MYXOSPOREAN INFECTION IN THE INTESTINE OF *CIRRHINUS MRIGALA* (HAMILTON, 1822) AT YEZIN FISHERY STATION, NAY PYI TAW, MYANMAR

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Abstract

Cirrhinus mrigala fingerlings were sampled monthly from Yezin Fishery Station, over 12 months of study period and examined for intestinal myxosporean parasites. Three myxosporean species, *Myxobolus* sp., *Thelohanellus* sp. and *Henneguya* species under phylum Cnidarian were recorded. Among all these parasites, the most dominant species was *Myxobolus* sp., followed by *Thelohanellus* sp. and *Henneguya* species. Spores of *Myxobolus* sp. elongated and ellipsoid in valvular view. It had 11.6µm±1.1µm in length and 7.6µm±0.8µm in width. The myxospores of *Thellohanellus* sp. were pyriform in shape, blunt at the anterior end measuring 16.0µm±1.3µm x 8.8µm±1.3µm in size. Spores of *Henneguya* species are oval in shape and measurement of its length and width were 27.5µm±1.7µm x 3.8µm±0.7µm in size. The highest prevalence and intensity of *Myxobolus* sp. infection were recorded in December 2018 (84%). The highest prevalence and intensity of *Thelohanellus* sp. infection were found in November (18%). However, *Henneugya* species was found only in December with low prevalence infection (4%). Cyst formation of *Myxobolus* sp. was found on the surface of intestine. To examine the histopathological changes of infected tissues, histopathological slides were prepared and checked under microscope. Histopathological changes such as proliferation of villi, necrosis of serosa, mucosa and submucosa as well as space in villi necrosis and fusion of villi were observed in the intestine of infected fish. Therefore, management practices and pond hygiene should be adopted in nursery operation systems and grow-out ponds for producing quality fish fry and successful harvesting.

Keyword: - myxosporean infection, intestine, histopathology, *Cirrhinus mrigala*

Introduction

In Myanmar, freshwater aquaculture depends mainly on carp culture practices and farming in earthen ponds depicts the major source of aquaculture production. Myanmar's foreign income from fishery exports reached more than US$535 million in 2018. Myanmar's farmed fish exports are dominated by *Labeo rohita*, along with two other species; *Cirrhinus mrigala* (Mrigal carp) and *Catla catla* (Belton *et al*., 2015). Mrigal carp is an important component of polyculture with other native and exotic carp species.

Disease has a serious impact on fish in both captive and natural environments. In cultured fish population, the parasites may involve in the serious outbreak of disease (Kayis *et al*., 2009). It is a major problem that carrying heavy infection of parasites of freshwater fishes in aquaculture. The water quality parameters and stocking density correlate with the development of fish parasites (Bhuiyan and Musa, 2008). In the high stocking density, if the fishes are stressed, the parasites multiply rapidly. Meanwhile the farmers had acquired as a result of research and development as well as their own experiences.

Myxozoan parasites are one of the economically important groups of microscopic parasites as they infect fish harvested for food and most commonly parasitize invertebrates (Kent *et al*., 2001and Lom and Dykova, 2006). They are common in juvenile carps in nursery ponds and high mortality rates caused by their infections in the organs of fish. Due to a site of gaseous exchange and rich blood supply, gills are prone to be more infected (Martins, *et al*., 1997). Myxosporeans infected in the organs of fish, where they may cause serious structural changes depend on the

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intensity of infections. Myxosporean parasitic infections caused economical losses in the carp nursery ponds (Sanaullah and Ahmed, 1980).

The complex life cycle of myxozoa involves myxospore and actinospore life stages within a fish host and an invertebrate host respectively (Markiw and Wolf, 1983 and Wolf and Markwi, 1984). The life cycle of myxozoa requires a tubificid worm *Tubifex tubifex* as an alternative host, in which the ingested spore further develops as actinospores. When actinospores were released from the tubificid, they enter into the fish and life cycle is completed (Lom and Dykova, 1992). Therefore, transmission of myxozoa among the fish are considered in Myanmar since freshwater fish farms are conducted in the earthen ponds.

