PARASITIC INFECTIONS IN CULTIVATED FRESHWATER FISHES A SURVEY OF DIAGNOSTICATED CASES FROM 1993 TO 1998

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SUMMARY: The present paper related fish diseases from 393 diagnosticated cases by the Aquaculture Center, UNESP, Jaboticabal, SP, Brazil for a period of 1993 to 1998. Fishes were from owners that reported agglomeration of animals at the pond edges, water inlets and progressive mortality. Parasitological diagnosis was performed and gill pieces were fixed in 10% buffered formalin solution to histopathological analysis. The main group of parasite responsible for mortality or changes in fish behaviour was monogenean helminths (36.6% occurrence) followed by *Ichthyophthirius multifiliis* (29.5%), *Piscinoodinium pillulare* (18.0%) and copepodid (15.2%) and adult *Lernaea cyprinacea* (13.0%). Bacteria (20.0%) and fungus (17.0%) were present as secondary infection. The most infected fish was pacu (*Piaractus mesopotamicus*) followed by tambacu hybrid; tambaqui (*Colossoma macropomum*); piauçu (*Leporinus macrocephalus*); tilapia (*Oreochromis niloticus*) and carp (*Cyprinus carpio*).

KEY WORDS: Occurrence, parasites, histopathology, cultivated fish, Brazil.

INTRODUCTION

Freshwater aquaculture have rapidly developed in Brazil. With the intensification of culture systems, diseases related to nutritional deficiency, secondary infection or parasitic infection have been responsible for economic losses (SNIESZKO, 1974; CECCARELLI *et alli*, 1990; KLESIUS & ROGERS, 1995; MARTINS *et alli*, 1997). MARTINS (1998) emphasized the importance of prophylaxis in the fish culture. Among the species being considered for aquaculture in Brazil, there is considerable interest in the pond culture of *Piaractus mesopotamicus* HOLMBERG, 1887 (pacu), *Colossoma macropomum* CUVIER, 1818 (Tambaqui), tambacu hybrid *(P. mesopotamicus* male x *C. macropomum* female), *Leporinus macrocephalus* GARAVELO & BRITSKI, 1988 (Piauçu), *Brycon cephalus* GUNTHER, 1869 (Matrinxã), *Cyprinus carpio* LINNAEUS, 1758 (Carp), *Oreochromis niloticus* TREWAVAS, 1983 (Tilapia) and *Clarias gariepinus* (african catfish). Studies on prevalence of parasites found in brazilian cultivated fish are scarce. Some authors reported fish parasite occurrency in the Northeast São Paulo (CECCARELLI *et alii*, 1990; MARTINS & ROMERO, 1996) and in the brazilian Northeast (BÉKÉKI, 1992). Parasite infections in cultivated fishes around the world were well described by MEYER (1970), MELLERGAARD & DALSGAARD (1987), POJMANSKA (1994), BUCHMANN *et alii* (1995), SHARPLES & EVANS (1995) and HUNTINGTON *et alii*, (1996).

The present work relates a survey of parasitic infections on freshwater cultivated fishes in the Northeast region of São Paulo State, Brazil. Parasitological and histopathological analyses were performed at Aquaculture Center, UNESP, Jaboticabal, SP for the period of 1993 to 1998.

MATERIALS AND METHODS

At the Pathology Laboratory of Aquatic Organisms at the Aquaculture Center, UNESP, Jaboticabal, SP, Brazil, 393 fishes were examined from 1993 to 1998. Original ponds had 240 to 22,000 m^2 and 1 to 8 fish/m^2 stocking density. Fishes were from owners that referred aglomeration of animals at the pond edges, water inlets and progressive mortality. Anamnesis and parasitological diagnosis were performed.

