Parasitic Infection in the Gills of *Cirrhinus mrigala* (Hamilton, 1822)

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Abstract

Cirrhunus mrigala is economically important fish species in Myanmar. A total of 50 *Cirrhinus mrigala* fingerlings were sampled monthly from Yezin fishery Station from September 2018 to August 2019 and parasitic infestation in the gills of fish were examined. Ectoparasites such as *Dactylogyrus* sp., under the phylum Platyhelminthes and *Trichodina* sp., and *Ichthyophthirius multifiliis* under the phylum Ciliophora were recorded. Among all these parasites the most dominant were *Dactylogyrus* sp. followed by *Trichodina* sp. and *Ichthyophthirius multifiliis. Dactylogyrus* sp. infested in the gills with the highest prevalence (80%) in March with the mean intensity 4.3. The highest prevalence of *Trichodina* sp. (66%) was found in October 2018 and the highest mean intensity was 2.8 in February 2019. However, *Ichthyophthirius multifiliis* was found only in September with the prevalence (50%) and enormously decreased to 12% in October. Hyperplasia and hypertrophy with presence of numerous inflammatory cells and an accumulation of blood cells were observed at the base of secondary gill lamellae of the fish. The management practices and pond hygiene should be adopted in nursery operation systems of the study area for producing quality fish fry for successful harvesting.

Introduction

Heavy infestation of parasites of freshwater fishes in aquaculture becomes major problem in biosecurity and production. Parasitic infestations, especially ectoparasites like monogenetic, digenetic flukes and protozoans are the most devastating groups affecting skin and gills (Lom and Dykova, 1992). Therefore, proper health management procedures should be followed with appropriate control measures to boost up the aquaculture production. In the high stocking condition, particularly if the fishes are stressed, the parasites multiply rapidly. The incidence of fish parasites correlate with stocking density and water quality parameters (Bhuiyan and Musa, 2008). Fishes may stress especially if poor water quality parameters in fish pond.

Monogenea are commonly found on skin or gills of aquatic vertebrates and most species are host and even site specific (Reed *et al.*, 2009). They are predominantly ectoparasitic on gills and skin of fishes (Bychowsky, 1957). They cause great damage to gill filaments of carps in hatcheries and grow-out ponds.

Trichodina species are one of the primary causes of disease in cultured fish, which leads to economic losses (Martins *et al*., 2010). *Trichodina* species can result in extremely high mortality rates, particularly in young fish. However, there are few studies of the relationship between the host and parasite in trichodinid-diseased fish. The ciliated protozoan *Ichthyophthirius multifiliis* (Ich) is a common parasite of freshwater fish (Jessop, 1995 and Matthews, 2005). All aquatic animals can be infected though species vary in their susceptibility to the disease. Stress can also bring about an outbreak in a fish population as it decreases the immune function of the host.

Observation of parasitic infection in fry and juvenile fish is greatly important to improve the Myanmar aquaculture system. Information of parasitic infection of fish fry should be collected before they have been sold from the hatchery to farmers. However, it has not been conducted regularly in hatchery. In the present study, parasitic infection in gills of mrigal was studied by maintaining them in earthen pond from fry to adult.

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The gills of fish are an important media for the infectious agents, because they are a rich source of blood and an important site for disease production. Since gaseous exchange takes place through the gills, they may easily become contaminated from external sources. Laglar *et al*. (1962) also reported that gaseous exchange and other solutes between blood and water takes place in the gill lamellae. Therefore, gills are an important organ rather than the other organs to study the parasitic infection in fish.

In the meantime the fish farmers are facing to infectious diseases of cultured freshwater carps in nursery and grow-out ponds. This study was conducted to identify the parasitic species of in the gills of *Cirrhinus mrigala* using morphological cues, to examine the prevalence and mean intensity of parasites in the gills and to evaluate the histopathological alterations caused by parasitic infestation in gills.

