Recent studies on parasitic infections of freshwater cultivated fish in the state of São Paulo, Brazil

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ABSTRACT. The present work relates diagnosticated cases of fish diseases in the state of São Paulo, Brazil between January/1999 and December/2000. During 1999, the monogenean was the most important parasite (72.9%), followed by *Piscinoodinium pillulare* (43.2%), *Henneguya piaractus* (34.2%), *Ichthyophthirius multifiliis* (23.4%) and copepodids of *Lernaea cyprinacea* (9.0%). However, in 2000, monogenean showed 78.9%, trichodinids 52.1%, *P. pillulare* 35.7%, *I. multifiliis* 29.8% and *L. cyprinacea* 11.9%. The most infected fish was *Piaractus mesopotamicus*, followed by *Leporinus macrocephalus*, hybrid "tambacu" and *Oreochromis niloticus*. This work showed the highest susceptibility of "tambacu" and *L. macrocephalus* to *P. pillulare* and *P. mesopotamicus* to monogenean *Anacanthorus penilabiatus*. The authors emphasize the importance of prophylaxis in farmed-fish, as well as adequate transport and water quality. The regular accompaniment of fish health, strategic applications of sodium chloride in the water and vitamin C supplementation in the ration to avoid the unbalance of the host/parasite/environment system.

Key words: Brazil, cultivated fish, diagnosis, parasites, prophylaxis.

RESUMO. Recentes estudos de infecções parasitárias em peixes cultivados no Estado de São Paulo, Brasil. O presente trabalho relata os casos de doenças diagnosticadas em peixes no Estado de São Paulo, Brasil, entre janeiro/1999 e dezembro/2000. Durante o ano de 1999, os monogenéticos foram os parasitos mais importantes, com ocorrência de 72,9%, seguidos de *Piscinoodinium pillulare* (43,2%), *Henneguya piaractus* (34,2%), *Ichthyophthirius multifiliis* (23,4%) e copepoditos de *Lernaea cyprinacea* (9,0%). No ano de 2000, os monogenéticos mostraram 78,9% de ocorrência, tricodinídeos 52,1%, *P. pillulare* 35,7%, *I. multifiliis* 29,8% e *L. cyprinacea* 11,9%. O peixe mais infectado foi *Piaractus mesopotamicus*, seguido de *Leporinus macrocephalus*, híbrido tambacu e *Oreochromis niloticus*. Este trabalho mostrou a maior susceptibilidade do tambacu e do *L. macrocephalus* ao *P. pillulare* e do *P. mesopotamicus* ao monogenético *Anacanthorus penilabiatus*. Os autores enfatizam a importância da profilaxia na criação, como o acompanhamento regular da saúde dos animais, aplicações de cloreto de sódio na água e suplementação com vitamina C para evitar o desequilíbrio do sistema hospedeiro/parasito/ambiente.

Palavras-chave: Brasil, peixes cultivados, diagnóstico, parasitos, profilaxia.

Introduction

Aquaculture in Brazil has presented a rapid development, especially with the intensive fish farms. However, nutritional deficiency, poor water quality, infectious and parasitic diseases may cause unbalance in the host/parasite/environment system, culminating in economic losses (Snieszko, 1974; Ceccarelli *et al.*, 1990; Klesius and Rogers, 1995; Martins *et al.*, 1997; 2000). In the state of São Paulo, Martins (1998) has disseminated the importance of prophylactic measures in fish farms, with the accompaniment of fish health, to avoid parasite reproduction. The most cultivated species in Brazil are "pacu" *Piaractus mesopotamicus* Holmberg, 1887; "tambaqui" *Colossoma macropomum* Cuvier, 1818; hybrid "tambacu" *(P. mesopotamicus* male x *C. macropomum* female); "piauçu" *Leporinus macrocephalus* Garavello and Britski, 1988; "matrinxã" *Brycon cephalus* Gunther, 1869; "carp" *Cyprinus carpio*

Linnaeus, 1758; and "red and black tilapia" *Oreochromis niloticus* Trewavas, 1983. Studies on parasites prevalence in cultivated fish are scarce. Some authors reported occurrences in the Northeast region of São Paulo (Ceccarelli *et al.*, 1990; Martins and Romero, 1996, Békési, 1992). Parasitic infections have been studied by Meyer (1970), Mellergaard and Dalsgaard (1987), Pojmanska (1994), Buchmann *et al.* (1995), Sharples and Evans (1995) and Huntington *et al.* (1996). Recently, Martins *et al*. (2000) related the main parasites in the cultivated freshwater fish in São Paulo State as well as their histopathological effects from 1993 to 1998.

