UNIVERSIDADE PAULISTA

Simultaneous multiple ectoparasitic infection in cultured Goldfish (Carassius auratus) associated with environmental conditions and management - Multiple parasitism in goldfish

Dissertação apresentada ao Programa de Pós-Graduação em Patologia Ambiental e Experimental da Universidade Paulista – UNIP para a obtenção do título de Mestre em Patologia Ambiental e Experimental.

CARLA RENATA SERANTONI MOYSES

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Aprovado em:

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Prof.(ª) Dr.(ª) Diva Denelle Spadacci Morena
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DEDICATÓRIA

Dedico este trabalho à minha família:

Ao meu pai, Enéas que me ensinou a aproveitar a vida e os momentos dentro da Universidade, o meu eterno professor;

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SUMÁRIO

Simultaneous multiple ectoparasitic infection in cultured Goldfish (*Carassius auratus*) associated with environmental conditions and management

Multiple parasitism in goldfish

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Abstract

Parasitic infections of fish are common and often debilitating the host, but multiple and simultaneous parasitism is rarely found. Its occurrence is usually associated with environmental and management issues. In this study, we describe the prevalence of multiple and concurrent parasitic infections in goldfish (*Carassius auratus*) raised in fish farms. Fish with skin damage (nodules and ulceration) were subjected to necropsy and examinations with fresh scraped of skin and gills, histopathological examination with HE and Giemsa stain, ultrastructural study by transmission and scanning electron microscopy. We identified in the skin the multiple parasitic infection by Gyrodactylidae, *Epystilis* sp., *Vorticella* sp., *Trichodina* sp., *Ichthyophthirius multifilis, Tetrahymena* sp. *and Ichthyobodo necatrix* associated with epithelial cell hyperplasia and epidermal sloughing. Although no gross lesions were observed in the gills, we identify a large number of parasites (Gyrodactylidae, *Piscinoodinium* sp., *Ichthyophthirius multifilis, Vorticella* sp. and *Trichodina* sp.) and microscopic lesions such as epithelial hypertrophy and hyperplasia, fusion of secondary lamellae, leukocyte infiltration, epithelial cells detachment, resulting in respiratory distress. In conclusion, multiple and simultaneous parasitism was very prevalent in goldfish with skin lesions, usually associated with ciliates and flagellate protozoan and it was associated with adverse environmental conditions and inadequate management.

Keywords: *Carassius auratus*, ciliates parasites, fish ectoparasites, goldfish, protozoan

INTRODUCTION

Aquarium fish trade is a very important sector in all over the world (1). The culture of ornamental fish is very popular in the pet market of Brazil, however, the fish production by farmers is carried on intensive livestock farms with rudimentary handling. Fish in aquaculture farms are often subjected to acute or chronic stressors such as handling, transportation, sorting, temperature changes, high rearing density and poor water quality, which affect it physiology. Thus, the growth, behavior, welfare and reproduction can be strongly affected by stressors. As a result, the presence of the disease is common cause high mortality and severe economic losses (2).

In fish cultured populations, parasites often cause serious outbreaks of disease, especially when the presence of dense populations of fish kept in particular environmental conditions may favors certain parasites species so that the parasite population increases to a very high level (3). Among fish parasites, protozoa are the most dangerous group that probably causes more diseases in fish cultures than any other ones (2). Ciliates, particularly sessilines such as *Apiosoma, Scopulata, Ambiphrya* and *Epistylis* are obligate parasites, which utilize gills and skin merely as a substrate for attachment, causing massive destruction. Even moderate infection of these parasites on small fish may prove a fatal disease, since the infection may cause the fish to stop feeding (4).

The aquatic parasites usually classified in six groups include Protozoa, Platyhelminthes, Nematode, Acanthocephala, Annelida and Arthropoda. Protozoans can be ectoparasites or endoparasites depending on their species and are the most common parasites encountered in cultured fish (4,5). With some exceptions (e.g. *Ichthyophthirius multifiliis, Chilodonella* and *Ichthyobodo*) the external protozoans are

not obligate fish parasites, but may occur on a variety of surfaces including logs and plants or even as parasites on other fish ectoparasites (4).

Goldfish (*Carassius auratus*) was one of the first fish species to be domesticated and is the most commonly kept aquarium fish. It is closely related to the less colorful carp, *C. aerates*, which is native to Eastern Asia and was domesticated in China more than a thousand years ago. The freshwater sub- tropical goldfish inhabits rivers, lakes, ponds and ditches with stagnant or slow-flowing water (6). The fish pathology and parasitology is undeveloped in Brazil, despite its abundant fish populations and the huge pet market of aquaculture. Data on parasite and diseases caused by then in goldfish are not available in Brazil and besides, the multiple concurrent parasitism and its consequences have not been reported in the world. So the purpose of this study was to describe the multiple-parasitism in farmed goldfish (*Carassius auratus*) associated with inadequate management conditions.