In Myanmar, parasitic infections of freshwater fish were studied by Moe Kyi Han (2006), Pa Pa Win (2007), Sein Sein Myint (2007), Myint Myint Win (2012), Hnin Hnin Htay (2014), Su Su Mon (2014) and Yan Naung Tun (2019). However, examination of intestinal Myxoaporea in *Cirrhinus mrigala* is still required to improve the production. In turbot and gilthead sea bream aquaculture, it was reported that the myxozoan parasites invade the intestinal mucosa, causing a cachectic syndrome associated with intestinal barrier alteration. *Myxobolus* sp. cyst infection in the intestine can damage villi and mucosal epithelium causing myolitic (Maftuch *et al*., 2017). Intestine infection leads to myolitic on the intestinal wall of fish (Marjoram and Bagnat, 2015). Moreover, developing and mature spores of plasmodia in the wall of the intestine dispersed throughout the body by the blood stream (Molnar and Kovacs-Gayer 1985).

The intestine is an important organ for digesting and absorbing nutrients to growth fish. The parasite extracts energy and nutrients from the host fish so, which are supported the general health and reproductive effort and also impair mating and gonad maturation (Reddy and Benarjee, 2014). Therefore, investigation on intestinal myxosporeans in *Cirrhinus mrigala* is required from the biosecurity point of view and diseases control strategies. In the present study intestinal myxosporean parasitic infections in *Cirrhinus mrigala* were investigated to understand on fish health status of carp hatchery in Myanmar. Besides, histopathological changes of infected fish were examined to reveal on the impact of parasites on fish physiology.

Materials and Methods

Sample collection

Fish samples, *Cirrhinus mrigala* were collected initially one month old fingerlings from nursery pond at the Yezin Fishery Station, Nay Pyi Taw, Myanmar. *Cirrhinus mrigala* fingerlings were cultured in experimental pond (8.3mx33.3m) at Yezin Fishery Station using extensive culture system. The fifty host fishes, *Cirrhinus mrigala* were collected on regular basis once a month during the study period. Fish were collected for 12 months from September 2018 to August 2019. The fish samples were brought in live condition to the laboratory at Department of Aquaculture and Aquatic Diseases, University of Veterinary Science or laboratory Aquatic Bioscience, University of Yangon with oxygen and water filled plastic bags. The fish samples were kept temporarily in a small aquarium (45.72 × 91.44 × 91.44 cm3) and aeration was given in the laboratory.

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 external symptoms of the whole fish were checked under stereomicroscope before dissecting the fish. The body of the fish then was cut to examine the present of different parasites in the intestine. The whole intestine was transferred on to the petridish with 0.9% saline. Then, intestine was cut into

1cm each, put on glass slide, added with 10 µl of 0.9% saline, covered with coverslip and examined under light microscope (Olympus – CX 31).

Identification of parasites

The identification of myxosporean parasites were conducted according to the guidelines of Lom and Arthur (1989), Lom and Dykova (1992) and Kalavati and Nandi (2007). Identification was made on the basis of various morphological structures of spore such as shape, size, and number of polar capsules, length of polar filaments, number of coils of polar filaments, presence or absence of intercapsular process, presence of any iodinophilous vacuole and number of nuclei in the sporoplasm, length of caudal appendage etc. They were measured and photographed using the light microscope (Olympus CX 31) under 100X magnification.

Data analysis for parasites

Prevalence of parasitic infection was calculated in accordance with the following methods (Bush *et al*., 1997).

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

Mean intensity of infection was classified four stages according to Bachere *et al*. (1982) and 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

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

Preparation of histopathological slides

To understand the histopathogical changes of infected tissues of intestine, the tissue infected with cyst of parasites were cut and fixed in 10% neutral buffered formalin. After fixation for 48 hours, the tissues 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 (Thermo Scientific Microm hm355s) 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 lesions were examined and photographed at different magnifications with the help of binocular light microscope (Olympus – CX 31).

