Investigation on Some Diseases of Shortfin Eel *Anguilla bicolor bicolor* McClelland, 1844 From Fattening Concrete Tank

Ca Tin Hoi[1], Khin Mar Kyi[2], May Thu Rein Oo[3], Kay Lwin Tun[4]

Abstract

Shortfin eel specimens *Anguilla bicolor bicolor* were collected from the fattening concrete tank. Those specimens with white patches were considered infected with parasites and the others without the white patches were considered non-infected. A total six fin eels were observed for the present work, of these, three infected eels and the other non-infected eels were investigated for fungal, parasite and histopathological changes of infected eels. For the study of fungal infection, mucous from skin was obtained from infected fin eel; it was inoculated and cultured in the Sabouraud Dextrose Agar (SDA) culture medium. According to the fungal development and its morphology, it was identified as genus: *Aspergillus*. In the case of non-infected fin eels the fungus was not found. Similarly, in the study of infected fin eels three kinds of parasite were observed and recorded, these genera were: *Myxobolus*, *Myxidium* and *Trichodina* respectively. In the examination of histopathological study, in order to find parasites, samples were taken from seven places of external and internal organs. These were skin, gill, intestine, liver, spleen, swim bladder and gall bladder. Out of seven organs, some changes of structure were found only in the skin. In the non-infected eels, adipose tissue layer under the skin was wider and in the infected eels it was narrow, showing it was thin infected layer and not fatty, indicating, it was not normal. In addition to these, the other symptom was some damages of small oval scales in the infected eels. In other organs, no differences were found between two samples of eels. These finding were fully supported by histological slides with labels. This finding was first record in Myanmar.

Key words: Anguilla bicolor bicolor, parasite, fungal infection, histopathology

Introduction

Eel *Anguilla bicolor* is a catadromous fish that have high economic value due to the high nutrient content. *Anguilla* eels are important food fish which include longfin eel, shortfin eel, and Japanese eel (Monticini, 2014). In the genus *Anguilla*, there are 19 species of freshwater eel (FishBase, 2019).

Freshwater eels of the family Anguillidae are globally distributed, inhabiting in the fresh waters, brackish estuaries and coastal waters of more than 150 different countries (IUCN, 2014). They are easily recognizable by snake-shape elongated body. Depending on the habitat, their body coloration is greenish brown or black color. They spend most of their life time in the freshwater but sometimes returning to the sea for breeding (Regan, 1922).

In Myanmar, *Anguilla bicolor* is found along the Ayeyarwaddy region (Thanda Tun, 2004). *Anguilla bicolor* is one of the imports items for export market (MPEA, 2018). They have been tried to culture in captive condition due to the demand of export market. *A. bicolor* has been cultured in Laboratory of Aquatic Bioscience as experimental ponds since 2017 (Thiri Aung, 2017).

Eels have been caught for trade and consumption. Demanding for fish availability in the global market has been increasing drastically nowadays (Nijman, 2015). Eels are produced mainly in three Asian countries such as Indonesia, Philippines and Myanmar (Muthmainnah *et al*., 2016).

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Disease is one of the most serious limiting factors in aquaculture. Diseases are related to nutritional deficiency, secondary infection or parasitic infections (Southgate, 1993). Many disease outbreaks of captive fish stocks are associated with stressful conditions such as poor water quality, excessive crowding or inadequate nutrition (Banrie, 2013). In eel farming, diseases can be caused by under certain stressful conditions and also high stocking densities (Haenen *et al*., 2011).

Secondary infections can be caused by fungi, bacteria or viruses, which ultimately cause the death of fish. Parasitic infections are not only the direct cause of death of fish but also the presence of wounds in the fish's body (Amrullah *et al.*, 2019).

Fungi have the ability to grow on and in both invertebrate and vertebrate animals (Carris *et al.*, 2012). Fungal infections are mainly caused by immune suppression. They can also attack all the ages of fish. Poor management of fish ponds can also increases the chances of fungal infection *Aspergillus* species in fish (Niaeem *et al.*, 2015).

