DETECTION OF MYXOSPOREAN PARASITES INFECTED IN THE KIDNEYS OF *CIRRHINUS MRIGALA* (HAMILTON, 1822)*

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Abstract

Cirrhinus mrigala, Mrigal carp was sampled monthly and examined myxosporean parasitic infection in kidneys of fish, over 12 months of study period. *Myxobolus* sp. under the phylum Cnidarian was recorded. Spores of *Myxobolus* sp. elongated and ellipsoid in valvular view, measurement 11.6µm±1.1µm in length and 7.6µm±0.8µm in width. The highest prevalence of *Myxobolus* sp. infection were recorded in August (82%) with highest mean intensity of infection (3) when the fish was one year old. Among the infected fish, only 16.7% of infected fish showed cysts formation on the kidneys through the study period. The histology slides of infected tissues were examined under light microscope to understand the histopathological changes of infested tissues. Histopathological changes such as abnormalities of convoluted tubules, dilation of blood vessels, hypertrophy and deformities of glomerulus, and congestion of blood cells caused by *Myxobolus* infection were observed. Dilation in the capillaries and vacuolar degeneration in the epithelium of renal tubules were observed. The infested kidney tissue showed the prominent circular vacuolar spaces filled with damaged cells necrosis in the tissue. To improve quality fish fry production and successful harvesting, therefore, management practices and pond hygiene should be adopted in nursery operation systems and grow-out ponds.

Introduction

Disease has a serious impact on fish in both captive and natural environments in worldwide. 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 load of parasites in cultured fishes, out of which myxosporean parasites are emerging as a major group in aquaculture. In order to increase the production and to get the profits, application of the knowledge in the control of diseases is essential in fisheries sector (Snieszko, 1983).

Examination of parasitic infections in *Cirrhinus mrigala* is still required to improve production for local market. Among the fish parasites, myxosporeans are diverse and widely distributed metazoan parasites known both marine and freshwater fish (Kent *et al*., 2001; Canning and Okamura, 2004; Lom and Dykova, 2006). They are small parasites (< 100 µm) and more than 2200 species are reported across the world (Liu. 1981).

The complex life cycle of myxozoa requires a tubificid worm 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). Myxospoean parasites are one of the economically important groups of parasites as they infect the fish and most commonly parasitize invertebrates (Lom and Dykova, 2006). They are common in nursery ponds and high mortality rates caused by their infections. These parasites infested in the organs of fish, where they may cause serious structural changes depend on the intensity of parasites. Myxosporean parasitic infestations caused economically losses in the carp nursery ponds (Sanaullah and Ahmed, 1980). Parasitic diseases are the most serious limiting factors because fish pathogens can easily be transmitted in a restricted water body in fish ponds.

Different myxosporeans infect various organs of fish. *Myxidium* species infection abundantly found in gallbladder and intestine while *Myxobolus* and *Thellohanellus* species occur

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in gills and skin (Kay Lwin Tun, 2016). Infections of Myxosporea to internal organs of fish show more serious damage than those to external organs such as skin and scale (Tun *et al.*, 2017). The kidneys are one the important organs in excretion and regulation of the water balance within the fish body. Myxosporean infection in kidney of fish has been reported in marine and freshwater fish. Severe lesion of the glomeruli capsules and fat deposits on the kidney tubules of *Hypophthalmicthys molitrix* (Yu and Wu, 1992). Similarly, diffused histozoic myxidian parasite in the caudal kidney of cultured eel in Taiwan (Liu, 1981). Degenerative and necrotic changes in the kidney tubules in carps caused by enzootic nature of myxosporeans (Mishra, *et al*., 1982). Moe Kyi Han (2006), Sein Sein Myint (2007), Pa Pa Win (2007) and Shwe Sanda, *et al*. (2020) reported incidence of Myxosprean in Manadaly environs. However, the classification of myxosporean parasites is currently based on morphology of spore.

