Report of *Tetrahymena* sp. and *Dactylogyrus* sp. infestation in GloFish tetra (*Gymnocorymbus ternetzi)*: Diagnosis and treatment

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Received: March 2021 Accepted: April 2021

Abstract

The cultivation and propagation of ornamental fishes have been increasing in the last few years in Iran. GloFish is a registered and trademarked brand of genetically engineered fluorescent fish. GloFish fluorescent fish are available in a variety of tropical fish species. In an ornamental fish farm of GloFish tetra (*Gymnocorymbus ternetzi),* mortality of 20 to 30 per day was reported in juveniles and 4 to 6 per day in adult fish. Several fish were sent for sampling while they had been kept in their aquarium water. Wet smears were taken from the skin and gills. After the autopsy, the internal organs were examined for parasitic infestation under a light microscope. No internal parasites were observed. External parasites, including *Dactylogyrus sp.* in the gills and *Tetrahymena sp.* in the skin were detected. According to references, treatment with salt bath and formalin 37% were performed. A reduction in mortality was observed during the treatment period. After two weeks, re-sampling was performed and the samples were negative for *Tetrahymena sp.* and *Dactylogyrus sp.* As a result, Salt baths and formalin were effective for the treatment of *Tetrahymena sp.* and *Dactylogyrus sp.* in GloFish tetra.

Keywords: *Gymnocorymbus ternetzi*, *Tetrahymena* sp., *Dactylogyrus* sp., Salt, Formalin

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Introduction

The GloFish® is a genetically modified Betta trademark fluorescent fish with several different species: zebrafish, which was the first GloFish on the market, tetra, tiger barb, rainbow shark, and recently added betta. These types of fish have bright bodies under the light of white LEDs (www.glofish.com).

Today, diseases caused by facultative parasites have become extremely important in the aquaculture industry (Leibowitz and Zilberg, 2009).

Tetrahymena sp. is ciliated protozoa and mainly includes free-living holotrichous, which feed on bacteria and organic materials from natural habitats (Lom, 1995) that become an optional parasite in many aquatic animals, including fish, under conditions such as being kept in an enclosed water system and being injured on the body (Nigrelli *et al*., 1956). These parasites infect fish under adverse environmental conditions and cause lethargy, white lesions to the surface of the body, and erosion of the fins (Lom J. (1995; Imai *et al*., 2000; Hatai *et al.,* 2001; Leibowitz *et al.,* 2005). *Tetrahymena sp.* can cause mild superficial infection of the skin and even systematic infection, presumably by physical tissue disruption using cilia (Leibowitz, 2010).

Metazoans, which include monogeneans, cestodes, digenean, trematodes, acanthocephalans, and nematodes, are important parasites of ornamental fish that can cause irritation, atrophy of tissues, obstruction in the digestive system, and deprive the fish of natural food (Leibowitz and Zilberg, 2009; NIOF, (2011). *Dactylogyrus sp.* is an important pathogenic monogenean that shows a predilection for specific parts of their hosts' gill apparatus (Turgut, 2006). The infection causes thickening of the gill epithelium, impaired respiratory function, negative effects on growth, and even death (Thoney and Hargis, 1991).

In this study, the diagnosis and effective treatment of the parasitic diseases *Tetrahymena sp.* and *Dactylogyrus sp.* in GloFish were carried out.

Materials and methods

In GloFish tetra (*Gymnocorymbus ternetzi*) of an ornamental fish farm, 20-30 deaths per day in juveniles and 4-6 deaths per day in adults were reported. Loss of appetite, emaciation, and swimming near the surface were other clinical signs reported in these fish. From each aquarium, a few samples of sick fish were referred, in aerated plastic bags containing aquarium water, for examination. Fish were euthanized by inserting a sharp needle into the brain through the upper part of the eye (Turgut, 2006) and wet mounts from the skin and gill were taken. Skin scrapings were gently collected from the lateral side of the fish using a glass coverslip and examined for parasitic infestation under a light microscope (Leibowitz *et al*., 2005). Then, after the autopsy, wet mounts from internal organs were taken to check for internal parasites. *Tetrahymena sp.* infection was observed in the juvenile fish aquarium, and

Dactylogyrus sp. contamination was observed in the adult fish aquarium.

Treatment

To eliminate both types of parasites, a similar treatment process was used, with a salt bath and formalin 37%. For this purpose, salt with a dose of 3g per liter of water and formalin 37% with a dose of 2ml per 100 liters of water (Bassleer, 2004) were added to both aquariums at the same time. The water temperature of the aquariums was 25 degrees Celsius, and they had filters and aerators. At the beginning of the treatment, the fish in both aquariums were deprived of food for 24 hours, and the water was changed 3 days later.