Parasites are found in fish culture farms are *Trichodina*, *myxidium*, and *monogenia copepods*. *Trichodina* is one of the most common ciliates present on the skin and gills of fish. Low numbers are not harmful, but when fish are crowded or stressed, and water quality deteriorates, the parasite multiplies rapidly and causes serious damage (Klinger and Floyd, 1998).

Myxozoans are economically important group of microscopic metazoan parasites, which cause disease in a large variety of commercially important fishes (Boreham *et al.*, 1998). Nowadays, research on myxozoan fish parasites are get momentum in the field of ichthyoparasitology. Many myxosporeans which have been reported from freshwater as well as marine fishes are highly pathogenic (Lom, 2006).

Histopathology has been used to study the cellular basis of infectious and non-infectious diseases. Histopathology typically involves a biopsy, which is a procedure involving taking a small sample of tissue. Examination of tissue for the identification of infectious organisms is a very important diagnostic tool. Histology is utilized over many years like human and veterinary pathology (Ashley, 1975, Ribeln and Migaki, 1975).

A fish skin lesion is generally a change in color or an opening in the skin or fins of a fish. Lesions can occur on the surface of the skin, and they can go deeper into the muscle or organs of a fish (Hale *et al*., 2016). In this study, in the fattening concrete tanks behind the laboratory of Aquatic Bioscience, shortfin eels with disease symptoms are occurred especially on their skin. Other lesions originate beneath the surface of a fish’s skin and push outward, spreading through to the surface. There are many causes of lesions. Skin lesions can develop when a fish is wounded by another animal or is injured from nets or traps. In this study, *Anguilla bicolor bicolor* cultured in fattening concrete tank, has been found disease symptom such as white patches on the skin. Not done in Myanmar.

The present research is thus conducted with the following objectives;

- to investigate the fungal infection on the skin of infected eel
- to study the external and internal parasites of shortfin eels culture in fattening concrete tank
- to compare the histopathology differences of skin and internal organs of non-infected and infected *Anguilla bicolor bicolor*

Materials and Methods

Study area

Anguilla bicolor bicolor was cultured in the fattening concrete tank behind the Laboratory of Aquatic Bioscience, Department of Zoology, University of Yangon (Fig. 1 and Plate 1).

Study period

Study period lasted from December 2018 to August 2019.

Collection of fin eel specimens

The total specimens of six shortfin eels were collected from the fattening tank; which were three individual with infected eel while three individual with non-infected eel. The total length and body weight of infected eels and non- infected eels were measured (Plate 2, A, B and C). The infected eel unknown had symptoms of external skin lesion in head, tail and abdomen region (Plate 2D).

Equipment and Media Preparation of Fungal Culture

For culture, 65g of Sabouraud Dextrose Agar (SDA) were dissolved in 1L of distilled water. Sabouraud Dextrose Agar 20mL was poured into the pertidish and prepared for fungal culture (Plate 4).

Fungal Isolation

Small pieces of specimens were taken from infected area of mucus from the skin lesions with the help of sterile inoculation loop and put on Sabouraud Dextrose Agar plate. Each plate was labelled with sample number, date and name of organism. The agar plates were incubated at 27°C - 30°C for three days in inverted form until a circular fungal mat developed. Isolation was done in Laminar flow air cabinet to avoid contamination. A piece of fungal was take placed on the glass slide, added with distilled water and examined unstained microscopically for fungal hyphae. After three days of culture period, mat from the petridish were collected for sub culture and cultured again (3 replications) on Sabouraud Dextrose Agar (SDA) at room temperature for 7 days.

Identification of eels and diseases

Shortfin eels, parasites and histology were identified according to Hine *et al*. (2000) and Mumford *et al*. (2007) and supplemented by Abdelmonem, (2009), Monticini (2014), and Mokhtar (2017). Fungal were identified Robert *et al*. (2007), Samson *et al*. (2011) and Ajello (2013).