The gills and other internal organs were excised and maintained into Petri dish with 0.65% saline solution. Pieces of organs were mounted between a glass microscope slide and coverslip. Wet mounts were studied microscopically. A scraping of body surface mucous in skull-tail direction was performed to search for parasites with the aid of enthomological microscope. Parasites were treated and identified according to THATCHER (1991), MARTINS & URBINATI (1993), BOEGER *et alii* (1995), MARTINS & ROMERO (1996) and MARTINS & SOUZA (1997). For histopathological analyses, parasitized tissues were fixed in 10% buffered formalin and embedded in paraffin-block, sectioned

(6 mm) and stained in haematoxilin-eosin for the microscopical analysis.

RESULTS

The owners had reported that fishes generally gathered near the pond edges or near the water inlets. Feeding activity decreased with loss of appetite and fish became lethargic, swam erratically with loss of equilibrium; progressive increase in mortality occurred. According to owners information, they rarely cleaned fish nets. Mortality, high organic matter contents and high stocking density were the main reasons for economic losses. In three cases chicken bowels were fed to the fish and then mortality started to occur. From 1993 to 1998 fish mortality caused by parasites, specially dinoflagellates resulted in 23,000 dead fishes, out of which 3,000 died in the period of May 1 to 15, 1996. The data showed that a great number of diagnosticated cases occurred more frequently in the cold season from May thru July 1996 and June thru July 1997, when the water temperature was at 17 to 24°C (Tables III, IV, V). Decrease in diagnosticated cases in 1998 probably due to spreading of prophylaxis methods (Figs. 1 and 2) was observed.

The main group of parasites responsible for changes in fish behaviour or mortality was monogenean helminths (36.6% occurrence) followed by *Ichthyophthirius multifiliis* FOUQUET, 1876 (29.5%), dinoflagellates *Piscinoodinium pillulare* (SCHÄPERCLAUS, 1954) LOM, 1981 (18.0%) and copepodid (15.2%) and adult of *Lernaea cyprinacea* LINNAEUS, 1758 (13.0%). Bacteria (20.0%) and fungus (17.0%) were present as secondary infection accompanied by other parasites (Table 1). Separate analyses of the main fish species cultivated is shown in Table 2. The data revealed the importance of monogenean *Anacanthorus penilabiatus* BOEGER, HUSAK & MARTINS, 1995 to pacu (*P. mesopotamicus*) followed by *Henneguya piaractus* MARTINS & SOUZA, 1997 and *I. multifiliis*. Tambaqui (*C. macropomum*) showed occurrency of monogenean followed by fungal infections and *Trichodina* KAHL, 1933. Tambacu hybrid is the most important cultivated fish presenting a great number of cases with monogenean followed by *P. pillulare*, *I. multifiliis* and *L. cyprinacea*. Piauçu (*L. macrocephalus*) presented mainly monogenean and *L. cyprinacea* infections. Tilapia (*O. niloticus*) presented more frequently bacteriosis caused by inadequate water quality or fed with chicken entrails. However, carp (*C. carpio*) showed their susceptibility to *L. cyprinacea*.

Histopathology

***I. multifiliis* (Ciliophora)**

This cosmopolite and nonspecific parasite was observed on the surface or gill filament of fish. In the gills, trophonts were present in the primary lamellae, involved by a simple layer of epithelium cells. Parasite cells were surrounded by epithelial cells in young fish infection. Hyperplasia, necrosis and inflammatory infiltrate with oedema were present in advanced infection. Hyperplasia of the goblet cells was an important observation in the epithelium gill. Severe infection showed dense lymphocyte infiltrate in the primary and secondary lamellae.

***P. pillulare* (Dinoflagellida)**

Was a very common parasite that occurred in the gills or surface of many fish species. Circulatory changes as ecchymosis of the caudal peduncule and operculum as well as congestion and petechiae were observed. Histopathology showed a great number of trophonts between the secondary lamellae embedded or not with rhizocysts into the epithelium which penetrate firmly in the epithelial cells. Degeneration and focal epithelial necrosis were observed. The primary lamellae showed congestion, focal interstitial haemorrhages, subepithelial oedema, severe hyperplasia of epithelial and goblet cells and inflammatory infiltrate. The extremity of secondary lamellae presented fusion because of the hyperplasic process, originating cavities, in which the parasites were present. In other cases the extension of the hyperplasic process of the epithelial cells filled all interlamellar space. Such epithelium masses obstructed respiratory surface of the gills.