MATERIALS AND METHODS

Fish. Goldfish (*Carassius auratus*) was obtained from commercial aquaculture farms in São Paulo state, Brazil, where several fish species were growing together only separated by age. Farmers studied had high mortality of goldfish especially in winter. The farms evaluated in this study showed three negative factors in relation to the environment conditions: high population density in the tanks; abundant diet predisposes to the high content of ammonia, and slightly alkaline pH, was conducted an analysis of certain physical and chemical parameters of water of 20 tanks where these animals are 120 to 150 days before being sold. Among them are: pH, ammonia, nitrite and nitrate. The animals are exchanged the tanks every 30 days. The fish are removed with the emptying of the tanks, is placed cal with chicken

manure or quail, both without specified quantity. The food is a mixture of powdered feed with protein levels to 48%, granulated feed and 1 liter of oil. The mixture is stored in a drum and is offered to the animals three times a day, without quantity. Thirty fish with whitish hyperplastic lesions or ulcerative skin lesions were objects of research by portraying the main problem of the fish farmers. Fish were collected in the winter season when the temperature ranges from 10 to 20 ºC. The animals were taken to the laboratory where they remained housed in tanks with a continuous flow water system at 22 ± 1ºC in the aquatic facility of the Paulista University and fed ad libitum for 24 h. Prior to necropsy, fish were anaesthetized by immersion in a solution of tricaine methane sulfonate 150 mg/L (MS222 Sigma) until stop breathing. Animals in the Aquatic Facility were maintained according to the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and all procedures were approved by the ethics committee of the Paulista University.

Collection and processing of specimens for light microscopy. After the collection of biometric data of each fish, skin area with damage was scraped to collecting material and fresh observation under a light microscope. Parasites were identified using morphological parameters (7). The body, fins, mouth, eyes and inner cappings were examined for the location of possible parasites. Subsequently, the gills, skin and other organs were removed and fixed in Bouin's solution for 8 hours and kept in 70% alcohol. These materials were embedded in paraffin for histological analysis and histological sections were stained routinely with Hematoxylin-Eosin (HE) and Giemsa.

Electron and Scanning Microscopy. For electron microscopy (TEM), small pieces of gills and skin lesions were fixed in 2% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2) at 4ºC for 10 h, and post-fixed in 1% OsO4 buffered for 2 h and then in 5%

uranyl acetate overnight at 4 ºC. The fragments were dehydrated in an ascending ethanol series with propylene oxide and embedded in Spurr resin. Blocs were cut and semi-thin sections were stained with toluidine blue and photographed under a light microscope. Ultrathin sections were double stained with aqueous uranyl acetate and lead citrate and then observed under a ZEISS EM 109 TEM operated at 80 kV. Fragments of skin and gills were fixed and dehydrated as described for TEM and then processed in drying apparatus, subsequently mounted on copper stubs and gold coated. The fragments had been examined using JEOL JSM-65 MCA scanning electron microscope (SEM).

RESULTS

The intensive rearing, due to its high rate of settlement and artificial feeding, generates a large amount of waste, which may be in solid in suspension or dissolved (feces, unconsumed ration, flake, mucus, agents therapeutic)(8). The physical and chemical parameters of the water changes, so you must monitor it constantly and wastes removed as that may pose risks to the creation. Among the species dissolved in water from the metabolic activity of fish and degradation of proteins of unconsumed feed, the ammoniacal nitrogen, found in the form of NH_3 (ammonia) and NH_4^+ (ion ammonium)(9). The first is highly toxic; your concentration depends on the pH and the temperature of the water. Concentrations above 0.6 mg L^{-1} are lethal for ornamental fish. The limit recommended by the EIFAC (European Inland Fisheries Advisory Commission) is 0.025 mg L^{-1}(10). The result of the analysis of water for ammonia had an average of 0.31 mg/L with a standard deviation of ±0.37. For nitrite all tanks were with the same concentration of <0.002 being insignificant. For nitrate, obtained an average of 0.92 mg/L with a standard deviation of ±0.91, even if it's not

toxic nitrate is a quality parameter for finalizing the nitrogen cycle, being a source of food for algae and plants. For the average pH was 7.95 with standard deviation of 0.74. The pH levels can vary widely, from 6.5 to 8.5 point literatures. Levels slightly above or below may not take the animals to death, but are more debilitated and lethargic. Although there is a wide variation, ideally the pH stayed between 7.5 and 8.5 for nitrifying bacteria (nitrossomas) which could play its role.(8)

Lesions. All animals examined had elevated skin lesions and thickening of the dermis covered with mucus or with cotton wool spots appearance and necrotic tissue. In some cases the ulcerated lesions were hemorrhagic appearance. Skin lesions were located in the dorsal and lateral fish, sometimes with fins. The gills showed no pronounced macroscopic lesions, sometimes a slight swelling and mucus was observed. Some fish died by manipulating before anesthesia.

The fresh examination performed by scraping the skin lesions or gills and allowed the observation of various parasites (Fig.1). We identified in the skin Gyrodactylidae, *Epystilis* sp., *Vorticella* sp., *Trichodina* sp., *Ichthyophthirius multifilis, Tetrahymena* sp. *and Ichthyobodo necatrix*. In gills, we found Gyrodactylidae, *Piscinoodinium* sp., *Ichthyophthirius multifilis, Vorticella* sp. and *Trichodina* sp.

By histological examination of the gills we observed that the epithelium showed hyperplasia in different degrees and detachment of epithelial cells. There were some cases where the hyperplasia was more severe, resulting in the fusion of some secondary lamellae (Fig.2). Frequently, alterations such as blood congestion, hypertrophy of epithelial cells and lamellar disorganization were also observed. Some examples of more severe lesions found in the gill were lamellar aneurysms and hemorrhages with rupture of the lamellar epithelium. Inflammation of the epithelium

of primary and secondary lamellae was also observed, characterized by the presence of large numbers of granulocytes (Fig.3). Epithelium associated protozoan dome shaped structures with slightly concave, consistent with protozoa of the genus *Trichodina* sp. (Fig.4) aboral adhesive disc were observed or in the presence of Gyrodactylidae or *Ichthyophthirius multifilis* (Fig.2). In some cases protozoan *Ichthyophthirius* sp. was seen blocking a blood vessel, resulting in localized hyperemia and congestion, evident by vascular distension.