Investigation of parasites

At first for parasites, mucous scraped from fins, skin and gills removed from the branchial cavity and were placed in the petridish for microscopic examination. Tissues were placed on the glass slide, added with saline solution (0.9% Na CL solution) covered with coverslip and examined with compound microscope (Olympus - CX31). And then, internal organs, gallbladder, swim bladder, liver, intestine, stomach, brain and spleen were removed with the help of sharp scalpel, forceps and then kept in separate petridish (Plate 3).

Calculation of mean intensity and prevalence of parasites

Both ecto and endo parasites for the description account were measured by a calibrated ocular micrometer.

The following analyses were carried out after Margolis *et al.*, (1982):

$$\text{Prevalence} = \frac{\text{Total numbers of infected eels}}{\text{Total numbers of eel hosts examined}} \times 100\ \%$$

Calculation of intensity of myxosporean parasites

Intensity of infection was categorized into five stages according to Bachere *et al.*, (1982) and Culloty *et al.*, (1999).

Stage (I) : 1-20 parasites observed within 5-min of screening under 100 x magnification

Stage (II) : 21-40 parasites observed within 5-min of screening under 100 x magnification

Stage (III) : 41-60 parasites observed within 5- min of screening under 100 x magnification

Stage (IV) : 1-100 parasites in all field of region observed immediately in screening under 100 x magnification

Histology preparation for internal organs

To understand the histopathological changes of infected eel, 1 cm^3 thickness of tissues sample (gill, skin, intestine, liver, swim bladder, spleen, gall bladder) were taken and fixed in 10% neutral buffer formalin for 3 weeks isolated. Then the samples were placed in an automatic tissue processor for dehydration, clearing and infiltration. The tissues embedding were carried out in metal molds and covered with cassettes. The sections were stained with haematoxylin and eosin stains (Appendix II). Finally, the sections were mounted with Canada balsam and cover by a cover slip. Then the slides were examined under a light compound microscope (Olympus CX 31). Pathological observations were made from the slides and photographs and compare between infected and non-infected eels (Plate 5).

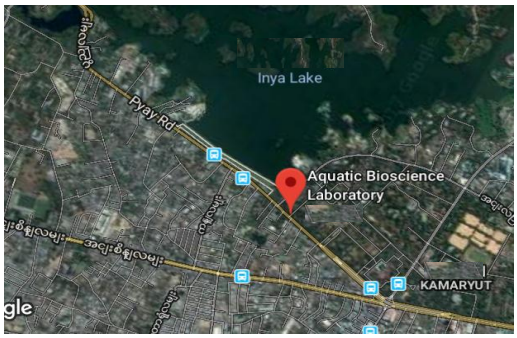


Fig. 1 Location map of the study area
(Source: Google Map 2019)