Infections with myxozoa have recently been described as a cause of clinically significant kidney disease in two species of anurans (Kayis *et al*., 2009). Histopathological changes were glomerular shrinkage, increased spaces between glomerulus and Bowman's capsule, increased tubular lumen in the kidneys of some carps (Ullah, *et al*., 2017). Examination of parasitic infections in kidneys of *Cirrhinus mrigala* in Myanmar is still required to improve production of mrigal fish. The present study was therefore undertaken to detect the myxozoan parasites infected to the kidneys of *Cirrhinus mrigala* in Yezin fishery station, one of the biggest *C. mrigala* hatchery in Myanmar and to evaluate the histopathological alterations caused by Myxosporean parasitic infestation in kidneys of *Cirrhinus mrigala*.

Materials and Methods

Study Area

Yezin Fishery Station is a government owned fish seed multiplication center, carrying out research and documentation of fish species in Zayarthiri Township, Nay Pyi Taw Region. It is situated at 19° 50' 14.9" N and 96° 16' 36.8" E about 19 km away from Pyinmana city, and it is located near the Yezin Dam and beside the Yangon-Mandalay Highway Main Road. One of the biggest *Cirrhinus mrigala* hatchery in Myanmar and also distributes fry/fingerling *C. mrigala* through the country.

Study Period

The research work was carried out from August 2018 to September 2019.

Sample Collection and Examination of Parasites

Cirrhinus mrigala fingerlings were cultured in experimental pond (8.3mx33.3m) at Yezin Fishery Station as extensive culture system. Thirty fish were collected monthly to examine the occurrence of parasites. A total of 30 fish samples were carried to the laboratory of Department of Aquaculture and Aquatic Diseases, University of Veterinary Science or laboratory Aquatic Bioscience, University of Yangon with oxygen filled plastic bags. The total length, standard length and body weight of each specimen were immediately measured and recorded. Fish were dissected and kidneys were collected to examine the parasites. The kidney is divided into 2 parts. One part was used for smear slide preparation and other for histological slides preparation. For smear slide preparation, the kidneys of fish were checked under stereomicroscope for the cyst formation of myxosporean. Kidneys were squeezed with cover slip with 1 drop of normal saline (0.9%). Occurrence of parasites was examined under light microscope, Olympus – CX 31.

Identification of parasites

Identification myxosporean parasites was conducted on the various morphological structures of spore such as shape, size, number of polar capsules, length and number of coils of polar filaments, intercapsular process presence or not, number of nuclei and iodinophilous vacuole in the sporoplasm, etc. according to the guidelines of Lom and Dykova (1992) and Kalavati and Nandi (2007). They were measured and photographed using the light microscope (Olympus CX 31) under x100 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 Culloty *et al*. (1999).

Stage (I): 1-20 parasites observed within five minutes of screening under x40 magnification

Stage (II): 21-40 parasites observed within five minutes of screening under x40 magnification

Stage (III): 41-60 parasites observed within five minutes of screening under x40 magnification

Stage (IV): 1-10 parasites in all field of region observed immediately in screening under x40 magnification

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

Fish were divided into three groups according to their total length and prevalence of infection among the group was compared. Fish sizes will group as 0-4 Cm, 4-8 Cm and 8 and above.

Preparation of Histopathological Slides

To understand the histological changes of infested tissues of kidneys, infected tissue with cyst formation were fixed 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 (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 lesions were examined and photographed at different magnifications with the help of binocular microscope with digital camera and attached monitor (Olympus – CX 31).

Results

Myxobolus sp. infection in the kidney of *Cirrhinus mrigala*

Myxobolus sp. was recorded in the kidneys of *Cirrhinus mrigala* collected from Yezin Fisheries Station. *Myxobolus* sp. was identified and according to Lom and Arthur (1989), Lom and Dykova (1992) and Kalavati and Nandi (2007).

Morphometry of *Myxobolus* parasites

Spores of *Myxobolus* sp., measured 11.6µm±1.1µm in length and 7.6µm±0.8µm in width and appeared elongated ellipsoidal in valvular view. Two polar capsules were slightly pyriform and unequal in shape with 4 to 6 filaments, larger 5.0µm±1.1µmx3.3µm±0.5µm and smaller 3.5µm±0.8µmx3.3µm±0.5µm in size (Plate 1). Sporoplasm was finely granular and occupied most of the extracapsular cavity of spore. Spores elongated and ellipsoid in valvular view with mucus envelope around the posterior end. Two polar capsules were slightly pyriform and unequal in shape with 4 to 6 filaments. Sporoplasm was finely granular and occupied most of the extracapsular cavity of spore.