Result

No internal parasites were observed under the microscope. External parasites, including *Dactylogyrus sp.* on the gills and *Tetrahymena sp.* with their slow movement on the skin were detected. According to references, treatment with salt bath (dosage= 1-3g/lit 3-5 days (Bassleer, 2004)) and formalin 37% (dosage= 2ml/100 lit, 12-48 h (Bassleer, 2004)) was performed separately. A reduction in mortality was observed during the treatment period. After two weeks, re-sampling was performed and the samples were negative for *Tetrahymena sp.* and *Dactylogyrus sp.* Therefore, Salt and formalin 37% were effective for the treatment of *Tetrahymena sp.* and *Dactylogyrus sp.* in GloFish tetra. One of the commonly used disinfectants to eliminate infectious agents in aquaculture is formalin, which is an aqueous solution of formaldehyde stabilized with methanol. Formalin may also have negative effects on water and fish quality such as damage to the gills and changes in the mucous cells (Leal *et al*., 2018). Formalin is used as a bath treatment to control external parasitic infections of fish, including most protozoa and some monogenic trematodes, and it effectively kills parasites on the gills, skin, and fins (Francis-Floyd, 1996).

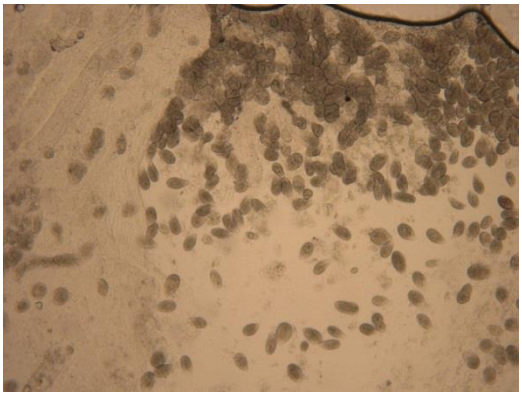

Figure 1: *Tetrahymena* sp. *X*10

Salt kills a wide range of pathogens, including protozoa and worms. It is less harmful to fish than formalin or malachite green and is cheap and readily available. For these reasons, the salt bath is the recommended treatment in the freshwater aquaculture industry and all types of aquarium fish (Lio-Po and Lim, 2002; Srivastava *et al*., 2004; Schelkle *et al*., 2009; Schelkle *et al*., 2011).

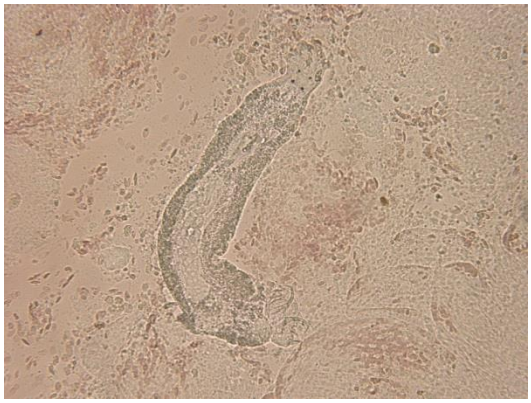

Figure 2: *Dactylogyrus* sp. *X*20

Conclusions and discussion

Common treatments against protozoan infections are effective against superficial and non-systemic infections with *Tetrahymena sp.* (Leibowitz *et al*., 2005). A combination of immune stimulants and salt baths is effective in treating the disease (Ponpornpisit *et al*., 2001). The conditions that cause *Tetrahymena sp.* infection are still not well known, but most reports state that skin damage is the main contributing factor to this infection (Leibowitz *et al*., 2005). Since *Tetrahymena sp.* and many other ciliated protozoa can tolerate low levels of dissolved oxygen in the water and high content of organic matter can be considered indicators of the existence of stressful aquatic environments (Bharati *et al*., 2001). Management measures such as removing dead fish, optimizing water quality and culture conditions, reducing density, and generally avoiding stress will reduce the risk of contracting *Tetrahymena sp.* (Leibowitz *et al*., 2005). One of the most important factors determining the presence and frequency of monogenic parasites is water temperature (Koskivaara *et al*., 1991). *Dactylogyrus sp.* shows a sharp increase in the spring and early summer when many of their host fish are spawning (Chubb, 1977).

In the current study, the investigated farm was very poor in terms of health conditions due to the presence of various insects, rodents, birds, and dogs in the farm area, breeding hall, and food storage. In addition, tools and equipment had a limited number that was used jointly between the aquariums. There were no suitable disinfectants on the farm and the entry of different people was permitted without observing the hygiene principles.

So, according to the parasitic contamination found in this study, it is considered necessary to follow biosecurity principles. These principles include quarantine, prevention of rodents, birds, and insects from entering fish breeding halls and food warehouses, disinfecting the halls and equipment and separating nets, hoses, and other equipment needed by each aquarium.

Parasites are transported around the world in the aquarium trade. Treatment of fish before export or on arrival in an importing country against known parasites minimizes the risk of future parasite entry and further parasite spread (Evans Bede, 2001).

References

Bassleer, G., 2004. The new illustrated guide to fish disease in ornamental tropical and pond fish, pp. 255-257.

Bharati, V.R., Khan, R.N., Kalavati, C. and Ramam, A.V., 2001. Protozoan colonization on artificial substrates in relation to water quality

in a tropical Indian harbour. *Journal of Environmental Sciences*, 13, 143–147.