Materials and methods

Sample collection

Fish samples, *Cirrhinus mrigala* were collected initially one month old fingerlings from nursery pond at the Yezin Fishery Station. It is a government owned fish seed multiplication center, located at Zayarthiri Township, Nay Pyi Taw. The research work was carried out from September 2018 to August 2019. The fifty host fishes, *Cirrhinus mrigala* were collected monthly during the study period. The fish samples collected in live condition to the laboratory with oxygen and water filled plastic bags.

Examination of parasites

The fishes were examined immediately after collection. The total length, standard length and body weight of each specimen were immediately measured and recorded. The gill arches were removed from the branchial cavity and placed into the petridishes containing normal saline water for microscopic examination. Tissues were transferred on to the clean glass slide, added with saline solution covered with coverslip to examine the different species with compound microscope (Olympus – CX 31).

Identification of parasites

In case of monogeneans, gill arches were placed into the petridishes containing distilled water and softly scrapped to dislodge monogeneans from the gill filaments. Monogeneans with distilled water were removed on to a clean glass slide with a fine pipette, water was absorbed on the slide by using filter paper, a drop of picric acid was added and covered with coverslip to identify the monogeinean parasites. Identification of monogeneans was based on the sclerotized hard parts of haptor, copulatory organs, marginal hooks and supporting bar according to Yamaguti (1963).

For identification of Ciliophora parasites, *Trichodina* sp., tissue samples from gills and body surface of fishes were taken and placed on the clean glass slide and covered with coverslip to examine the presence of parasites by using light microscope. The identification of *Trichodina* parasites was carried out mainly based on the diameter of adhesive disc, denticulate ring, clear area and number of denticles according to Lom and Dykova (1992) and Asmat *et al*. (2005) using the light microscope (Olympus CX 31). Length and width of *Ichthyophthirius multifiliis* parasite were measured and identified following the description and figures of Lom and Dykova (1992) and Asmat *et al*. (2005). All parasites were measured with micrometer for description and identification under light microscope.

Data analysis for parasites

Prevalence, mean intensity and abundance of parasitic infection were calculated in accordance with the following methods (Bush *et al*., 1997).

$$\textbf{Prevalence (\%)} = \frac{\text{Number of infected host}}{\text{Total number of host examined}} \text{ X } 100$$

Intensity of infection was classified four stages according to Culloty *et al*. (1999).

Stage (I): 1-20 parasites observed within five minutes of screening under 40 x magnification

Stage (II): 21-40 parasites observed within five minutes of screening under 40 x magnification

Stage (III): 41-60 parasites observed within five minutes of screening under 40 x magnification

Stage (IV): 1-10 parasites in all field of region observed immediately in screening under 40 x magnification

$$\textbf{Mean Intensity} = \frac{\text{Total Number of parasites recovered}}{\text{Total number of infected fishes}}$$

Preparation of histopathological Slides

The naturally parasitic infested gills were taken for histological observations. For histological study, collected samples were preserved and fixed for 24 hours in 10% neutral buffered formalin. After fixation, the samples were cut in order to obtain a size of 1 cm^3. The prepared tissues were dehydrated through a graded series of ethanol, cleared in xylene, and infiltrated in the paraffin. Sections were cut at 5µm in thickness on a microtome (TBS SHUR/Cut 2500) fitted with a sharpened microtome knife. These sections were then stained with Hematoxylin-Eosin. The permanent mounting of the slides was made by DPX (distyrene, plasticizer and xylene). Histopathological changes were examined and photographed at different magnifications with the help of binocular microscope with digital camera and attached monitor (Olympus – CX 31).

Results

A total of 50 *Cirrhinus mrigala* were collected monthly and ectoparasitic infection in gills of the fish were examined. During the study period, one monogenean, *Dactylogyrus* sp., and two ciliophora, *Trichodina* sp., and *Ichthyophthirius multifiliis* were recorded.

Morphometry of infected *Dactylogyrus* sp. in gills

Anterior part of the body of *Dactylogyrus* sp. composes of two pairs of head organs with the length 296.4µm ± 75.1µm. Copulatory complex is composed of tubular cirrus with enlarged initial end with the length 28.5µm ± 2.4µm. Haptor consists of one pair of anchor, one supporting bar, 89µm ± 14µm in length and seven pairs of marginal hooks. Anchor is solid, stout with well develop V shaped roots with smoothly curved point, 52.3µm ± 6.8µm in length. Marginal hooks consist of 14 of nearly uniform size, 11.9 µm ±2.7 µm in length (Plate 1).