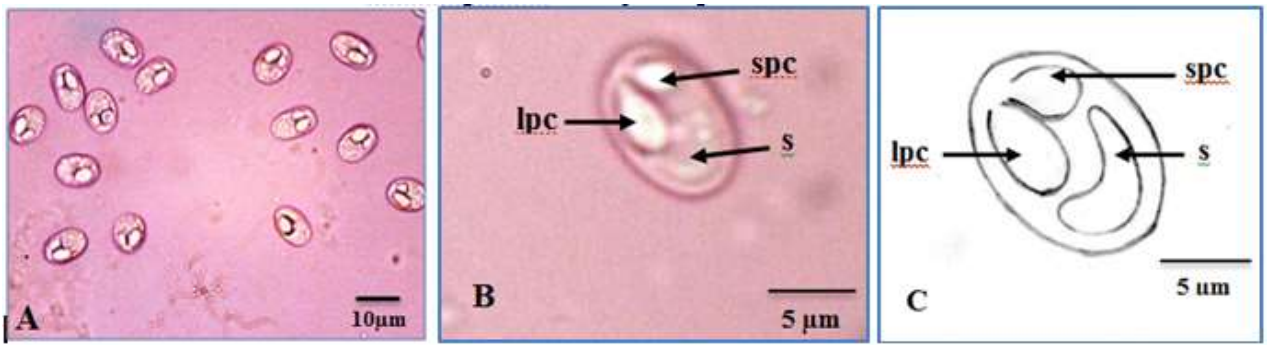

Plate 1 *Myxobolus* sp. recorded in the kidneys of *Cirrhinus mrigala* (A) *Myxobolus* sp. infected in the kidneys of *Cirrhinus mrigala* (B) Detail morphology of *Myxobolus* sp. (C) Diagramatic presentation of *Myxobolus* sp. (lpc = large polar capsule, spc = small polar capsule, s = sporoplasm)

Prevalence and mean intensity of *Myxobolus* sp. infections

The white nodule, cyst, was found on the surface of the kidney. The size of the cysts was 0.5 ± 0.08 mm (n=10). Only 16.7% of infected fish showed cysts on the kidney through the study period. Prevalence of *Myxobolus* sp. infection was examined 8%, 2%, 14%, 6% and 26% in December 2018, January, February, March and April 2019, respectively (Fig.1). The highest prevalence of *Myxobolus* sp. was 82% in August and followed by 64% in July, 54% in June and 62% in May 2019. The mean intensity of *Myxobolus* sp. was Stage 3 in July and August and the remaining months fluctuated between Stage 1 and Stage 2 respectively (Fig. 2). The total highest prevalence infection (51.6%) was observed on fish over 8.1cm group. The total lowest prevalence infection was observed on fish, under 4cm group in the present study (Fig. 3).

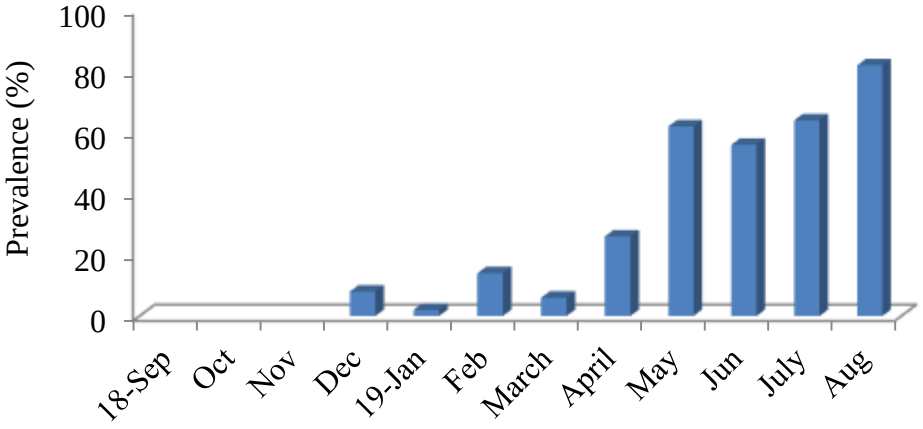

Figure 1 Prevalence of *Myxobolus* sp. in the kidneys of *Cirrhinus mrigala* during the study period, from September 2018 to August 2019.