Environmental parameters examination

Environmental parameters such as pH, Dissolved oxygen (DO), Ammonia and Nitrite from fish pond were examined by using portable water Test Kits. Water temperature was measured with a thermometer.

Results

Growth rates of *Cirrhinus mrigala*

A total of 50 fish was sampled monthly from Yezin Fishery Station. The initial weight of fish was 0.8 g (±0.2 g) while it gradually increased during the study period (Fig. 1). In the end of experiment, the mean body weight of fish reached to 14.7 g (±2.1 g).

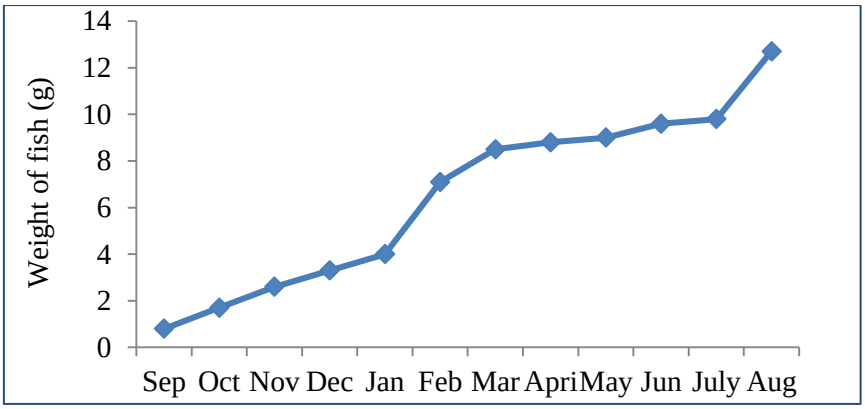

Figure 1 Growth weight of *Cirrhinus mrigala* recorded from Yezin Fishery Station

Morphology and morphometry of myxosporean parasites

Three myxosporean parasites, *Myxobolus* sp., *Thellohanellus* sp. and *Henneguya* sp. were found in the intestine of *Cirhinus mrigala*.

Spores of *Myxobolus* sp., was 11.6µm±1.1µm in length and 7.6µm±0.8µm in width with elongated and ellipsoid in valvular view. Mucus envelope was found around the posterior end. Two polar capsules were slightly pyriform and unequal in shape with 4 to 6 filaments, larger 5.0µm±1.1µm x 3.3µm±0.5µm and smaller 3.5µm±0.8µm x 3.3µm±0.5µm in size (Plate 1, B). Sporoplasm was finely granular and occupied most of the extracapsular cavity of spore.

The myxospores of *Thellohanellus* sp. were pyriform in shape, blunt at the anterior end measuring 16.0µm±1.3µm x 8.8µm±1.3µm in size (Plate 2, B). Polar capsule was elongated pyriform measuring 7.1µm±0.9µm x 4.9µm±0.6µm in size.

Henneguya sp. spores are oval in shape and the measurement of length and width were 27.5µm±1.7µm x 3.8µm±0.7µm in size. It has two equal polar capsules inside, measuring 3.5µm±0.6µm x 1.2µm±0.5µm in size. Length of caudal appendage was 12.5µm±1.3µm long (Plate 3, A).