Significant epithelial cell hyperplasia and hypertrophy with eventual exhaustion of mucous cells and epidermal sloughing was seen (Fig.3). Because the enzymes released by some parasites, there was extensive tissue damage with areas of necrosis and impairment of muscles under the skin of the affected regions (Fig.3). The parasite lies within an interstitial tissue space, which contains cellular debris and proteinaceous tissue fluid. A large number of invading parasites were seen in the skin (Fig.3). The epithelium immediately surrounding the parasite was hyperplastic, the cells were degenerating, appear hydropic and necrotic with pyknotic nuclei. Inflammatory infiltrate was characterized by the presence of lymphocytes, macrophages and neutrophils in the dermis.

Ciliates

Sessile peritrichs. The peritrichious ciliates *Epystilis* sp. and *Vorticella* sp. were present in large numbers in the skin lesions fact that it is extremely unusual and most often occurs in fish predisposed by debilitating environmental or infectious factors, or both in this case. *Epistylis* is colonial ciliates with bell-shaped or conical body (Fig.1, 5). A non-contractile stalk bears several or many zooids. *Vorticella* sp. is a sessile peritrich was characterized by its inverted bell-shaped body and occurred solitarily on

a retractile stalk (Fig.1). *Apiosoma* sp. is a solitary ciliates with a scopula that is circular directly attached to the substract (Fig.5).

Mobile peritrichs. *Trichodina* sp. appear as saucer-shaped, hemispheric, dumbbell-shaped, and sac-like or flattened cylindrical organisms in cross-section, or as round discs in oral or aboral view (Fig.1, 4).

Hymenostomatan ciliates. *Ichthyophthirius multifilis* is a large in size (about 0.1-1.0 mm) contains a horse-shaped macronucleus (Fig.1, 2) was often seen in the gills associated with lesions. *Tetrahymena* sp. showed pyriform shape or ovoid with a prominent oval macronucleus visible in H-E stained sections.

Flagellates. *Piscinoodinum* sp. trophonts was attached to gill with ovoid, pyriform or sac-like appearance, with a single eccentric nucleus but without visible flagella. In smears of fresh gills, trophonts showed chloroplasts (Fig.4 c-d). *Ichthyobodo necatrix* was only observed in the fresh smears of skin and gill lesions. The free-swimming stage was oval to kidney-shaped with size ranging to 10-15 μm in length (Fig.1).

DISCUSSION

Disease in fishes is closely linked to environmental stress. In cultured fish populations, parasites often cause serious outbreaks of disease (8). The presence of dense population of fish, as was observed in the farms analyzed in this study, kept in particular environmental conditions may favors certain parasite species so that the parasite population increases to a very high level. Furthermore, the abundant supply diet increases the level of ammonia in water and basified to pH and these factors favor the development of parasites in fish observed in this study. It should also be pointed out as a stressor form of management for the sale of animal specimens.

Weekly, a large number of specimens are collected and taken to the trade sales (7). Animals that are not sold back to the creation and are reintroduced in the breeding tank without any quarantine or separation. The stress of capture and transport can determine changes that depress immune responses, contributing to meeting multiparasitism in goldfish, including parasites that usually saprophytic (11). At the same time we observe that although large numbers of parasites were present in the skin and gills fish, death did not occur acutely and is characterized by chronic and slow processes, a fact which also suggests an animal adaptation to parasitism.

Fish gills participate in many important functions such as respiration, osmoregulation and excretion and remain in close contact with external environment and it is particularly sensitive to changes in the quality of the water (9, 10). Gills are generally considered as a good indicator for water quality and it will be a model for studies of environmental impacts. In this study it became clear that unfavorable environmental conditions were prevalent for the presence of multi-parasites in fish, especially because many of the parasites observed are saprophytes and can live symbiotically with fish in good health. Although no macroscopic lesions were found, many histopathological changes of gills associated with parasites were observed, a fact that reinforces the potential of gills as markers of water quality. Another important finding of this study was to demonstrate that the gills had intense parasitic infection and severe histopathological changes, though not revealed significant macroscopic changes. Thus it is clear that the microscopic examination of gills scrapes and histopathology is essential for the identification of parasites.

Certain parasites found in this study, e.g. *Trichodina, Apisoma* and *Epistylis*, which are peritrichous ciliates, are believed to be primarily bactivorous filter feeders

relying on bacteria present in water or mucous for nutrition. Presumably, their mode of feeding is innocuous to the host. *Trichodina* adhesive discs attach to the epithelial surface thereby presumably impairing host cell respiration, energy production, and cell survival. In a heavily infected fish, their adherence and suction on the epithelium may cause enough damage to produce the clinical signs of anorexia, lethargy and weight loss (15) in accordance with the signals by nodes found in this study with massive infection.

Epistylis sp. and the related species *Vorticella* sp. are sessile and stalked ciliated protozoans generally found attached to vegetation or crustaceans (16). *Epistylis* sp. is sessile, colonial ectocommensal ciliate attacking the surface of fish skin and gills (7) and *Vorticella* sp. have inverted bell-shaped body and occurred solitarily on a retractile stalk. *Vorticella* sp. was reported for the first time from gills of *Carassius auratus* in Brazil in the present study. They frequently affect goldfish and many species of bottom-dwelling freshwater fish. *Epistylis* sp. first appeared on the tips of both dorsal and pectoral fin spines. Then, *Epistylis* colonies spread down the spines and eventually covered much of the anterior region of the body. Cutaneous lesions observed in fish studied had exactly this location, so *Epistylis* sp. was major pathogens in this study. This ciliophora is potentially harmful if it is in large number especially to gill tissue where gas exchange may be impeded by the large numbers of parasites physically covering gill, the infected fish show lethargy and death, which reduces their productivity (17).