***H. leporinicola* (Myxozoa)**

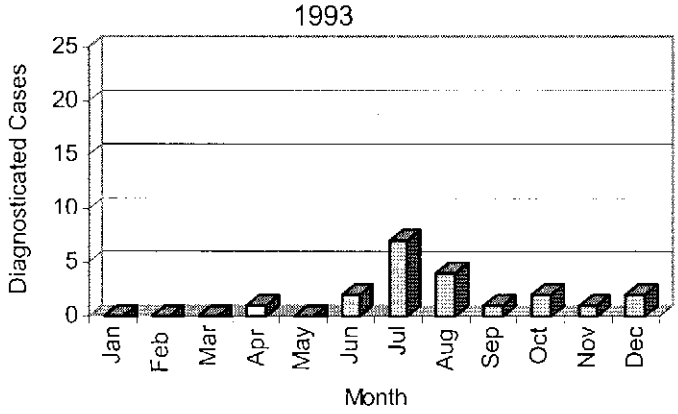
A common host of *H. leporinicola* was *L. macrocephalus* (piauçu). The parasite was found in the gill filaments of fish. Hemorrhages and severe inflammatory foci in the gill epithelium, where the cysts were located were reported. Occasionally, injuries were also noted on the primary and more frequently on the secondary lamellae. Moreover, the cysts were involved by two layers of elongated cells (fibroblast-like-cells) and by intense mononuclear inflammatory infiltrate. The cysts pushed the lamellae reducing the respiratory efficiency. Hyperplasia and displacement of the respiratory epithelium were also observed.

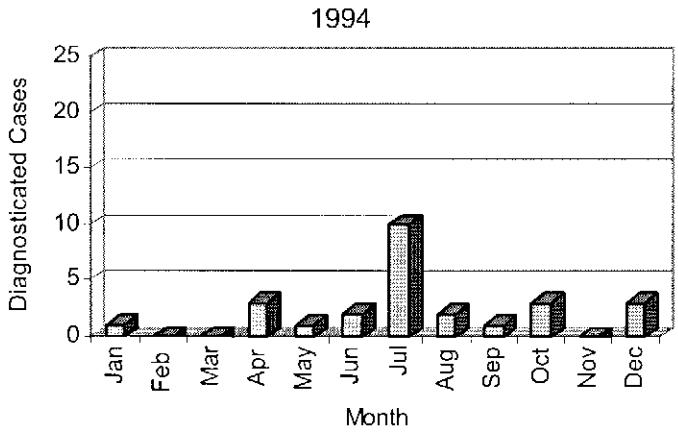
***H. piaractus* (Myxozoa)**

Myxosporidian were observed in several cases of mortality in *P. mesopotamicus* (pacu) associated or not to other parasites. In the gills, hemorrhages were observed in the most intense infections. Cysts were surrounded by two layers of elongate fibroblast-like cells and an inflammatory mononuclear infiltrate. Larger cysts displaced the adjacent lamellae. Hyperplasia of the basal cells and displacement of the respiratory epithelium was observed. Sometimes, sub-epithelial oedema with displacement of the gill epithelium and congestion of the sinusoid capillaries were observed. Hyperplasia of the goblet cells at the end of the secondary lamellae was frequently observed as well.