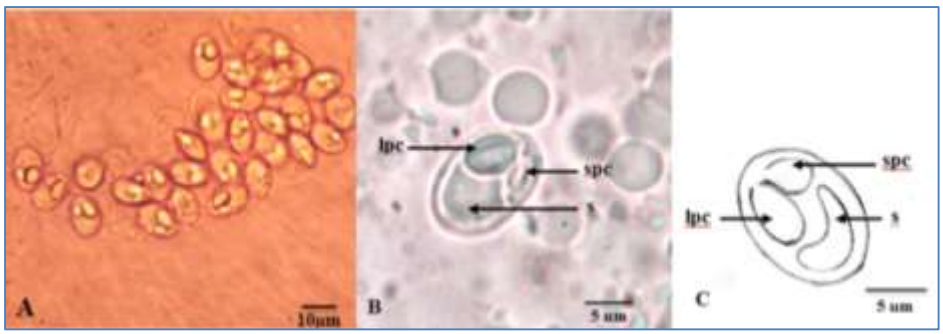

Plate 1 *Myxobolus* sp. recorded in the intestine of *Cirrhinus mrigala* from Yezin Fishery Station (A) Plasmodia of *Myxobolus* sp. (B) Detail morphology of *Myxobolus* sp. (C) Line drawing of *Myxobolus* sp. (lpc = large polar capsule, spc = small polar capsule, s = sporoplasm)

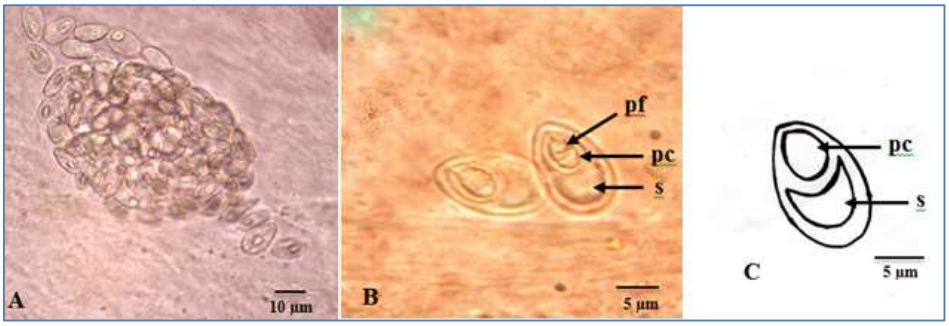

Plate 2 *Thellohanellus* sp. recorded in the intestine of *Cirrhinus mrigala* from Yezin Fishery Station (A) Plasmodia of *Thellohanellus* sp. (B) Detail morphology of *Thellohanellus* sp. (C) Line drawing *Thellohanellus* sp. (pc = polar capsule, s = sporoplasm)

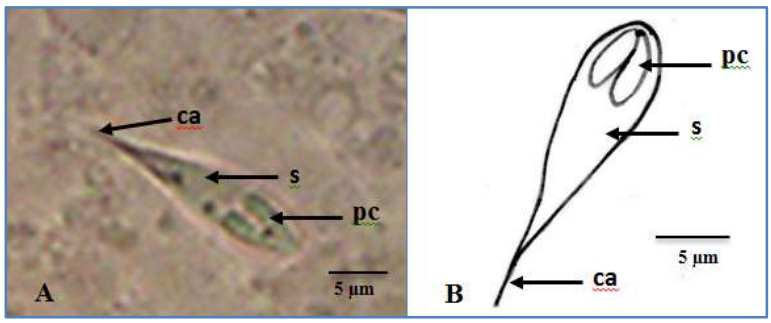

Plate 3 *Henneguya* sp. recorded in the intestine of *Cirrhinus mrigala* from Yezin Fishery Station (A) Detail morphology of *Henneguya* sp. (B) Line drawing of *Henneguya* sp. (pc = polar capsule, s = sporoplasm, ca = caudal appendage)

Prevalence and mean intensity of myxosporean parasites

The prevalence and mean intensity of three myxosporeans fluctuated during the study period (Fig. 2 and 3). It is clear that the prevalence of *Myxobolus* sp. in the intestine of fish was 62% in November 2018 and it noticeably increased to 84% in December 2018. Then, it sharply decreased to 52% in January 2019. The prevalence fluctuated around 40% in April, May, June, July and August 2019. *Myxobolus* sp. was found in the intestine with the highest mean intensity 3.5 in January 2019 and followed by 3.4 in December 2018. However, the lowest mean intensity 1.2 was recorded in May and July 2019 (Fig. 3).