Were summarized 72 species and one subspecies of *Apiosoma* (18), which may be considered ectocomensal or ectoparasite. To date, there are few studies that demonstrate the pathogenic effects of *Apiosoma* in goldfish or other fish species. We

observed the presence of *Apiosoma* together with other parasites in the gills and as a result of parasitism simultaneously several pathological changes were described. This genus belongs to the peritrichious sessile ciliates survive alone and adhere to the gills by scopula (19).

Ich is one of the most common diseases of freshwater fish and virtually all freshwater fish are susceptible to infection and up to 100% mortality may occur (15). Stress plays a major role in Ich epidemia. In current study, all fish examined had *Ichthyophthirius multifilis* in the examination of the skin and gills, although typical whitish nodules distributed throughout the body have not been observed, many parasitic specimens were seen in skin lesions and histopathological lesions of the gills, increased mucus production and gill filaments hyperplasia which is in agreement to results previously observed (20). The epithelial cell erosion and ulceration that has been resulted from the entrance and exit of the parasite damage determine the host's skin that favors the multiplication of the parasites (12). The mechanical action of the trophont of *Ichthyophthirius multifilis* is responsible for the tissue damage. In the gills, there is a more marked epithelial hyperplasia and the trophonts tend to migrate towards larger blood vessels.

Tetrahymena sp. is a saprozoic ciliate protozoan that feeds on organic matter and bacteria in natural habitats (21). *Tetrahymena* sp. is causative agents of tetrahymenosis or “tet disease”, also known as guppy killer disease in tropical aquarium fish, which causes severe economic losses in commercial fish farms worldwide. Ornamental fish species reported to be infected with *Tetrahymena* sp. include zebrafish (*Danio rerio*), angelfish (*Pterophyllum scalare*), neon tetra (*Paracheirodon innesi*) and others (21, 122). Susceptibility to this parasite increases

in fish that are wounded and/or weakened by stress conditions, such as high ammonia level, high organic load, extreme water temperature, non-optimal shipment conditions or a disease (6). In our study, *Tetrahymena* sp. was found only in part of the animals associated with skin lesions and probably environmental conditions identified predisposed to infection by the parasite.

The bodonid flagellates *Ichthyobodo necator* (formerly *Costia necatrix*) is significant cause of morbidity and mortality in aquaculture fishes. This parasite is often restricted to protected areas on fish gills, pectoral and pelvic fins, and on areas adjacent to the dorsal fin (23,24). Nevertheless, *Ichthyobodo* induces significant epithelial cell hyperplasia and hypertrophy with eventual exhaustion of mucous cells, and epidermal sloughing (3,223,224). The location and type of injury were similar to those described by us in goldfish. The large number of parasites *Ichthyobodo necator* was observed in skin lesions, but mostly they were seen in scrapings from the gills or skin lesions, contributing to the number of lesions that were observed.

Piscinoodinum sp. pathogenicity is high (7). In heavily infected fish, symptoms are signs of discomfort, a golden, velvety hue on the body surface, spreading opercula, folding of fins and eventually emaciation. There may be petechiae in the skin and even slight inflammation. The gill lamellae may be fused, and epithelial hyperplasia may involve entire gills filaments. Eventually, there is epithelial cell degeneration and necrosis. The presence of *Piscinoodimun* and *Ichthyobodo* certainly contributed to the presence of lesions on gills and skin, since the both flagellates possess great potential pathogenic.

Gyrodactylidae are the most common and prevalent ectoparasites which can produce severe parasitic disease in aquaculture (25). Morbidity and mortality caused

by excessive parasite loads of dactylogyrids are common in cultured fishes and have also occurred in wild fishes (25, 26). Fish appear to co-exist with their specific monogeneans, in natural habitats as well as in culture conditions, even when infestations are intense. We observed high amount of Gyrodactylidae lesions of pel and gill of fish studied in exams fresh and light microscopy. A few monogeneans, notoriously gyrodactylids, are, however, pathogenic to their host fish, usually to younger fish and in intensive culture conditions (25, 26). Histopathological changes in the gills are hardly detectable in most instances even in relatively intense infections. The fish infected with *Dactylogyrus* showed clinical symptoms including the lethargy, unilateral swimming and erosion on gill filament and scale loss (26), such changes have been described by the owner of the farm. Gill filament fusion, secondary filament hyperplasia and aneurism were reported in fishes which were infected by *Dactylogyrus* sp. (27, 28), which is in agreement with results obtained from this study.

CONCLUSION

Multiple and simultaneous parasitism was very prevalent in goldfish with skin lesions, usually associated with ciliates and flagellate protozoan. We attribute this parasitism to the adverse environmental conditions and inadequate management.

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REFERENCES

1. De Silva SS, Turchini GM. 2008. Towards Understanding the Impacts of the Pet Food Industry on World Fish and Seafood Supplies. J. Agric. Environ. Ethics. **21**:459-467.

2. Ashley PJ. 2007. Fish welfare: current issues in aquaculture. Appl. Anim. Behav. Sci. **104**:199-235.

3. Roberts HE, Brian Palmeiro B, Scott Weber E. 2009. Bacterial and Parasitic Diseases of Pet Fish. Vet. Clin. Exot. Anim. **12**: 609–638.

4. Woo PTK, Bruno DW. (eds.). 2011. Fish Diseases and DisordersViral, Bacterial and Fungal Infections. Second Edition, vol. 3. CABI, Wallingford, OX.