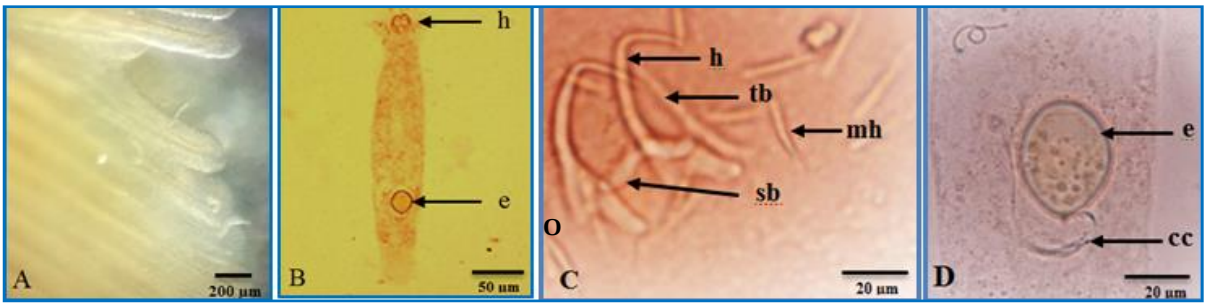

Plate 1 *Dactylogyrus* sp. infected in the gills of *Cirrhinus mrigala* (A) *Dactylogyrus* sp. attached on the gill filaments (B) *Dactylogyrus* sp. infested to the gills of *C. mrigala* (h = haptor, o = ovary) (C) Haptor of *Dactylogyrus* sp. (h = haptor, tb = eggtranverse bar, sb = supporting bar, marginal hook) (D) Ovary and copulatory organ of *Dactylogyrus* sp. (o = ovary, cc = copulatory complex).

Morphometry of infected *Trichodina* sp. in gills

Trichodina sp. is composed of massive denticles with broad curving blades, 44µm ± 4.8µm in diameter adhesive disc. It as medium-sized and disc-shaped and the center of the adhesive disc is a clear with 10.1µm ± 1µm in diameter. The denticulate ring consists of 18 to 22 denticles (Plate 2, A).

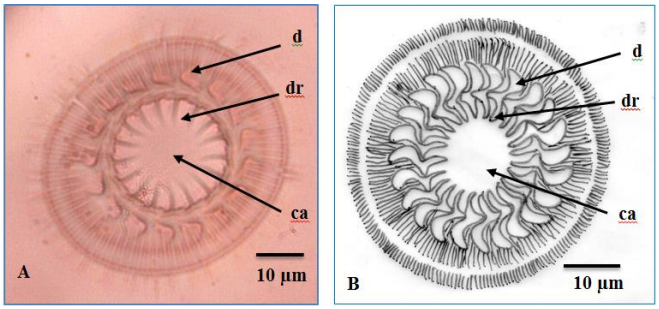

Plate 2 Ciliophora parasites, *Trichodina* sp. infested gills and skin of *Cirrhinus mrigala* (Wet mount) (A) *Trichodina* sp. infested to the gills of *C. mrigala* (B) Line drawing of *Trichodina* sp. infested to the gills of *C. mrigala* (d = denticle, dr = denticulate ring, ca = clear area).

Morphometry of infected *Ichthyophthirius multifiliis* in gills

Ichthyophthirius multifiliis, body is possessed subspherical to ovoid, uniformingly covered with cilia arranged in meridional rows with large and horsesho-shaped macronucleus measuring 28.4µm ± 5.1µm in length and 15.7µm ± 1.4µm width (Plate 3, B).