The highest prevalence of *Thellohanellus* sp. was 18% and it was found in November. It gradually decreased to 10% in December 2018, followed by 2% in January and February 2019. After that, it marginally increased to 4%, 6% and 8% in March, April and May respectively and minimally decreased to 6% in June and July 2019. *Thellohanellus* sp. was not recorded in September, October 2018 and August 2019. The mean intensity of *Thellohanellus* sp. was 4 in November, December 2018 and April 2019 but it was only 1.0 in the other months during the study period (Fig. 3).

During the study period *Henneguya* sp. was found only in December 2018 with low prevalence (4%) and mean intensity of infection was (1) (Fig 3).

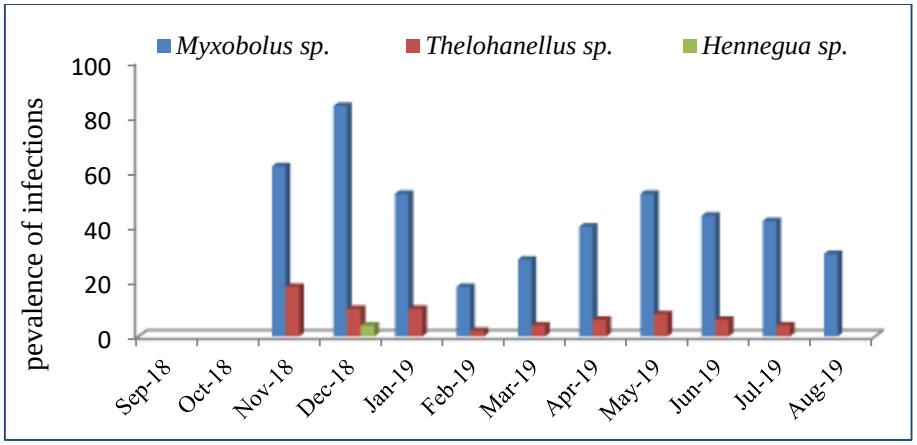

Figure 2 Prevalence of myxosporean infections in the intestine of *Cirrhinus mrigala* during the study period

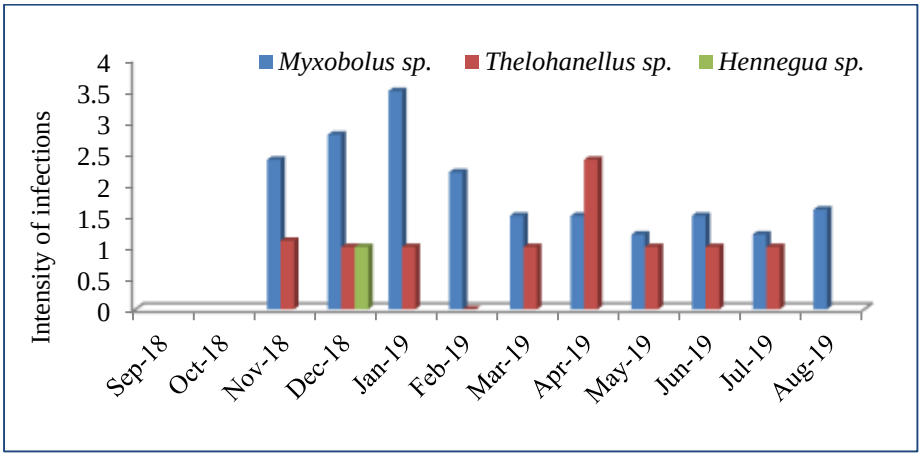

Figure 3 Mean intensity of parasitic infestations in the intestine of *Cirrhinus mrigala* during the study period

Mean body weight of infected and uninfected *Cirrhinus mrigala*

Mean body weight of infected and uninfected *Cirrhinus mrigala* was compared to understand the effect of parasitic infection on growth of fish. The weight of infected fish is slightly decreased than that of uninfected fish from February to August 2019.