5. Purivirojkul W, Boonsoong B. 2012. A new species of *Tetraphyllidean* (Onchobothriidae) cestode from the brown-nbanded mabbooshark *Chiloscyllium punctatum* (Elasmobranchii: *Hemiscyllidae*). J. Parasitol. **98**:1216-1219.

6. Sharon G, Pimenta Leibowitz M, Kumar Chettri J, Isakov N, Zilberg D. 2014. Comparative study of infection with *Tetrahymena* of different ornamental fish species. J. Comp. Path. **150**:316-324.

7. Lom J, Dykova L (ed). 1992. Protozoan Parasites of Fishes - Developments in Aquaculture and Fisheries Science*,* 26. Elsevier Sci., Amsterdam.

8. REN, J. S.; Stenton-Dozey, J.; Plew, D. R.; Fang, J.; Gall, M. An ecosystem model for optimising production in integrated multitrophic aquaculture systems. Ecol. Model. Vol. 246, p. 34-36, 2012.

9. BOYD, C. E. Water quality in warmwater fish ponds. Agricultural Experiment Station: Alabama, 1984.

10. TIMMONS, M. B.; Ebeling, J. M.; Wheaton, F. W.; Summerfelt, S. T.; Vinci, B. J. Sistemas de Recirculación para La Acuicultura. Fundación Chile: Santiago, 2002.

11. Barber I. 2007. Parasites, behaviour and welfare in fish. App. An. Beh. Sci. **104**:251–264.

12. Mazon AF, Pinheiro GHD, Fernandes MN. 2002. Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa.* Braz. J. Biol. **62**:621-631.

13. Camargo MMP, Martinez CBR. 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotrop. Ichtyol. **5**:327-336.

14. Hoffman GL. 1970. Parasites of North American freshwater fishes. Univ. California Press, Los Angeles, CA.

15. Noga EJ. 1996. Fish disease: diagnosis and treatment. St. Mosby, St. Louis, MO.

16. Saglam N, Sarieyyupoglu M. 2002 A study on *Tetrahymena pyriformis* (Holotrichous) and *Epistylis* sp. (Peritrichous) found on freshwater leech, *Nephelopsis obscura*. Pak. J. Biol. Sci. **5**:497-498.

17. Paperna I. 1991. Diseases caused by parasites in the aquaculture of warm water fish. An. Rev. Fish Dis, **1**:155-194.

18. Li M, Wang J, Zhu D, Gu Z, Zhang J, Gong X. 2008. Study of *Apiosoma piscicola* (Blanchard 1885) occurring on fry of freshwater fishes in Hongze, China with consideration of the genus *Apiosoma*. Parasitol. Res. **102**: 931–937.

19. Reda ESA. 2011. A Review of some ecto- and endo-protozoan parasites infecting *Sarotherodon galilaeus* and *Tilapia zillii* from Damietta branch of River Nile, Egypt. J. Amer. Sci. **7**:362-373.

20. D6ickerson HW. 2012. *Ichthyophthirius mutifiliis*. In Woo PTK, Buchmann K. (ed) Fish Parasites – Pathobiology and protection Cabi, Oxfordshire, OX.

21. Ponpornpisit A, Endo M, Murata H. 2000. Experimental infections of a ciliate *Tetrahymena pyriformis* on ornamental fishes. Fish. Sc. 66:1026-1031.

22. Astrofsky KM, Schech JM, Sheppard BJ, Obenschain CA, Chin AM, Kacergis MC, Laver ER, Bartholomew JL, Fox JG. 2002. High mortality due to *Tetrahymena* sp. infection in laboratory maintained zebrafish (*Brachydanio rerio*). Comp. Med. **52**:363-367.

23. Reavill D, Roberts HE. 2007. Diagnostic cytology of fish. Vet. Clin. Exot. Anim. **10**:207–34.

24. Buchmann K, Sigh J, Nielsen CV, Dalgaard M. 2001. Host responses against the fish parasitizing ciliate *Ichthyophthirius multifiliis*. Vet. Parasitol. **100**:105–116.

25. Thilakaratne ID, Rajapaksha G, Hewakopara A, Rajapakse RP, Faizal AC. 2003. Parasitic infections in freshwater ornamental fish in Sri Lanka. Dis. Aquat. Organ. **54**:157–62.

26. Baker DG, Kent ML, Fournie JL. 2007. Parasites of fishes. p. 69–116. In: Baker DG. (ed). Flynn's parasites of laboratory animals. 2 ed. Blackwell, Hoboken, NJ.

27. Scott M. 1985. Dynamics of challenge infections of *Gyrodactylus bullatarudis* Turnbull (Monogenea) on guppies, *Poecilia reticulata* (Peters). J. Fish Dis. **8**:495-503.

28. Shinn AP, Hansen H, Olstad K, Bachmann L, Bakke TA. 2004. The use of morphometric characters to discriminate specimens of laboratory reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). Folia. Parasitol. **51**:239–252.

