/44) of fish from Mairinque were infected by nematodes and 40.9% (18/44) by diplomonad flagellates. Like metacercariae, nematodes also present a complex life cycle that requires two or more intermediate hosts, and because of the absence of other hosts during the life cycle, they are relatively uncommon in cultured fish. However, fish fed live and wild-caught arthropods can become infected, enabling them to be either intermediate or final hosts for nematodes (Woo, 2006; Noga, 2010). Crustaceans and arthropods were part of the diet of fish from Mairinque, which most likely explains the infection. Diplomonad flagellates are comprised of various *Spironucleus* species that are mostly found in the intestine and are directly transmitted from fish to fish. Many cases of diplomonad flagellates in aquarium fish are mixed infections that involve other bacterial opportunists (Gallani et al., 2016) or parasites (e.g., *Capillaria* nematode infections in angelfish) (Noga, 2010).

Host-parasite interactions can be affected by inadequate handling, suboptimal water quality, inadequate nutrition, and stress (Piazza et al., 2006; Martins et al., 2011; Hoshino et al., 2018). The prevalence rates found in this study might have been influenced by the lack of control of the fish populations, water quality, high stocking density, inadequate water temperature, and a lack of quarantine measures for introduced fish. The farms from the municipalities of Fortaleza and Mairinque had a veterinarian to assist fish management and health, and raised fish in fiberglass tanks, a material that facilitated cleaning and disinfection of water environment. Conversely, the counties of Cascavel, Iguape, Jacareí, Patrocínio do Muriaé, São Francisco do Glória and Timbó did not had a technician present to ensure an effective biosecurity program to maintain healthy animals, thus reducing the risk of infectious disease in the facility. Additionally, they had pond-raised tanks that not only enabled the contact of fish with hosts that could disseminate parasites, but also made cleaning and disinfection more complicated.

The absence of clinical signs in fish on farms allows for the trading of infected fish and the spreading of parasites, or other harmful organisms, along the aquatic trade chain. Parasites may be normally present on fish skin, fins, and gills, or in internal organs without putting the host at risk. However, when fish are exposed to environmental or behavioral stressors, the potential damage from those organisms increases, enabling host susceptibility, causing imbalance between host, parasite and environment (Martins et al., 2002). Therefore, the manifestation of these diseases only occurs when the parasites encounter favorable situations for their proliferation.

Cestodes were found in fish from only two farms: São Franscisco do Glória 8% (5/57) and Cascavel 2,7% (1/37). Although we didn't identify genus and species of the parasite, the only *Ciclassoma salvini* analyzed from Cascavel presented a cestode very similar to the Asian fish tapeworm *Schyzocotyle acheilognathi* commonly found in native freshwater fishes from Asia. This cestode has low host specificity and for that reason it has been registered parasitizing more than 200 cultured and wild fish species, besides amphibians, reptiles and birds from different regions of the world. With a high pathogenic potential, *S. acheilognathi* may cause mortalities in highly infected fish. This cestode has already been found in Brazil (Souza et al., 2018).

Apart from host damage, depigmentation, skin ulceration, scale loss, excessive mucus production, gill lesions and reduced growth of fish, parasite infection also increases the mortality ratio by favoring secondary bacterial infections (Xu et al., 2012). Thus, prophylactic measurements adopted in farms and the ornamental fish industry are important tools to minimize the effects of parasitism (Martins et al., 2011; Cardoso et al., 2020b).

5. Conclusions

Ornamental fish commercialized in Brazil have high infection ratios and in order to provide the market with better and healthier fish, it is necessary to improve handling and maintenance of fish in the field in wholesaler facilities and pet stores. As showed in this study, few fish presented signs of infection when they were in the farms. Even though they had parasites, they might have not yet developed clinical signs. Therefore, monitoring and treatment of diseases in fish farms is essential to prevent the spread of diseases in the aquatic trade chain and to prevent aquarists from abandoning this hobby due to a decrease om the availability of healthy animals. More detailed research, including the use of molecular biology to identify parasites that can infect a range of ornamental freshwater fish, is necessary to enrich this initial study. In addition, this study can serve as a basis for new research that has a more adjusted design.

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