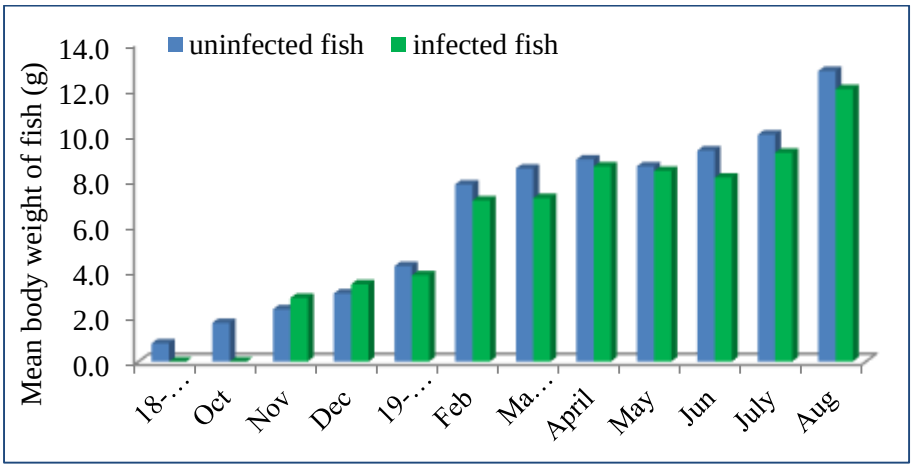

Figure 4 Mean body weight of *Cirrhinus mrigala* during the study period

Histopathological analysis of intestine

Cyst formations in intestines were found only in the fish that was infected with *Myxobolus* species. Pathogenesis in intestine due to the infection of *Myxobolus* sp. was observed. Pathological findings in the intestine of host included severe degenerative and necrotic changes in the intestinal mucosa and submucosa. Proliferation of mucus cells in the intestinal mucosa and intestinal cells damage in the form of necrosis occurred in infected fish.

Cyst of *Myxobolus* sp. had been found in the intestine (Plate 4, A). Hemorrhages and atrophy were found in the epithelial layer of intestine due to the abundance of *Myxobolus* parasites (Plate 4, B). Vacuolization and necrosis were found in the epithelial layers that were followed by hemorrhages (Plate 4, C). In some cases, massive atrophy and hemorrhages were also found in mucosa and circular layer of smooth muscle regions. In the present study, marked histopathological changes in the intestine of *Cirrhinus mrigala* have been observed, proliferation of villi, and necrosis of serosa, mucosa and submucosa as well as space in villi necrosis and fusion of villi.

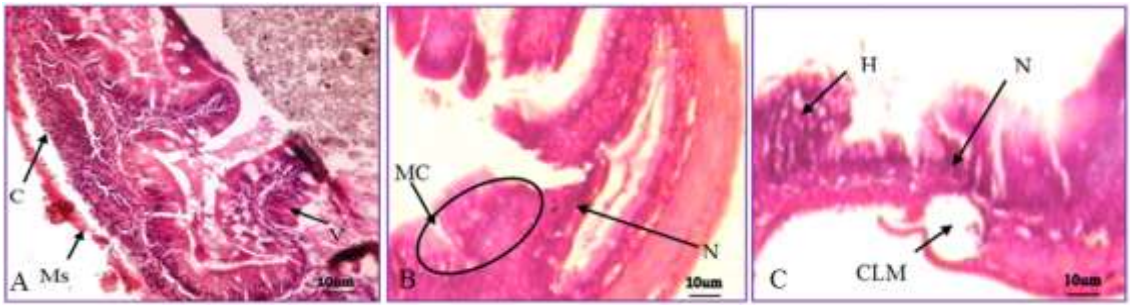

Plate 4 Pathogenesis in intestine of *Cirrhinus mrigala* caused by myxospoean infections
(A) *Myxobolus* sp. cyst formation in smooth muscle (C=Cyst of *Myxobolus* sp., Ms=Muscularis, V=Villi) (B) Haemorrhage and aggregations of inflammatory cells in the mucosa and submucosa (MC=Mucus cell, N=Necrosis) (C) Degenerated serosa and muscularis

(H=Haemorrhage, VCLM = Circular layer of smooth muscle, N= Necrosis)