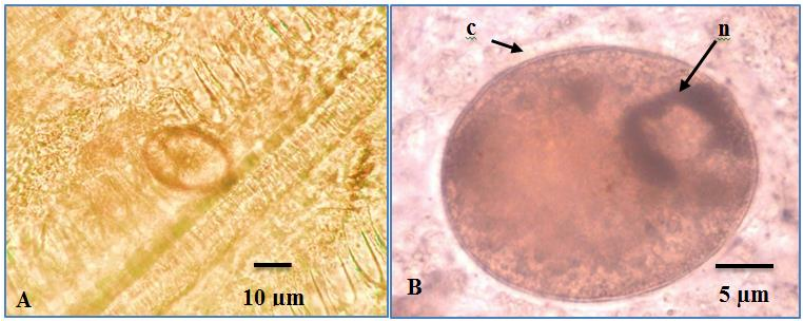

Plate 3 Ciliophora parasites, *Ichthyophthirius multifiliis* infested gills and skin of *Cirrhinus mrigala* (Wet mount) (A) *Ichthyophthirius multifiliis* infested to the gills o of *C. mrigala* (B) *Ichthyophthirius multifiliis* with distinct large horseshoe-shaped macronucleus present in the gills of *C. mrigala* (c = cilia, n = nucleus).

Prevalence and mean intensity of *Dactylogyrus* sp.

Dactylogyrus sp. was recorded only in the gills of *Cirrrhinus mrigala*. Prevalence of *Dactylogyrus* sp. was lowest (8%) in November 2018 and highest prevalence (80%) was found in March 2019 during the study period. After March 2019, it fluctuated significantly from April to August 2019 such as 44%, 34%, 60%, 48% and 62% respectively.

The highest intensity of infestation level 6.7 was recorded in February 2019. It is clearly seen that *Dactylogyrus* sp. was found out with the mean intensity round 3.0 in September, October, December 2018 and May, June, July 2019 and the other months were infected to 5.2 in January 2019, 4.3 in March, 4.1 in April and 4.5 in August 2019.

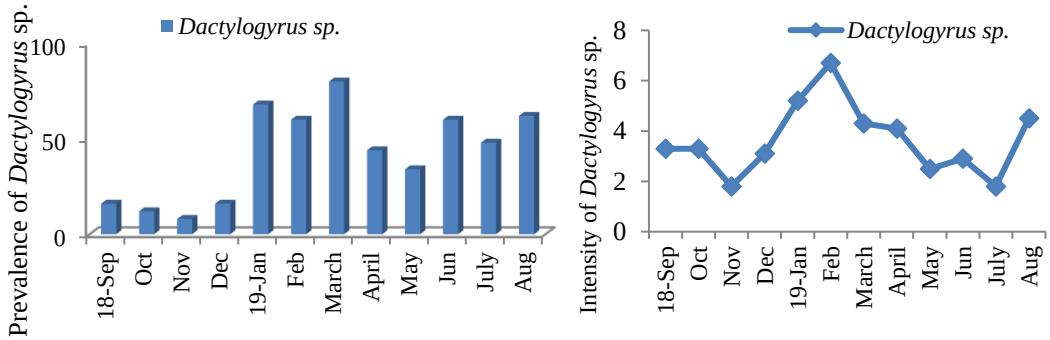

Fig. 1 Prevalence and mean intensity of *Dactylogyrus* sp. infected to *Cirrhinus mrigala*

Prevalence and mean intensity of *Trichodina* sp.

Trichodina sp. in gills and skin of *Cirrhinus mrigala* was found throughout the study period (Fig. 3). *Trichodina* sp. was collected in the gills with the percentage 40% in September 2018 and it was markedly increased to 66% in October 2018 however it was dramatically decreased to 28% in November 2018 and followed by 4% in December 2018. After that, it was gradually climbed to 14% in January 2019, 18% in February 2019.

Trichodina sp. infested in the gills with the highest mean intensity 2.8 in February 2019 while it fluctuated slightly between 1.0 and 1.5 in the other months during the study period.

Prevalence and mean intensity of *Ichthyophthirius multifiliis*

During the study period *Ichthyophthirius multifiliis* was found only in the months of September and October 2018. *Ichthyophthirius multifiliis* infested in the gills of the fish with the percentage 50% in September and 2% in October 2018. They were found out in the gills of *Cirrhinus mrigala* only in the two months, September and October 218. The mean intensity of *Ichthyophthirius multifiliis* in *Cirrhinus mrigala* was between 1.0 and 1.3 during the study period.