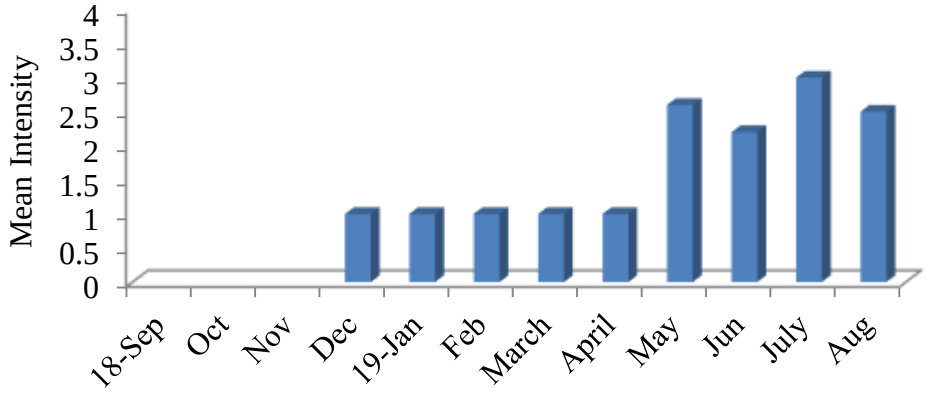

Figure 2 Mean intensity of *Myxobolus* sp. in the kidneys of *Cirrhinus mrigala* during the study period, September 2018 to August 2019.

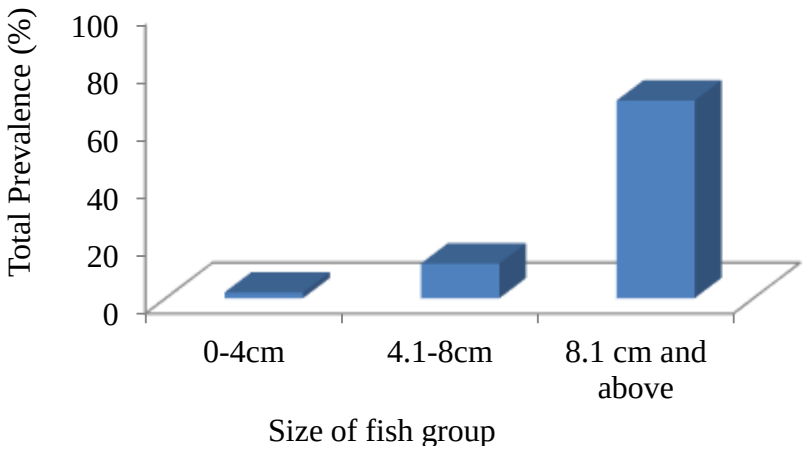

Figure 3 Total prevalence of *Myxobolus* sp. infection in the kidneys of *Cirrhinus mrigala* in relation to fish sizes.

Histopathological study of the kidneys

Pathogenesis in kidneys caused by *Myxobolus* sp. included black pigmentation, deformity of Bowman's capsule, necrotic in renal tubules and cysts of *Myxobolus* sp. in the infested kidneys of the fish (Plate 2, A). Most of the large plasmodia including mature spores and pathological lesions were also encountered in the fish kidneys. Aggregations of inflammatory cells were seen between renal tubules and the inflammatory response is indicated by the red colored tissue because of the effect of excessive erythrocytes (Plate 2, B). Pronounced change in the kidneys of fish included increasing gap between glomerulus and Bowman's capsule and shrinking of tubular lumen were observed under microscope in cross sections (Plate 2, B). The myxosporean infested kidneys showing distinct canalculi within the tissue, proliferation of Bowman's capsule and in some places necrotic renal tubules were also noted (Plate 2, C). In infested kidney cells, nucleus looks blurred due to *Myxobolus* sp. infestations so the membrane cannot be seen clearly. Dilation in the capillaries and vacuolar degeneration in the epithelium of renal tubules were observed (Plate 2, C). The infested kidney tissue showed the prominent circular vacuolar spaces filled with damaged cells necrosis in the tissue. The necrotic in kidney cells caused by parasitic infestations and abnormal increasing the haematophoitic tissue were seen in the kidney of fish (Plate 2, D). The kidney tissue showing deformity of Bowman's capsule and distal and proximal renal tubules were observed. In renal tubules, swelling of epithelial cells and large vascular formation were observed.