Monogenean helminths (Monogenea)

Monogenean infection on the surface or gills caused by a small number of parasites was not responsible for serious changes, but inflammatory reaction and hyperplasia of epithelial cells were related. Frequently monogeneans were found anchored with their hooks in the medium and basal region of secondary lamellae. A great number of parasite (about 3,150 to 10,000 per fish) provoked an extensive hyperplasia of the primary





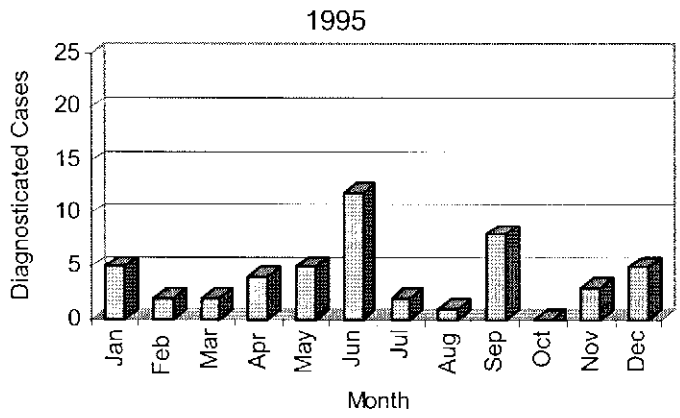
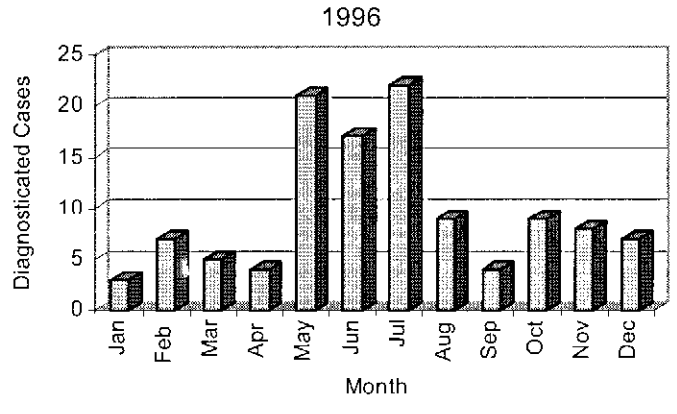
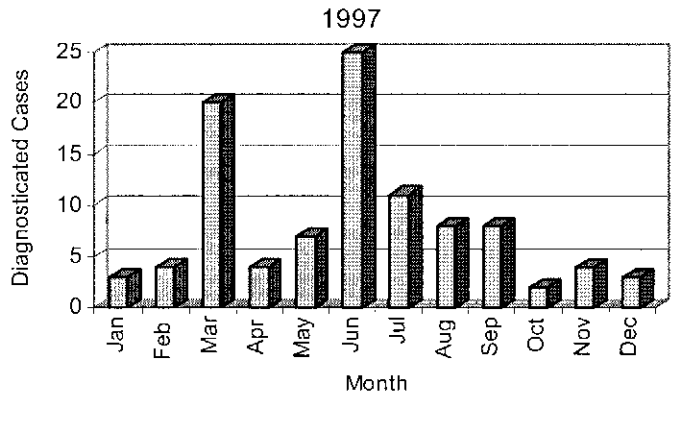


Figure 1 - Number of diagnosticated cases per year at the Aquaculture Center, UNESP.





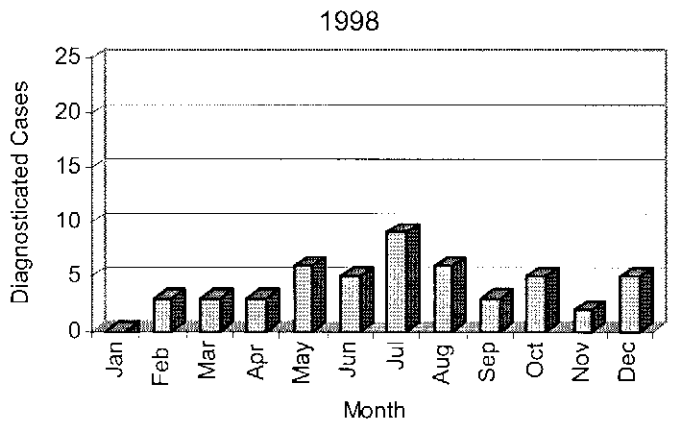


Figure 2 - Number of diagnosticated cases per year at the Aquaculture Center, UNESP.

lamellae with necrosis, oedema and epithelial displacement. It was possible to relate sporadic hemorrhages in the gill filament after death.