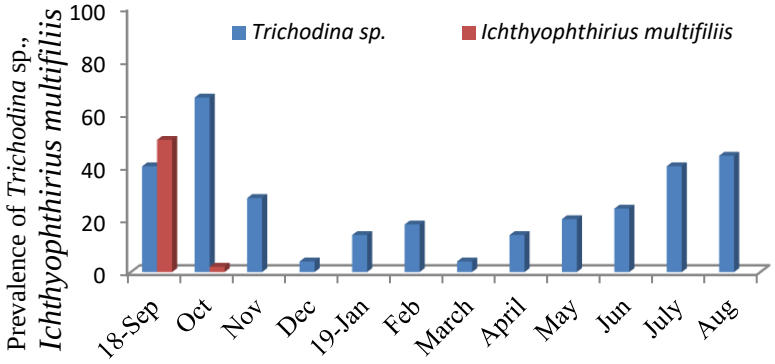

Fig. 2 Prevalence of *Trichodina* sp. and *Ichthyophthirius multifiliis* infected to *Cirrhinus mrigala*

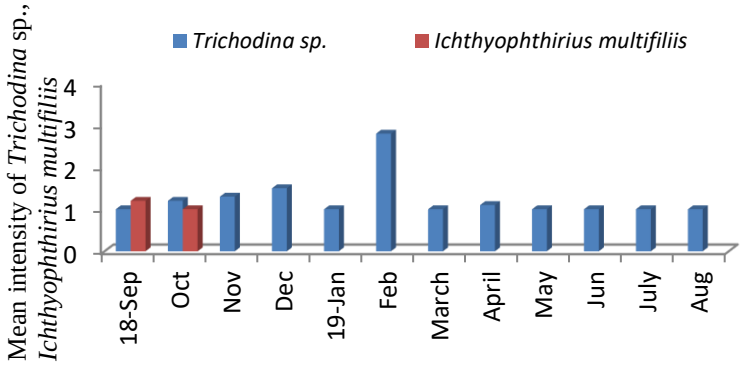

Fig. 3 Prevalence and mean intensity of *Trichodina* sp. and *Ichthyophthirius multifiliis* infected to *Cirrhinus mrigala* recorded from Yezin Fishery Station

Histopathological analysis of the gills

The critical damage due to parasitic infestations was not recorded. However, some fish infected with *Dactylogyrus* sp. showed internal damage to the gill tissues. *Dactylogyrus* sp. attached the gills and damaged the gill tissues. In primary and secondary gill lamellae, pillar cell and inter lamellar region were lost and degenerated due to the parasitic infestations. Marked proliferation of mucous cells and hemorrhage between gill filaments were observed. Split gill lamellae, hemorrhage, necrotized epithelial cells, having parasitic infestations were observed. Dilation and congestion in blood vessels of gill filaments and edema and shortening in secondary lamellae were also found out in the gills. Several secondary gill lamellae were clubbed with the accumulation of inflammatory cells and curling the secondary gill lamellae. Degenerative and necrotic changes in the epithelium of gill filaments such as epithelium lifting and curling of the secondary lamellae were found out.