Plate 1 Study site: Shortfin eel, fattening concrete tank

A. Catching the eel with net

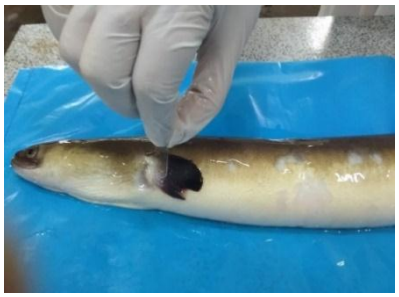
B. Measurement of eel

C. Weighing eel with the digital balance

D. Infected eel with white patches

Plate 2 Procedure for identification of infected eel

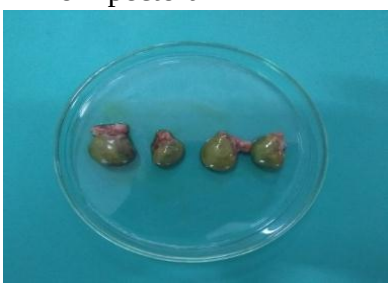
A. Mucous scraped from skin



B. Mucous scraped from pectoral fin

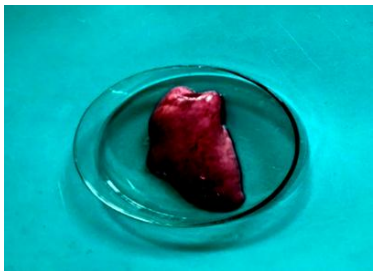
C. Dissected internal organs

A. Gill pieces



A. Gall bladder

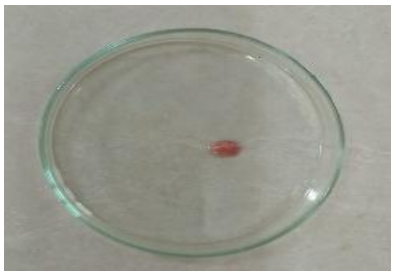
A. Swim bladder



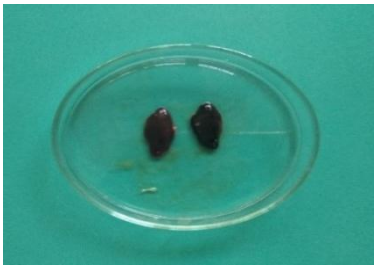
D. Liver

E. Intestine

F. Stomach



G. Brain



H. spleen

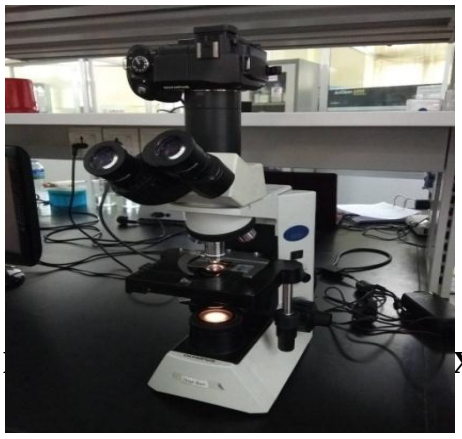
Plate 3 Examination of parasites from various external and internal organs

A. Fungal isolation from white part

B. Container with SDA

C. Autoclaved equipment

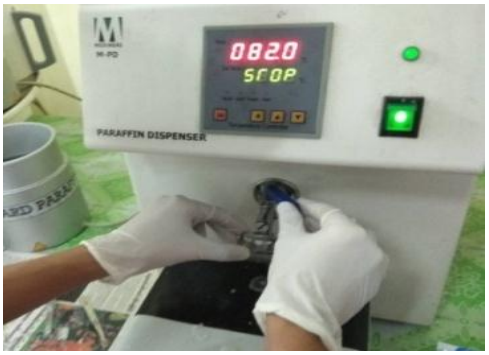
D. Pouring of media into petridish



X 31)

Plate 4 Preparation for fungal isolation

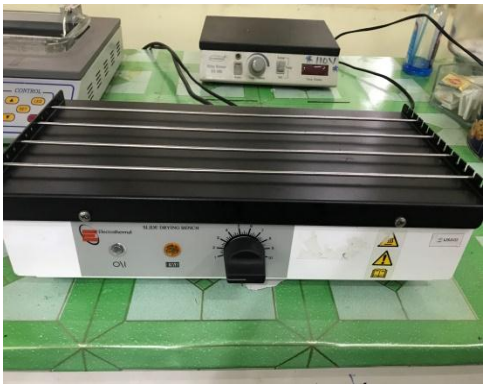
A. Automatic tissue processor



B. Paraffin dispenser

C. Semi- automatic rotary microtome

D. Water bath



E. Slide warmer

G. Staining jar

Plate 5 Histological procedure

Results

In the present study, three infected eels and three non-infected eels were collected from the fattening concrete tank. The size of total length and body weight of infected eels were (70.69±1.93) cm & (886.66±51.31) g. The size of the total length and body weight of non-infected eels were (72.39±3.36) cm & (913.33±66.58) g.