Environmental parameters in fish pond

Water temperature in fish pond varied from 20 Ċ to 28 Ċ during the study period (Fig. 4). The highest water temperature was 28 Ċ (April, 2019) in fish pond. The value of pH in studied pond ranged from 6.5 to 7.6 within twelve months. The highest level of dissolved oxygen (DO) was 7 ppm in September and the other months between 5 to 6 ppm in the fish pond. The concentration of ammonia in water was observed to be highest in March, April, May and June, 2019 (0.4 ppm) and the other months were 0.1 ppm and 0.2 ppm respectively. The value of nitrite in fish pond maintained the level at 0.05 to 0.1 ppm in the study period.

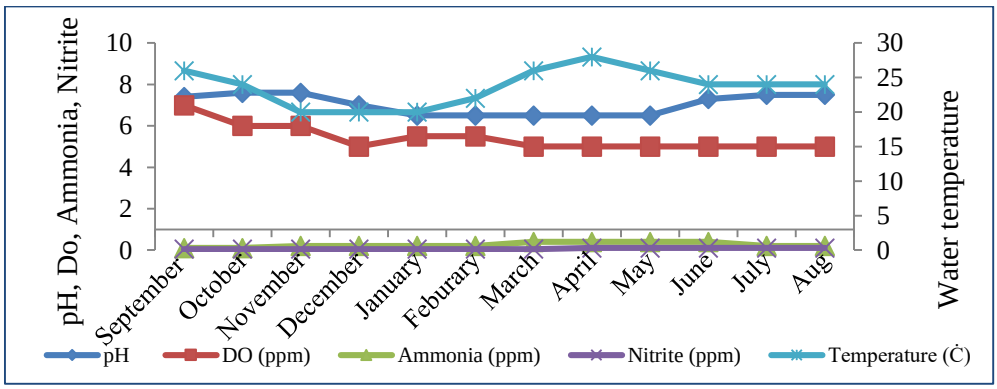

Figure 4 Environmental parameters in fish pond during the study period

Discussion

Three myxosporean parasites, *Myxobolus* sp., *Thellohanellus* sp. and *Henneguya* sp. were recorded in the intestine of *Cirrhinus mrigala* at Yezin Fishery Station. Identification of the parasites cannot be conducted into species level since only morphological study has been done in the present study. *Myxobolus* is the predominant species group within the phylum Cnidarian. Most of the species infect primarily fish, both freshwater and marine species all over the world, and a few numbers of species were found in amphibians. There are 112 nominal species were described for *Myxobolus* (Butschli, 1882).

The shape and dimension of *Myxobolus* sp. recorded in the present study is similar to *Myxobolus eirasi* infected in caudal fin of *Cirrhinus mrigala* and *Myxobolus guangzhouensis* infested in scales of *Cirrhinus mrigala* (Eiras *et al*., 2014). However, length of polarcapsule was slightly different. The shape and size of *Myxobolus* sp. detected in this study is similar to *Myxobolus* sp. 7 infected in gills and kidney of *Cirrhinus mrigala* that was recorded by Pa Pa Win (2007). However, the site of infection is differed from the present study.

Thelohanellus sp. recorded in the present study appeared similar to *Thelohanellus kalavatae* in caudal fin of *Cirrhinus reba* (Zhang *et al*., 2013). The dimension of *Thellohanellus* sp. infected in *Cirrhinus mrigala* of recorded by Pa Pa Win (2007) was nearly similar. However, the width of spore and size of polar capsule were slightly different.

Henneguya species was rear Myxosporadium species. *Henneguya* species was reported only in Mandalay Region by Shwe Sanda *et al*. (2020) from the gills of *Anabas testudineus* in Taungthaman Lake, Mandalay Region. In the present study, only one individual of *Henneguya* species was recorded during the study period. Therefore, it had low prevalence and intensity of infection. The species identification of *Henneguya* species was more difficult than the other two species.

Although the morphology of Myxosporean is the same, the species are assumed to be different if the host fish species and infection sites are varied. Molecular identifications of myxosporean parasites recorded in the present study are needed as further confirmation to identify the species level.