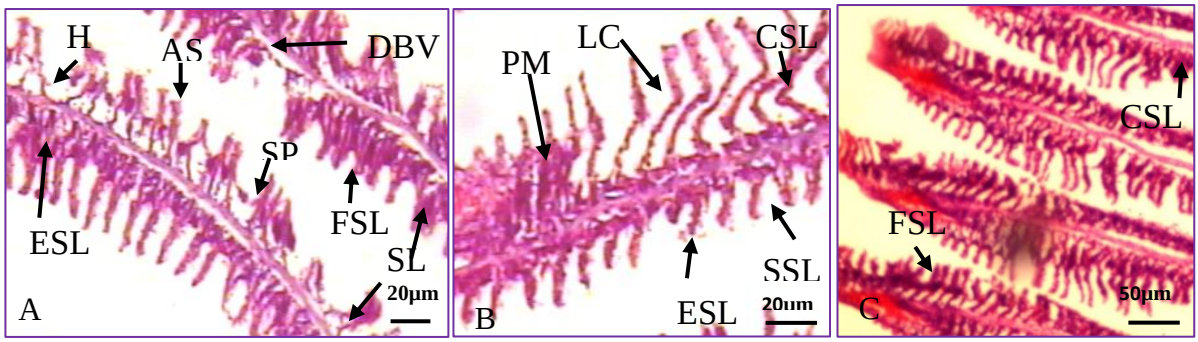

Plate 4 Longitudinal section of gill filaments in *Cirrhinus mrigala* showing *Dactylogyrus* sp. infested conditions. (A) Degenerative and proliferation mucus cells in the epithelium of gill filaments (H=Hemorrhage, ASL=Atrophy of secondary lamellae, EL=Epithelium lifting, FSL=Fusion of the secondary lamellae, DBV=Dilation of blood vessels, ESL=Edema of the secondary lamellae SL=Split lamellae, FSL=Fusion of the secondary lamellae). (B) Proliferation in the epithelium of primary and secondary lamellae (LCS= lamellae in club-shaped, PMC=Proliferation of mucus cells, CSL=Curling of the secondary lamellae, SSL=Shortening of the secondary lamellae, ESL=Edema of the secondary lamellae). (C) Degenerative and necrotic changes in the epithelium of gill filaments (CSL=Curling of the secondary lamellae, FSL=Fusion of the secondary lamellae).

Discussion

The present study was carried out to find out the incidence and parasitic infection in the gills of *Cirrhinus mrigala* with respects to different months. The different parasites were isolated and identified from the fish samples collected from Yezin Fishery Station, Nay Pyi Taw Region. During the study period, different types of parasites in the gills were observed such as *Trichodina* sp., *Ichthyophthirius multifiliis*, and *Dactylogyrus* sp. parasites.

During the study period *Trichodina* sp. was found in the skin with the highest in the month of October 2018 (54%) and the lowest in the month of January 2019 (2%) which was similar to the finding of Deva, 2016. Similarly, *Trichodina* sp. was collected in the gills with the highest in October 2018 (66%). Although they were found in all months during the study period. These findings deviated from the work of Ramudu *et al*., 2013. They reported that the highest prevalence of *Trichodina* sp. was December (91.3%) and the lowest was in October (19.04%).This might be considered due to water quality and density in fish ponds. Since the water level in pond decreased during dry season. Therefore, water level increases raining season while it decreases in dry season and as a consequence prevalence of ectoparasites increase in dry season, October to April. Ectoparasites are easy to transmit among the hosts in low level of water when it is compared to high level (Tun *et al*., 2009). Almost all extensive and semi-intensive cultured ponds in Myanmar depend mainly on rain for inlet water.

The denticulate morphology and dimensions of *Trichodina* sp. recorded in this study are similar to those reported by other authors (Lom and Dykova, 1992). *Trichodina nigra* (Lom, 1961) is exactly similar with the *Trichodina* sp. recorded in the present study.

Ichthyophthirius multifiliis was found only in the months of September (68%) and dramatically decreased to (12%) in October, but not encountered in the remaining months. *Ichthyophthirius multifiliis* infection was reported only during September-February when the water temperature is low comparison to other months (Banerjee and Bandyopadhyay, 2010). The monthly prevalence was heavy during December-February because of the biological factors of the host as well as the water quality are liable for infection (Banerjee and Bandyopadhyay, 2010). Ich infection can occur at any growth stages of fish, from one-day old

fry, fingerling, food-size to brood fish according to the water temperature and environmental conditions. The incidence of parasitic infestation in Nile tilapia decreased with increasing fish size (Lua *et al*., 1999).