ANEEX

TABLE 1-ANALYSIS OF WATER QUALITY				
TANK	AMMONIA (mg/L) NH_3	NITRATE (mg/L) NO_3	NITRITE (mg/L) NO_2	pH (UpH)
1	< 0,1	0,9	< 0,002	9,1
2	< 0,1	0,7	< 0,002	8,75
3	0,31	1	< 0,002	7,37
4	0,29	0,3	< 0,002	7,8
5	< 0,1	0,5	< 0,002	8
6	< 0,1	0,3	< 0,002	8,7
7	0,25	0,2	< 0,002	7,46
8	< 0,1	1,1	< 0,002	8,62
9	< 0,1	0,6	< 0,002	7,26
10	< 0,1	1,4	< 0,002	7,74
11	0,18	1,1	< 0,002	7,95
12	0,16	0,4	< 0,002	8,51
13	< 0,1	0,2	< 0,002	8,26
14	0,11	0,5	< 0,002	8,96
15	1,34	3,6	< 0,002	6,27
16	0,1	0,9	< 0,002	7,63
17	< 0,1	0,6	< 0,002	8,7
18	< 0,1	< 0,02	< 0,002	7,11
19	0,27	3	< 0,002	7,2
20	0,11	0,2	< 0,002	7,7
	0,31 ± 0,37	0,92 ± 0,91		7,95 ± 0,74

Figure legends

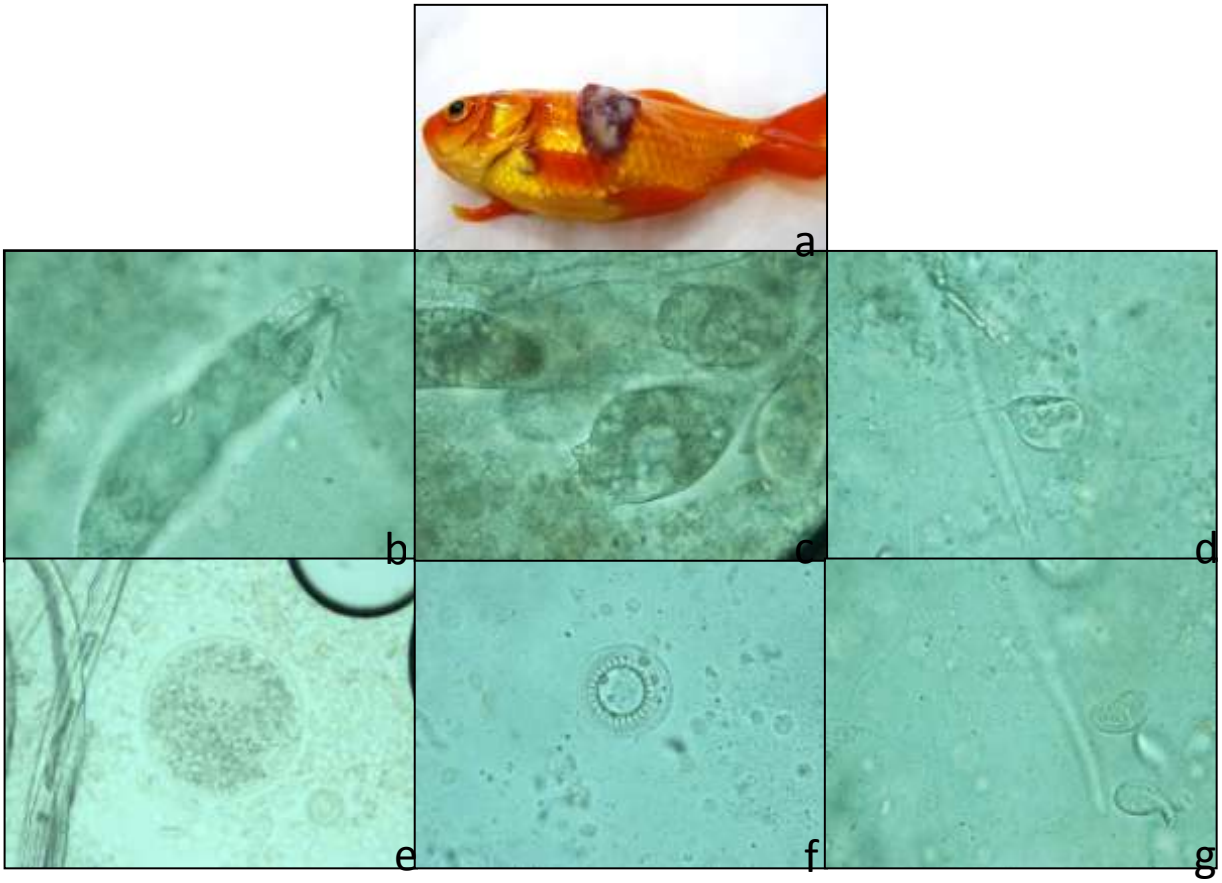

Figure 1. Parasites found on the skin or gills in fresh examination in goldfish (a) Skin lesion; (b) Gyrodactylidae; (c) *Epystilis* sp.; (d) *Vorticella* sp.; (e) *Ichthyophthirius multifilis;* (f) *Trichodina* sp.; (g) *Ichthyobodo necatrix*. (400x)

Figure 2. There is an extensive proliferation of branchial epithelium around the invading parasites and total lamellar fusion associated with space-occupying intralamellar monogenean Gyrodactylidae (a - Giemsa x 100, b - Giemsa x 400, c - Giemsa x 400) and trophonts of *Ichthyophthirius multifilis* are observed in secondary lamellae and blood vessel (arrow) (d - HE x 100, e - Giemsa x 400, f - Giemsa x 100).