According to observation, one fungal infection of *Aspergillus* species; three species of parasites: *Myxobolus*, *Myxidium* and *Trichodina* were found from infected eel *Anguilla bicolor bicolor*. However, neither fungal nor parasitic infections was recorded in non-infected eels.

Systematic Position of *Aspergillus* species Micheli, 1729

Phylum	-	Ascomycota
Class	-	Eurotiomycetes
Order	-	Eurotiales
Family	-	Trichocomaceae
Genus	-	*Aspergillus*
Species	-	*Aspergillus* sp. Micheli, 1729

Morphology of fungal colony

On Sabouraud Dextrose Agar, colonies are white at first granular to cottony, velvety, or powdery, showing various shades of green, most commonly a blue-green with a white apron at a margin. The pure culture of fungi was obtained from preliminary isolated fungi from fish. Pure culture produced uniform colonies of same colour, spreading all over the culture plate indicating the growth of *Aspergillus* sp. The growth of fungal colony is showed in (Plate 6 A).

Microscopic morphology of *Aspergillus* sp.

On SDA, colonies are typically blue-green with a suede-like surface consisting of a dense felt of conidiophores. Conidial heads are typically and uniseriate. Conidiophore stipes are short, smooth-walled and have conical-shaped terminal. Conidia are produced in basipetal succession forming long chains and are globose to subglobose, green and finely roughened (Plate 6 B).

Examination of ecto and endo parasites from *Anguilla bicolor bicolor*

Myxobolus species, *Myxidium* species and *Trichodina* species were collected from infected eels (Plate 7).

Systematic position of *Myxobolus* sp. Butschli, 1882

Host	-	*Anguilla bicolor bicolor*
Site of infection	-	skin
Phylum	-	Cnidaria
Class	-	Myxozoa
Order	-	Bivalvulida
Family	-	Myxobolidae
Genus	-	*Myxobolus*
Species (1)	-	*Myxobolus* sp. Butschli, 1882

Description of *Myxobolus* sp. Butschli, 1882

The myxozoan spores having two polar capsules, with or without iodinophilus vacuoles and generally two sporogenic nuclei. The spores were rounded in valvular view and biconvex in sutural view, and the shell valves were smooth and without projections.

Length of spore were measured 7.35±1.24µm (n=10) and width of spore 6.59±0.99µm (n=10). Polar capsules were little elongated, different in size, slightly converging anteriorly. Regarding the length of left polar capsule, it ranges from 3.45±0.65µm (n=10) and regarding its width it measures 1.91±0.3µm (n=10). Length of right polar capsule were 3.58 ±0.74µm (n=10) and width of right polar capsule 1.94±0.38µm (n=10). Distance between polar capsule

was 0.73±0.08µm (n=10) (Plate 7 A). Prevalence of parasite was 60% and mean intensity was 1 (Table 1).

Systematic position of *Myxidium* sp. Butschli, 1882

Host – *Anguilla bicolor bicolor*

Site of infection - gallbladder

Phylum - Cnidaria

Class - Myxozoa

Order - Bivalvulida

Family - Myxidiidae

Genus - *Myxidium*

Species (2) - *Myxidium* sp. Butschli, 1882

Description of *Myxidium* sp. Butschli, 1882

The spores were usually ellipsoidal in with rounded or bluntly pointed ends and almost spherical. Polar capsule are spherical and equal. Granular sporoplasm is located between polar capsules.

Length of spore were measured 11.25 µm ±1.3µm (n=10) and width of spore 78±1.4µm (n=10). Two polar capsules, situated at opposite ends of spore, near-spherical, equal sized. Regarding the length of left polar capsule 50µm (n=10) and regarding its width it measures 50µm (n=10). Length of right polar capsule were 50µm (n=10) and width of right polar capsule 50µm (n=10). Distance between polar capsule was 55±1.05µm (n=10) (Plate 7 B). Prevalence of parasite was 60% and mean intensity was 3 (Table 1).