Copepodids of *L. cyprinacea* (Copepoda)

Fishes presenting copepodids of *L. cyprinacea* showed moderate diffused epithelial hyperplasia affecting the interlamellar spaces. In severe infections hyperplasia affected the extremity of secondary lamellae. Congestion of the sinusoidal capillary, subepithelial oedema of secondary lamellae was observed in moderate infection. In several number of cases, *I. multifiliis* and monogeneans were present causing severe hyperplasia of epithelium and affecting all gill filaments with a great number of lymphocyte infiltrate. Congestion, telangiectasis and focal interstitial hemorrhages were observed.

DISCUSSION

In the year of 1993 some cases of mortality or changes in fish behaviour started to appear during the cold season. In 1995 more cases were observed even during the hot season. Such observations also pointed the significant increase of fish culture, including fishfarms and feefishing. The following year (1996) was marked with a great number of fish mortality caused by the presence of parasites and lack of prophylaxis by the owners. The present work demonstrated the importance of prophylaxis information that began to make effect after 1997 and that was observed in 1998.

Table 1 - Identified pathogens diagnosticated in cultivated fishes during a period of 1993 to 1998.

Etiological Agent	Occurrence (%)
Monogeneans (*Anacanthorus penilabiatus*, Ancyrocephalinae and others)	36,6
Ichthyophthirius multifiliis	29,5
Bacteria	20,0
Piscinoodinium pillulare	18,0
Fungus	17,0
Copepodids of *Lernaea cyprinacea*	15,2
Adult of *Lernaea cyprinacea*	13,0
Trichodina sp	9,9
Not identified agent	8,1
Ichthyobodo necator	4,8
Henneguya piaractus e *H. leporinicola*	4,1
Chilodonella sp	2,0
Myxobolus colossomatis, *Myxobolus* sp	1,3
Crustaceans Branchiura (*Dolops* sp and *Argulus* sp)	1,3
Nematodes (*Eustrongylides* sp, *Rondonia rondoni* and others)	1,3
Epistylis sp	1,0
Digenetic	0,7
Cestodes (Proteocephalidae)	0,5
Crustaceans isopods	0,5

BÉKÉSI (1992) related in the Brazilian Northeast plerocercoids, *Trichodina* and monogenean in analyzed tambaqui. In the carp the author observed *Argulus*, monogenean and *Trichodina*. In disagreement with BÉKÉSI (1992) monogenean helminths, *I. multifiliis*, *P. pillulare* and *L. cyprinacea* were the most found parasites. According to CECCARELLI *et alii* (1990) pacu was highly parasitized and *I. multifiliis* occurred in the cold season. Moreover monogenean infections were present all the year round with more incidence in the period of February thru September. In disagreement with data of CECCARELLI *et alii* (1990) that related tambaqui and pacu as the most infected fish, the present situation showed pacu and tambacu hybrid as the most infected fish.

In the United States, MEYER (1970) related more incidence of fish diseases during a period of April and July, because of the large number of fish that were handled during this time. The author reported *Trichodina*, *Scyphidia*, *I. multifiliis* and monogeneans as the most important parasites. According to MELLERGAARD & DALSGAARD (1987) and BUCHMANN *et alii* (1995) disease problems in Danish eel and trout farms involved mainly monogeneans *Pseudodactylogyrus* spp, *Gyrodactylus derjavini* and *G. salaris*. These experiences corroborated the data of the present work, pointing monogeneans as one of the most found parasites in fish farms. An estimation of annual losses (U$ 23

Table 2 - The main fish species and the occurrence of their etiological agent in the period of 1993 to 1998.