In this work, the authors carried out a survey of diagnosed diseases in cultivated fish from the state of São Paulo, Brazil, between 1999 and 2000. A new parasite species in "tilápia" was observed during the research, also, the host/environment relationship is discussed.

Material and methods

A total of 186 cases were diagnosed at the Laboratory of Pathology of Aquatic Organisms of the Aquaculture Center, Unesp, Jaboticabal, state of São Paulo, Brazil from January/1999 to December/2000. The animals arrived in the laboratory because the owners had related behavioral changes and mortality. The ponds where the animals were stocked measured 200 to 30,000 m^2 with 1 to 8 fishes/m^2 stocking density. The gills and other internal organs were excised and preserved into Petri dish with 0.65% saline solution. Pieces of organs were mounted between a glass microscope slide and coverslip for microscopic observation. A scraping of body mucous surface in the skull-tail direction was performed to search for parasites with the aid of enthomological microscope. The parasites were fixed and identified according to Thatcher (1991), Martins and Urbinati (1993), Boeger *et al.* (1995), Martins and Romero (1996) and Martins and Souza (1997).

Results

When the animals arrived at the laboratory, the owners had reported a decrease of feeding activity, with loss of appetite and the fish becoming lethargic, swimming erratically, with loss of equilibrium, usually gathering near the pond edges or water inlets, point-like or diffuse hemorrhages on the body surface, exophthalmia, swelling of the visceral cavity, changes in the normal body color and, in some cases, the presence of fungal infection by *Saprolegnia* sp. In the majority of cases mortality reached 3 to 200 fishes in just one day, related to poor water quality, caused by high stocking density, high organic matter contents or nutritional supplementation with refuse from other animals, such as feces or chicken bowels. In this study, the collected data showed the occurrence of fish diseases between May and September/1999 and May and July/2000, which made up in the colder season. Still, differentiated cases of mortality have occurred in February and March/2000 caused by monogenean and trichodinids. An increase of diagnosticated cases with the protozoan *Ichthyophthirius multifiliis* from autumn/1999 showed the highest level in winter/2000 (Figure 1).

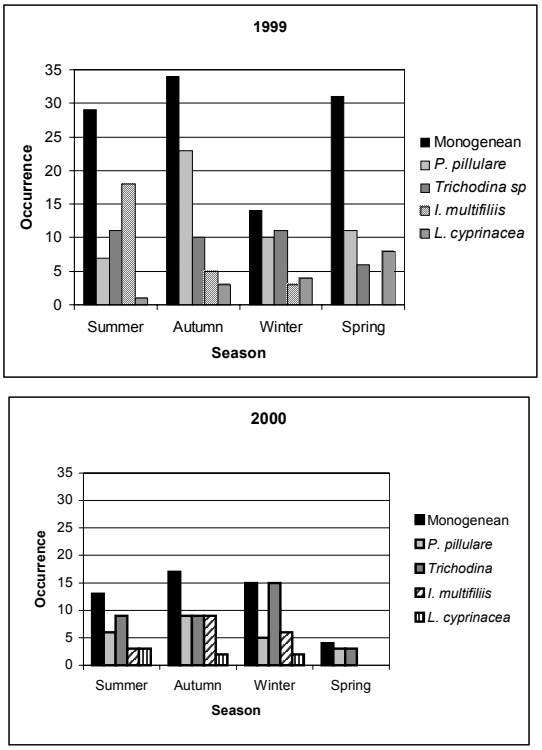

Figure 1. Occurrence of the principal parasites in cultivated fishes during the years 1999 (above), 2000 (below) and their respective season

In 1999, a total of 108 occurrences was diagnosed, being 25% in "pacu" *Piaractus mesopotamicus*; 21.3% in "piauçu" *Leporinus macrocephalus*; 19.4% in hybrid "tambacu"; 12% in "red and Taiwanese tilapia" *Oreochromis niloticus* and 10.2% in "matrinxã" *Brycon cephalus*. On the other hand, in 2000, a total of 78 cases was noted, being 20.5% in "piauçu"; 17.9% in "pacu"; 16.7% in "tilapia" and 15.4% in hybrid "tambacu".