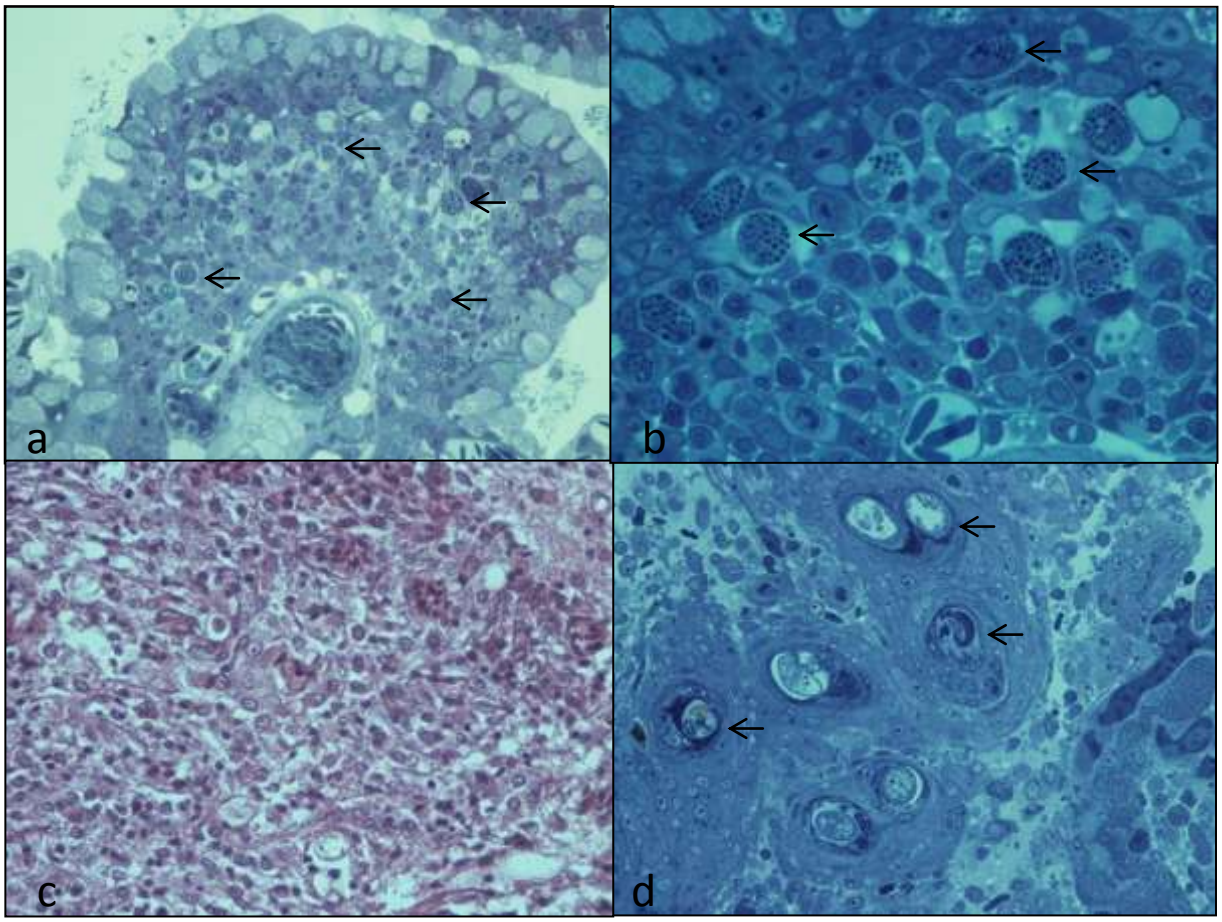

Figure 3. Inflammation of the epithelium of primary and secondary lamellae characterized by the presence of large numbers of granulocytes (a – Toluidine blue stain x 100, b - Toluidine blue stain x 400). Epithelial cell hyperplasia and hypertrophy and epidermal sloughing was seen (a – HE stain x 100). A large number of parasites in skin lesion surrounded by epithelial and inflammatory cells (d - Toluidine blue stain x 100).