Chubb, J.C., 1977. Seasonal occurrence of helminths in freshwater fishes Part. I. Monogenoidea. *Advances in Parasitology*, 15, 133–139. Doi: 10.1016/s0065-308x(08)60528-x.

Evans Bede, B., 2001. Parasites of ornamental fish imported into Australia. *Bulletin of the European Association of Fish Pathologists*, 21(**2**), 51 P.

Francis-Floyd, R., 1996. *Use of formalin to control fish parasites*. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Hatai, K., Chukanhom, K., Lawhavinit, O., Hanjavanit, C., Kunitsune, M. and Imai, S., 2001. Some biological characteristics of *Tetrahymena corlissi* isolated from guppy in Thailand. *Fish Pathology*, 36, 195–199. Doi:10.3147/jsfp.36.195

Imai, S., Tsurimaki, S., Goto, E., Wakita, K. and Hatai, K., 2000. *Tetrahymena* infections in guppies *Poecilia reticulata*. *Fish Pathology,* 35, 67–72. Doi: 10.3147/jsfp.35.67

Koskivaara, M., Valtonen, E.T. and Prost, M., 1991. *Dactylogyrids* on the gills of roach in Central Finland: Features of infection and species composition. *International Journal for Parasitology*, 21, 565–572. Doi: 10.1016/0020-7519(91)90061-B

Leal, J.F., Neves, M.G.P., Santos, E.B., and Esteves, V.I., 2018. Use of formalin in intensive aquaculture: properties, application, and effects on fish and water quality. *Reviews in Aquaculture*, 10(**2**), 281-295. Doi:10.1111/raq.12160

Leibowitz, M.P., 2010. Treatment development for systemic *Tetrahymena sp.* infection in guppies, *Poecilia reticulata Peters. Journal of Fish Diseases,* 33, 473–480. Doi: 10.1111/j.1365-2761.2010.01143.x

Leibowitz, M.P., Ariav, R. and Zilberg, D., 2005. Environmental and physiological conditions affecting Tetrahymena sp. infection in guppies, *Poecilia reticulata Peters*. *Journal of Fish Diseases*, 28(**9**), 539-547. Doi: 10.1111/j.1365-2761.2005.00658.x.

Leibowitz, M.P. and Zilberg, D., 2009. *Tetrahymena* sp. infection in guppies, *Poecilia reticulata Peters. Parasite Characterization and Pathology of Infected Fish,* 32, 10, pp. 845-855. Doi: 10.1111/j.1365-2761.2009.01062.x

Lio-Po, G.D. and Lim, L.H.S., 2002. Infectious diseases of warm water fishes in fresh water. In: Woo, P.T.K., Bruno, D.W., Lim, L.H.S. (Eds.), Diseases and disorders of finfish in cage culture. CABI Publishing, Oxon, U.K.

Lom, J., 1995. *Trichodinidae* and other ciliates (*Phylum ciliophora*) in fish diseases and disorders: Protozoan and metazoan infections (ed. by P.T.K Woo), CABIPublishing, New York. pp. 229–262.

Ministry of Scientific Research National Institute of Oceanography and Fisheries (NIOF), 2011.

Negative effects of parasites on fish farms production. Aquaculture Division Aquatic Pathological Lab (El-Qanater El-Khairia Research Station). Parasitol., Amina Ibrahim El-Sayed El-Mansz, 72 P.

Nigrelli, R.F., Jakowska, S. and Padnos, M., 1956. *Tetrahymena* as pathogenic epibiont in fishes and urodeles. *Japanese Journal of Protozoology*, 3 (**Suppl**.), 10.

Ponpornpisit, A., Endo, M. and Murata, H., 2001. Prophylacticeffects of chemicals and immunostimulants in experimental *Tetrahymena* infections of guppy. *Fish Pathology*, 36, 1–6. Doi:10.3147/jsfp.36.1

Schelkle, B., Doetjes, R. and Cable, J., 2011. The salt myth revealed: treatment of *gyrodactylid* infections on ornamental guppies, *Poecilia reticulata*. *Aquaculture*, 311(**1-4**), 74-79. Doi: 10.1016/j.aquaculture.2010.11.036

Schelkle, B., Shinn, A.P., Peeler, E. and Cable, J., 2009. Treatment of *gyrodactylid* infections in fish. *Diseases of Aquatic Organisms*, 86, 65–75. Doi: 10.3354/dao02087

Srivastava, S., Sinha, R. and Roy, D., 2004. Toxicological effects of malachite green. *Aquatic Toxicology*, 66, 319–329. Doi: 10.1016/j.aquatox.2003.09.008

Thoney, D.A. and Hargis, W.J., 1991. Monogenea (*Platyhelminthes*) as hazards for fish in confinement. *Annual Review of Fish Diseases*, 1, 133-153. Doi: 10.1016/0959-8030(91)90027-H

Turgut, E., 2006. Spatial distribution of *Dactylogyrus* (Monogenan) on the gills of the host fish. *Turkish Journal of Fisheries and Aquatic Sciences*, 6, 93-98.