The main group of parasites identified in 1999 were monogenean helminths *Anacanthorus*

penilabiatus Boeger, Husak and Martins, 1995 from "pacu", ancyrocephalids and gyrodactylids in "tilapia" (72.9% occurrence), followed by the dinoflagellate *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom, 1981 (43.2%); ciliated protozoan as trichodinids (34.2%); *Ichthyophthirius multifiliis* Fouquet, 1876 (23.4%); copepodids (9.0%) and adults of crustacean copepods *Lernaea cyprinacea* Linnaeus, 1758 (8.1%); myxosporean *Henneguya piaractus* Martins and Souza, 1997 in "pacu" (9.0%). In 2000, monogenean helminths accounted for 78.9%; trichdinids 52.1%; *P. pillulare* 35.7%; *I. multifiliis* (29.8%; adult 11.% and copepodids of *L. cyprinacea* 8.9%; finally followed by *H. piaractus* (6.9%) (Figure 1). An interesting fact started in 2000, with the intensification of "tilapia" cage culture. The first cases involving another lernaeid crustacean, the *Lamproglena* sp. Von Nordmann, 1832 (Crustacea, Copepoda) genus.

Discussion

The common practice of some fish farm owners in supplementing fish food with disposable products contributes to the poor water quality. This fact, allied to the water exit situated at the pond's surface, contributed to the accumulation of toxic substances and water eutrophication with algae blooms. This is especially true when the hot season arrives and accelerates the organic decomposition that often leads to a low dissolved oxygen content in the pond's water.

The parasites may be present on the fish surface, fins, gills or in the internal organs without harmful consequences to the host. But some conditions, such as changes in the aquatic parameters, climate, stress, introduction of some pathogen that was not present in the environment, are factors that can increase the host susceptibility to parasites and provoke an unbalance of the host/parasite/environment system (Coutant, 1998). The gill was the organ most attacked by the ectoparasites and little importance has been observed in relation to endoparasites such as nematodes, cestodes or digenean trematodes, at least in Brazil. Recently, personal sources revealed anisakid infection in the cultivated "piauçu" from Mato Grosso State, which is probably related to the intermediate and definitive hosts presents in that region. Ceccarelli *et al.* (1990) observed "pacu" as the most infected fish with *I. multifiliis* during cold season. On the other hand, the most important parasite in this work were monogenean helminths. Once more, the authors demonstrated that "pacu" and "piauçu" were the most infected fish. In the Northeast region of Brazil, Békési (1992) related plerocercoids, *Trichodina* and monogenean in *C. macropomum* and *Argulus*, monogenean and *Trichodina* in carp. Confirming the hypothesis that different regions in Brazil can present peculiar parasite behavior. In the present work, monogenean, *P. pillulare*, trichodinids, *I. multifiliis* and *L. cyprinacea* have great importance. According to Martins *et al*. (2000), an increase in diagnosticated cases was reported in 1995 and 1996. Then, in 1997 and 1998, a decrease in such cases has been observed, probably related to the dissemination of prophylatic measures and accompaniment of fish health, avoiding the proliferation of pathogens. The great number of "pacu", "piauçu" and "tambacu" parasitized coincide with the fact of these were the main cultivated fishes. Nevertheless, Tavares-Dias *et al.* (2001) related increase in the *A. penilabiatus* number in the gills of fish from a feefishing property situated in Franca, São Paulo, in spring and summer.

During the last years, the dinoflagellate *P. pillulare* has showed significant importance in cultivated freshwater fish in Brazil. Their low host specificity must be pointed out, because it enables them to affect a high number of fish species. From this point of view, severe mortality reached 3,000 fishes in 24 hours (Martins *et al*., 2001). The presence of *P. pillulare* on the body surface or gills has been associated to poor water quality, overcrowding or inadequate food supplementation. That was confirmed in this work and by Shaharon-Harrison *et al.* (1990).