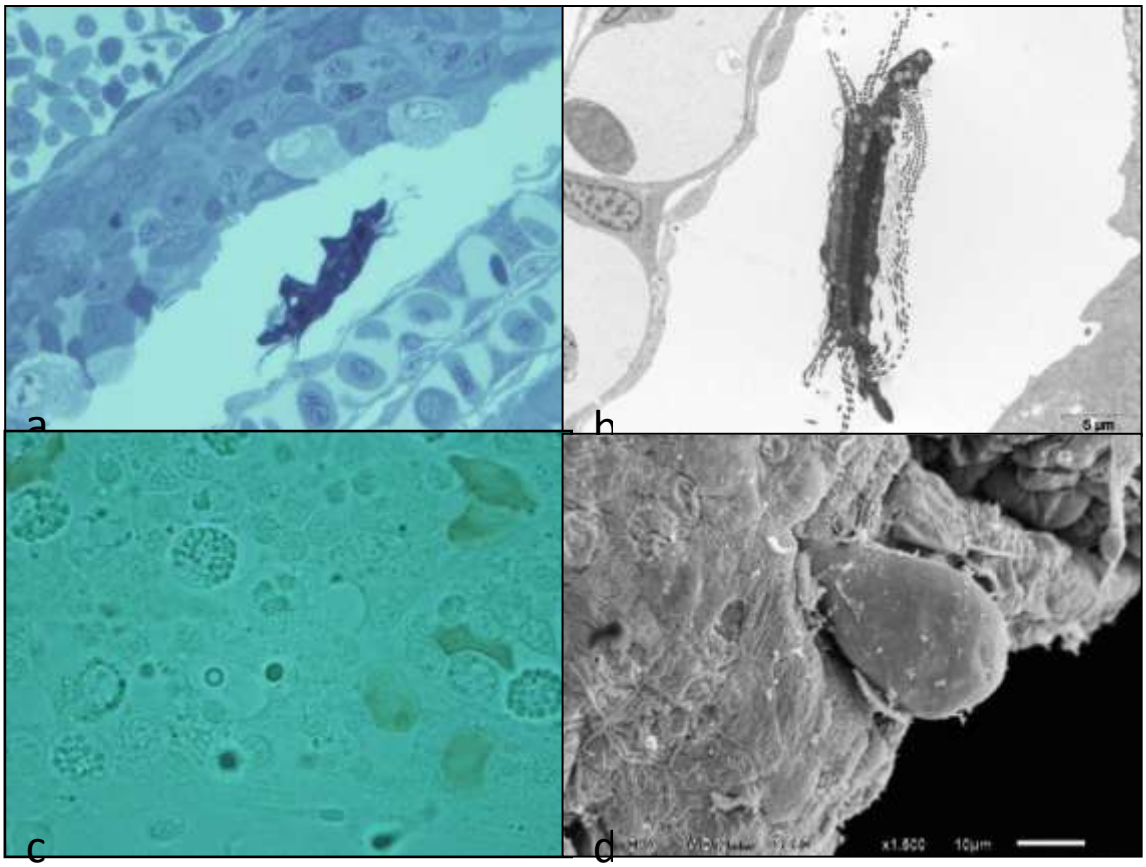

Figure 4. *Trichodina* sp. found in the space between the secondary lamellae ofthe gills of goldfish. (a) Toluidine blue stain x 1000; (b) Transmission electron microscopy of *Trichodina* sp. in gill; (c) *Piscinoodinium* sp. in fresh specimens; (d) Scanning electron microscopy of trophont of *Piscinoodinium* sp. attached to the gill of goldfish

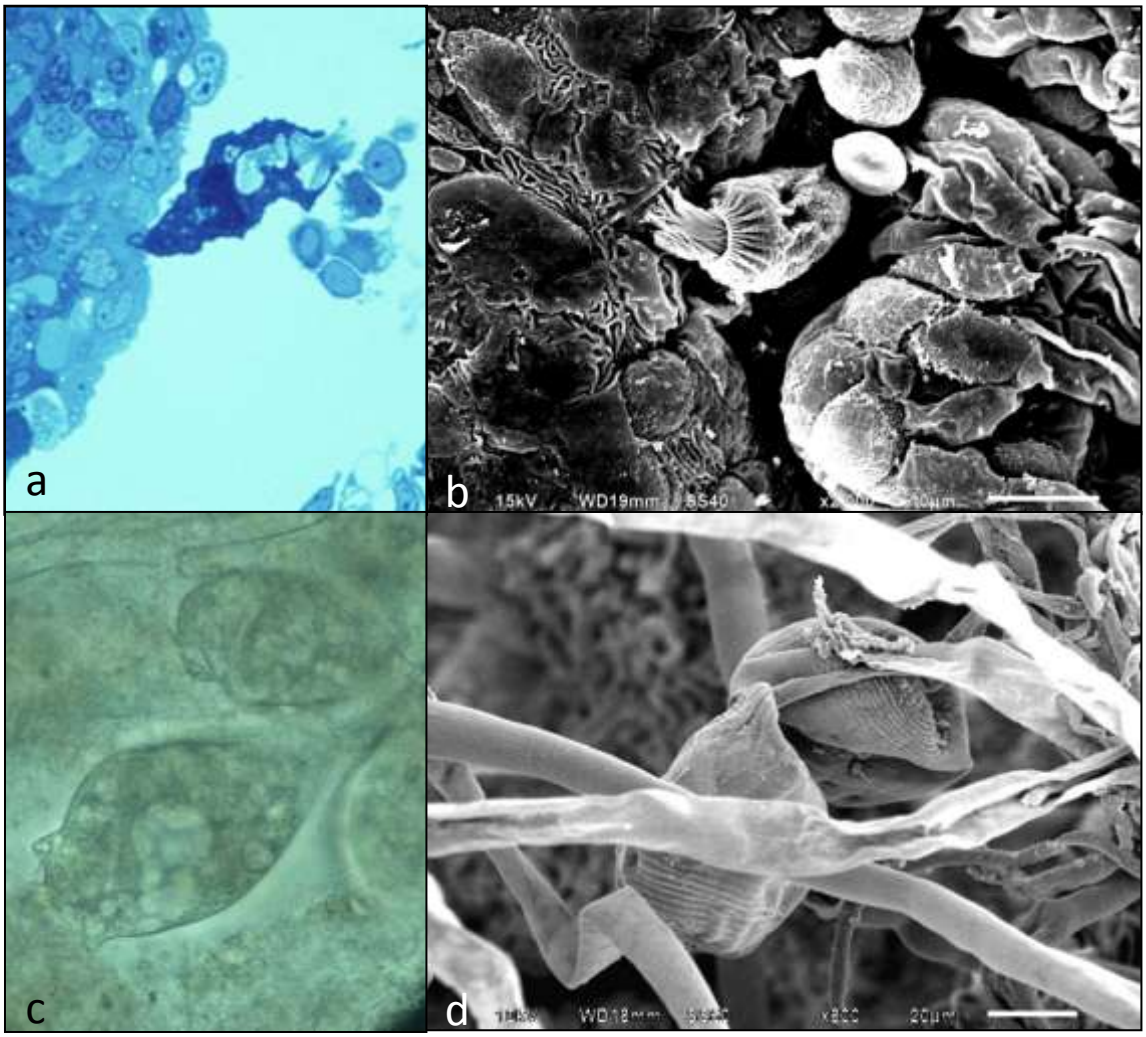

Figure 5. *Apiosoma* sp. attached to the gill of goldfish (a) Toluidine blue stain x 1000, (b) Scanning electron microscopy, (c) *Epistylis* sp. in skin smear the fresh and (d) Scanning electron microscopy.