In the present study, parasitic frequency of *Myxobolus* sp. was highest in December 2018 (84%) stated as "stage 4" and lowest in February 2019 (18%) stated as "stage 1". The result of the present study agreed with the work of Farhaduzzaman *et al*., 2010. They reported that the highest number of parasites was collected in December (94%) and lowest in February (15%). Because of the parasitic infection is greatly influenced by the seasonal especially in winter, which basically interfere with ecology and parasitic condition of the fish. Moreover, environmental parameters fluctuated very quickly during winter and summer seasons, fish becomes affected with diseases in these seasons.

The highest prevalence of infestation was found in *Myxobolus* species. High prevalence of infection was found from November 2018 to January 2019 when the fish was 3 to 4 months old. Tun *et al*. (2014) reported the prevalence of gallbladder myxosporean parasite, *Zschokella honjoi* infection in *Labeo rohita* and they found that the infection decreased when the size of fish increased. They postulated that *Zschokella honjoi* was released from the gallbladder after 6 months of infection. Since the fish in Yesin Fishery station was cultured in earthen pond, it is difficult to estimate the mortality due to infection. The older fish seems to be more resistant than the younger fish. In addition, Brown *et al*. (2016) suggested that adaptive immunity in fish is low during winter. Therefore, parasite was found starting from November when the temperature decreased.

Myxobolus cerebralis infects cartilage tissue and causes a whirling behavior (tail chasing swimming), a black tail, and skeletal deformities of affected fish. Whirling disease was previously

known as a hatchery disease, but recently, it has been recognized as one of the causes for the decline of natural rainbow trout population (Hedrik *et al*., 1986). Therefore, *Myxobolus* sp. recorded in the present study might be threatening species for fish hatcheries. In addition, it can have impact on natural population of *Cirrhinus mrigala* in near future.

The mean body weight of infected fish is lower than the uninfected one. Shanchita and Hossain, (2015) reported that the internal parasites can cause physiological damage, cell proliferation and reproductive damage, necrosis in epithelial tissue and mucosal destruction of the intestine due to parasitism of myxosporean parasites in the intestine. Tissue damage such as necrosis and proliferation of mucus cells were recorded in the intestine of fish. The parasite extracts energy and nutrients from the host which are not supported the general health and reproductive effort and also impair mating and gonad maturation (Reddy and Benarjee, 2014). Therefore, the poor absorption of nutrients can cause due to damage of intestinal wall of fish infected by parasites. It can cause malnutrition that led to the reduction of growth of fish. *Cirrhinus mrigala* is important aquaculture species in Myanmar for both local consumption and export market. They have been cultured in earthen ponds which will be one of the factors for disease transmission of Myxosporean since Tubifex in the earthen pond acts as an alternative host in the lifecycle species of Myxozoa. The present finding will support the fishery sector for the management of parasitic infection in earthen pond culture system for *Cirrhinus mrigala.*

Conclusion

The result of the present study indicated that the *Cirrhinus mrigala* is infected with three myxosporean parasites namely *Myxobolus* sp., *Thelohanellus* sp. and *Henneguya* species. High prevalence of infection was recorded when the fish were young ages. Epithelial tissue and mucosal destruction of the intestine caused by parasitism of myxosporean parasites were found. It can cause malnutrition and retards growth of the fish. Therefore, management practices and pond hygiene in nursery operation systems are suggested for producing quality fish fry for successful harvesting.

Acknowledgements

I would like to thank Dr. Win Ohnmar Kyaw, Professor and head of Department of Aquaculture and Aquatic Diseases, University of Veterinary Science for her encouragements to carry out this study. Profound indebtedness are due to U Khin Maung Maw, Director General, Department of Fisheries, for allowing me to conduct the study at Yezin Fishery Station; U Soe Paing, Assistant Director, Yezin Fishery Station, Department of Fisheries, for supporting fish specimens and fish pond to conduct this work at Yezin Fishery Station, Nay Pyi Taw.

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