In the present study, *Dactylogyrus* sp. was recorded in the gills of *Cirrhinus mrigala*. *Dactylogyrus mrigali* was first discovered from the gills filaments of *C. mrigala* from hybrid of *C. mrigala* and *Labeo rohita* from Bhavanisagar reservoir in India (Gussev, 1976). The species obtained from *Cirrhinus mrigala* of this study is similar to that described by previous authors. During the study highest infestation with *Dactylogyrus* sp. in the month March (80%) and the lowest in November (8%). This finding argued with the work of Ramuduu and Dash, 2013, who reported that both *Dactylogyrus* sp. and *Gyrodactylus* sp. showed decreased prevalence from August to September and followed by October and November, but again increased in the month of December (41.14% and 7.24%) respectively. They assumed that it is due to the different environmental and culture condition between Myanmar and India.

The gills are organs in many important functions of fish, such as gases exchange and excretion. In normal gill histology, the primary gills lamella gives rise to a number of secondary gill lamellae to increase the respiratory surface. The secondary gill lamella comprises a middle vascular layer surrounded on both sides with epithelium. Besides, the present of pillar cells is the most characteristic feature of the secondary gill lamella. These cells have a cylindrical body with a central large nucleus in non parasitic infested gills. Furthermore, the epithelium present in between and around the secondary lamellae also consists of two kinds of specialized cells, the mucus gland cells and acidophil granular cells.

However, pathogenesis in the gills caused by parasitic infestations was found out in the present study. The various parasites have the ability to cause internal damage to the gill tissues. This study shows erosion, necrosis in epithelial tissue, dilation in the gill tissue of fishes, which were infected with monogenean parasites. The histological damages in the gills inhibit the normal physiological functions of the gills. Proliferation and swelling of gill epithelium significantly reduced the oxygen uptake capacity of gills (Hughes, 1972 and Molnar, 2002). Fish change gases by taking oxygen-rich water through their mouths and pumping it over their gills. Therefore, gills closely contact with the external water environment and the primary target organ to attach the contaminants. The gills showed degeneration and necrosis of arch leading to the formation of vacuum space and the primary and secondary gill lamellae were totally damaged to a great extent due to the parasitic infestations. Ramudu and Dash, 2015 reported that the gills of the *Cirrhinus mrigala* showed complete degeneration of gill arch with excessive proliferation of primary lamellae. Therefore, the present finding is similar to the report of Ramudu and Dash, 2015.

In *Labeo rohita*, swelling of gill lamellae, hyperplasia in the tissues of lamellae, dilation of blood vessels of gill filaments with shortened lamellae and hyperplasia in the tissues of arch were observed (Moe Kyi Han, 2006). In some fish, damage of gill arch, dilation of blood vessel, congestion of blood capillary by blood cells, epithelial layer lifting and hyperemia of blood cells observed by Pa Pa Win, (2007). The present observations agree to Hassan, (1999) who reported the epithelial lining cells of the secondary lamellae with proliferative changes.

Split gill lamellae, hemorrhage, necrotized epithelial cells, having parasitic infestations were observed. In addition, dilation of gill filaments, marked proliferation of mucous cells and hemorrhage between gill filaments were also found out. Ramudu and Dash, (2013) reported that the gills were totally damaged due to mass infestation of parasites, the swelling might be due to the presence of toxins released by the parasites. Likewise, Deva, (2016) found out that

the gills of *Labeo rohita* were also infected with parasite showing inter-lamellar epithelial type plasmodium.

The present findings showed excessive proliferation of gill filaments with complete loss of primary and secondary lamellae which were similar with results found in *Catla catla* reported by Dey, *et al*. (1988). High prevalence and intensity of monogeneans have resulted the proliferation and abnormal changes on gill filaments as discussed by Mohu, *et al*., 2012. The proliferation and abnormal changes on gill filaments prohibit the oxygen intake of the fish that is leading to suppress the growth rate of fish. Regular water exchange should be conducted to reduce the intensity of parasites in the hatchery to prevent the disease transmission from the hatchery to the farms.

Conclusion

The gills of *Cirrhinus mrigala* collected from Yezin Fishery Station were infested with three kinds of parasites species. High prevalence of infection was recorded during the cold season. Histopathological changes were found at the tip of the gill lamellae and several primary gill lamellae were clubbed with the accumulation of inflammatory cells especially at the base of the primary gill lamellae. Thus, an important action to prevent the spread of these parasites should be taken hatchery to the farmers.

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