The main genus of myxosporean parasites in Brazilian cultivated fish is *Henneguya* Thélohan, 1892 and *Myxobolus* Bütschli, 1882, with special reference to *H. piaractus* often associated with other parasites, increasing greatly the host tissue response and diminishing the gill filaments absorption surface. Histhological observations revealed reduction in respiratory efficiency, behavioral changes, lethargy and fish agglomeration in the pond edges and water inlets (Martins *et al.*, 1997). This was also related by Hibiya (1982), Lom and Schubert (1983), Miyazaki *et al.* (1986), Ferraz de Lima *et al.* (1991), Stephen and Ribble (1995), Bastos *et al.* (1996) and Martins and Romero (1996).

When the Hungarian carp was introduced in Brazil, *L. cyprinacea* found a favorable environment for development, being present in a great number of cultivated and native fish species. Gabrielli and Orsi (2000) reported the increase in cases associated to *L. cyprinacea* in the North region of Paraná. The authors verified their incidence during almost one year in "matrinxã"; from October to February in

"pacu" and from September to April in "piauçu". Moreover, they related the presence of parasites in 4 out of 7 native fish species examined. This fact, allied to the lack of transport care, lack of quarantine and diagnosis before the entry of fish in a fish farm, are factors that have contributed significantly to their dissemination. Despite the efforts to eliminate adults of *L. cyprinacea* from culture, another problem exists: the presence of copepodids in the pond's water and in the water utilized during the transport. Tavares-Dias *et al.* (2001) observed high susceptibility of "pacu" to copepodids of *L. cyprinacea* during spring season.

The appearance of *Lamproglena* in gill filaments of cultured "tilapia", specially in the first arches, may be responsible for the lowering of the host resistance and for the increasing of secondary infections, which is emphasized in this work. About 28 species have been documented, 13 in Africa, 14 in Asia and one in Europe (Kumarie *et al.*, 1989). Thereafter, other descriptions were found (Douellou and Erlwanger, 1994; Marx and Avenant-Oldewage, 1996; Ho and Kim, 1997; Saglan, 1998). Leong (1986) observed high incidence of *L. minuta* between July and October in *Puntius binotatus* from Malaysia. Since then, trichodinids and monogenean parasites have been observed in "tilapia" (Vargas *et al.*, 2000). This recent problem in reared "tilapia" encouraged several studies on their identification, distribution and histopathological analyses.

The occurrence of fish diseases in the United States was related to fish transport and lack of careful handling during the hot season (Meyer, 1970). On the other hand, in Brazil, the principal fish mortality can be seen in the cold season (between May and August) when the water temperature reaches 17ºC, while in the hot season reaches the 30ºC. Mellergaard and Dalsgaard (1987) and Buchmann *et al.* (1995) related diseases in eel and trout caused by monogenean *Pseudodactylogyrus* spp, *Gyrodactylus derjavini* and *G. salaris*, respectively, corroborating recent data from this laboratory. Estimated annual losses with infectious and parasitic diseases in channel catfish were US$ 23 million (Klesius and Rogers, 1995). Ciliated protozoans as *I. multifiliis* were responsible for 10 to 20% mortality in trout from Denmark when the water temperature was 16 to 20ºC, although gyrodactylids were abundant at lower temperatures, according to Buchmann and Bresciani (1997).

Supplementation with 300-500 mg vitamin C/kg dry ration for 30 days before handling or winter has contributed to the host increased response against the parasites normally present in the aquatic environment. Moreover, strategic applications of 40 to 100g sodium chloride/m^3 (three times with two days interval between each application) have controlled parasitic infections with *P. pillulare*, *Trichodina*, monogenean and *Argulus/Dolops* crustacean. Recently, Martins *et al*. (2002) were able to reduce 80% monogeneans in the gills of *P. mesopotamicus* with the addition of 1.5-2.0g garlic/kg dry ration fed for 30 days.

In conclusion, the comparison between studies realized in 1995 to 1998 and 1999 to 2000 revealed little alteration in the kind of parasite that can be found in the Brazilian cultivated freshwater fish in the available region. It is necessary to remind that these occurrences may be related to the climatic changes, such as temperature, as well as to the species of cultivated fish.

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