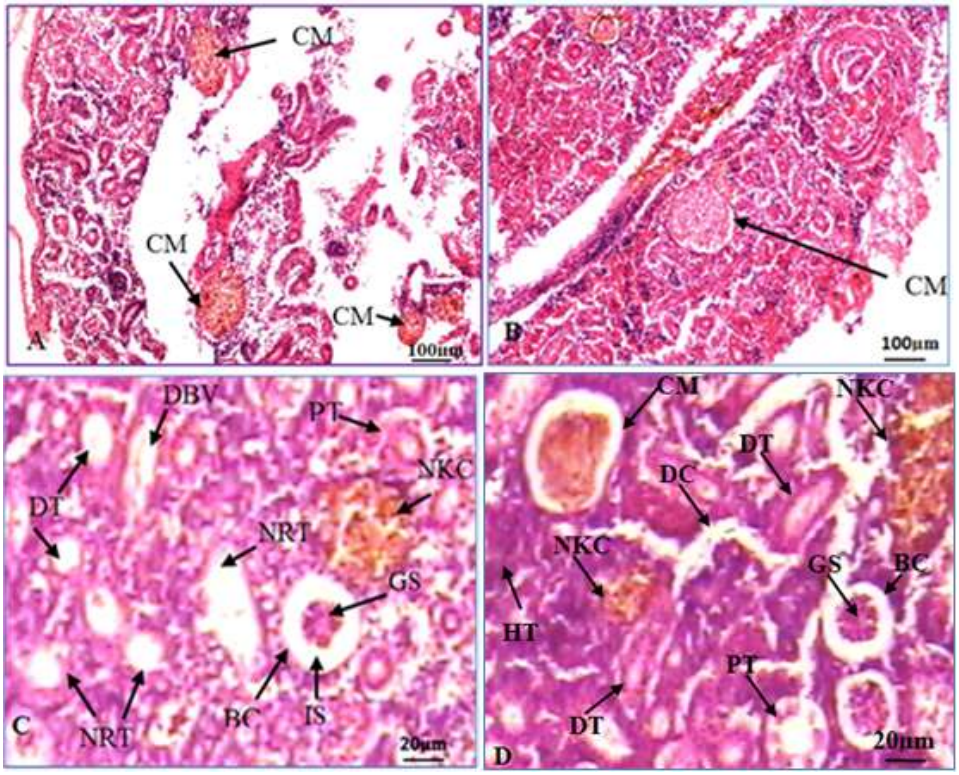

Plate 2 Pathogenesis in kidneys of *Cirrhinus mrigala* showing infested conditions under histopathological finding

(A) Fish kidney attached by *Myxobolus* sp. cyst and aggregations of inflammatory cells (CM=Cyst of *Myxobolus* sp.)

(B) Cyst in the kidneys tissue containing mature spores of *Myxobolus* sp. (CM=Cyst of *Myxobolus* sp.)

(C) Dilation in the capillaries of renal tubules (NRT=Neurotic renal tube, DBV=Degenerative blood vessels, DT=disorganized tubules, NKC=Necrotic kidney cell, GS=Glomerular Shrinkage, IS=Increase Space between glomerulus and Bowman's capsule, BC=Bowman's capsule, PT=Proximal tube)

(D) Deteriorated and necrotic kidney caused by *Myxobolus* sp. infestations (DT=disorganized tubules, PT=Proximal tube, NKC=Neurotic Kidney cell, GS=Glomerular Shrinkage, BC=Bowman's capsule, HT=Hematopoietic tissue, DC=Distinct canalculi, CM=Cyst of *Myxobolus* sp.)