Etiological Agent	Number of Infected Fishes							
	Pacu	Tambaqui	Tambacu	Piauçu	Matrinxã	Tilapia	Carpa	Bagre
Bacteria	19	10	34	2	4	8	3	4
Fungus	16	14	36		3	2	1	2
I multifiliis	22	12	45	2	3	4	2	3
Trichodina sp	19	2	9	5	1	5	2	1
Chilodonella sp	1		2					
Epistylis sp				1		2	1	
I. necator	7	2	8	4				
P pillulare	12	6	47	7		2		
H. piaractus and *H. leporinicola*	23		4	1				
M. colossomatis	13		2	1				
Monogeneans	76	19	70	18		5	13	
*L cyprinacea**	13	7	39	14	1		7	
Branchiura	3	1	5					
Nematodes	1		1					1
Digenetic			1					
Cestodes							1	

* Copepodids and adult of *L. cyprinacea*.

million) with infectious disease in channel catfish was reported by Klesius and ROGERS (1995).

Ciliates as *I. multifiliis* caused 10 to 20% mortality in Danish trout farms when the temperature increased from 16 to 20°C; although gyrodactylids occurred more abundantly at lower temperatures (BUCHMANN & BRESCIANI, 1997).

In the present work, at least in the region studied comprehending São Paulo State, the authors observed monogeneans as the most important reported parasite in fish farms and feefishing. Followed by *I. multifiliis*, *P. pillulare and L. cyprinacea*. Dinoflagellates (*P. pillulare*) may cause severe mortality reaching 3,000 fish in 24 hours in an area of 2,000 m^2 of fish ponds, as related by MARTINS (1998). This fact confirm observations made by SHAHARON-HARRISON *et alii* (1990).

Myxosporidian parasites that were present more frequently in pacu can increase together with other parasites the action upon the host. Severe histopathological consequences were described in pacu parasitized only with *H. piaractus*, that caused 100% mortality (MARTINS *et alii*, 1997). Histopathological observation revealed that the presence of parasite in the gills reduce gill/water contact area diminishing respiratory efficiency. This explains the observation of host behaviour, such as lethargy and agglomeration near the pond edges and water inlets. Similar descriptions on histopathology were reported by HIBIYA (1982), LOM & SCHUBERT (1983), MIYAZAKI *et alii* (1986), FERRAZ DE LIMA *et alii* (1991), STEPHEN & RIBBLE (1995), BASTOS *et alii* (1996), MARTINS & ROMERO (1996) and MARTINS *et alii* (1997).

In conclusion, in Brazil monogeneans, *I. multifiliis*, *P. pillulare, L. cyprinacea* and trichodinids were present in the cultivated fishes causing mortality. The authors contemplate prophylaxis and water quality as the essencial factors in the medical management.

SUMÁRIO

O objetivo do presente trabalho foi relatar doenças que ocorreram em 393 casos diagnosticados pelo Centro de Aqüicultura da UNESP, Jaboticabal, SP, Brasil no período de 1993 a 1998. Os proprietários relatavam aglomeração dos animais nas bordas dos viveiros, entrada da água e mortalidade. Foi realizado diagnóstico parasitológico e fragmentos das brânquias foram fixados em formalina a 10% tamponada para análise histopatológica. Os principais grupos de parasitos responsáveis por mortalidades ou alterações comportamentais nos peixes foram os helmintos monogenóides (36,6% de ocorrência) seguidos de *Ichthyophthirius multifiliis* (29,5%), *Piscinoodinium pillulare* (18,0%), copepoditos (15,2%) e adultos de *Lernaea cyprinacea* (13,0%). Bactérias (20,0%) e fungos (17,0%) estavam presentes como infecção secundária. O peixe mais afetado foi o pacu (*Piaractus mesopotamicus*) seguido do híbrido tambacu; tambaqui (*Colossoma macropomum*); piauçu (*Leporinus macrocephalus*); tilapia (*Oreochromis niloticus*) e carpa (*Cyprinus carpio*).

PALAVRAS-CHAVE: Ocorrência, parasitos, histopatologia, peixe cultivado, Brasil.

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