Discussion

The present study was conducted to assess the incidence and parasite infestation in the kidneys of *Cirrhinus mrigala* with respect to different months. *Myxobolus* sp. was isolated and identified from the fish samples collected from Yezin Fishery Station, Nay Pyi Taw Region. *Myxobolus* sp. is the predominant species group within the phylum Cnidarian. Most of the species infect primarily fish, both freshwater and marine species and a few numbers of species were found in amphibians (Lom and Dykova, 2006).

Myxobolus species were recorded in the kidneys of *Cirrhinus mrigala.* A total of 112 nominal species were described for *Myxobolus sp.* (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* infected in scales of *Cirrhinus mrigala* (Eiras *et al*., 2014). However, length of polarcapsules 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* cultured in Mandalay, Kantawgyi Lake which is reported by (Pa Pa Win, 2007).

Yokoyama *et al.*, 2014 discussed that although of Myxosporean are simlar, the species are assumed to be different if the host fish species and infection sites are different. *Myxobolus* can be identified by the morphological characters of the spores and the location and size of plasmodia. However, this technique is inconsistent due to many other biological features, such as life cycle, morphology of myxospores and actinospores or host and tissue preferences. Moreover, the morphological classification is artificial and does not reflect phylogenetic relationships reacquired from molecular data according to recent analyses (Fiala, 2006). The classification of myxosporeans recorded in the present study should be expended to phylogenetic analyses because molecular biological methods have become increasingly applied in parasitological studies.

High prevalence infection was found from August 2019 when the fish was about 1year 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. However, their finding is based on the Myxozoan parasites recorded in the external organs such as skin, gills and fins. For internal organs, Myxosporeans parasite should need infection time to penetrate to the target organs. Gastrointestinal myxosporeans, *Enteromyxum* spp., known to cause severe disease in numerous species of cultured marine fishes globally showed high prevalence of infection when the fish are more than 1year old (Yanagida *et al*., 2006). It is assume that Myxospreans infection in internal organs takes time.

In the present study, the kidneys showed distinct black melanin pigmentation, necrosis of nephric tubules, vacuole formation and enlargement of Bowman's capsule. Similar, small early developmental stages of the myxosporean parasites, dilation of blood vessel and hyperplasia of blood cells were observed in the kidneys of mrigal collected in Kantawgyi Lake (Pa Pa Win, 2007). The necrosis of the renal tubules affects the metabolic activities and promotes metabolic abnormalities in fish (Yokote, 1982). Therefore, it is a target organ in many diseases due to the

affinity of the organ for circulating particulate antigens. Kidneys are important organs in excretion and regulation of water and salt concentrations within the fish body. Pathological signs in kidneys such as necrotic kidney tubules, hemorrhage and vacuolation were observed. In this finding, the kidneys showed the prominent circular vacuolar spaces filled with damaged cells and necrosis of the tissue.

Since the fish in Yesin Fishery Station was cultured in earthen pond, it is difficult to estimate the mortality due to infection. However, according to the damage of kidney reveled in the histological studies of the present finding indicated the negative effect of *Myspobolus* sp. to Marigal fish cultured in Yezin Fishery Station. Therefore, *Myxobolus* sp. recorded in the present study are threatening species for fish hatcheries. In addition it can have impact on natural population of *Cirrhinus mrigala* in near future. *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.* Management practices and pond hygiene should be adopted in operation systems of Yezin Fishery Station.

Conclusion

The kidneys of *Cirrhinus mrigala* collected from Yezin Fishery Station is infected with *Myxobolus* species. High prevalence of infection was recorded from May to August, 2019. Black pigmentation, distinct canalculi within the tissue, proliferation of Bowman's capsule and in some places necrotic renal tubules were noted in the infected tissue. Therefore, management practices and pond hygiene should be adopted in operation systems of Yezin Fishery Station for the production of more hygienic and successful yield.

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