Systematic position of *Trichodina* sp. Ehrenberg, 1831

Host - *Anguilla bicolor bicolor*

Site of infection - gill

Phylum - Ciliophora

Class - Ciliata

Order - Mobilina

Family - Trichodinidae

Genus - *Trichodina*

Species (3) - *Trichodina* sp. Ehrenberg, 1831

Description of *Trichodina* sp. Ehrenberg, 1831

The characteristic feature of *Trichodina* sp. is its skeletal ring with radially arranged denticles that are readily apparent when viewed .These organisms have a saucer-to-bell shaped body that is about 48.23±6.26µm in diameter and denticles have 18-20. In numerous 35.04±3.81µm denticulate rings, a highly developed basal adhesive disc, and an adoral zone of cilia arranged in a spiral and are motile. They have a sucking disc which they use to attach to their host (Plate 7 C). Prevalence of parasite was 100 % and mean intensity was one (Table1).

Histological comparison of internal and external organs of non-infected and infected *Anguilla bicolor bicolor*

Comparison of histological observations of gills in non-infected and infected *Anguilla bicolor bicolor*

Histological observations on gills of *Anguilla bicolor bicolor* showed the normal gill structure. Gills situated in branchial chamber on either side of the body in fishes. Each gill has a gill arch with double row of elongated laterally projecting gill filaments. These filaments are flat and leaf like and join at the base on gill rackers by a gill septum. Numerous semicircular, leaf like projections are lined up along both sides of the primary gill lamellae called as secondary gill lamellae.

In the present study, non-infected and infected gills structures are similar. Therefore, histological structures of gills were not differences (Plate 8).

Comparison of histological observations of skin in non-infected and infected *Anguilla bicolor bicolor*

Anguilla eels skin comprising a multilayered, stratified epidermis and a compact, collagenous dermis and hypodermis or subcutaneous layer. The epidermis and dermis are separated by a basement membrane. The type of mucus-producing cells is in the epidermis. Small oval scales present, embedded in skin and arranged in a basket-weave pattern. The myomeres are separated from each other by collagenous myosepta.

Adipose tissue layer is found in the layer between the skin and the muscles. Adipocytes fat cells contain a large lipid droplet and connective tissue septa. Adipose tissue layer is divided into two subtypes, white and brown fat. The present work is white fat. White fat is widely distributed and it represents the primary site of fat metabolism and storage (Plate 8).

In the present study, the histological structures of skin are differences in both non-infected and infected eel. Healthy adipose tissue layer is wider in non-infected eels while narrow and separated muscular layers were found in infected eels. Small oval scales are damage in infected eels (Plate 8).

Comparison of histological observations of intestine in non-infected and infected *Anguilla bicolor bicolor*

The histological sections of the intestine of the *Anguilla bicolor bicolor* exhibited simple columnar epithelium, lamina propria, submucosa, tunica muscularis and serosa as well defined layers in the order from internal to external surface of the intestine. The epithelial cells appeared as long and thin columnar cells and the mucosa layer is folded into "villi" fingerlike projections. Villus is seen only in small intestine and it contains absorptive cells (enterocytes). A few goblet cells were located between the epithelial cells. The serosa consists of connective tissue. The mucosa was highly branched and folded. The lamina propria consisted of loose connective tissue, without the presence of glands, but with some striated muscle fibers from the circular muscle layer toward the folds. The muscle layer was formed by striated muscle fibers.

In the present study, the histological structures of intestine are the same. Therefore, there is no difference (Plate 8).

Comparison of histological observations of liver in non-infected and infected *Anguilla bicolor bicolor*

The histological sections of liver from *Anguilla bicolor bicolor* showed the typical structure of hepatic tissue that contains hepatic cells (liver cells or hepatocytes), sinusoids. Bile is secreted by hepatocytes. Hepatic cells were located among sinusoids forming cord like structure known as hepatic cell cords. A large number of blood sinusoids were found in the hepatic mass of these cords. Sinusoids, which are capillaries travelling between hepatocytes. In the present study, the histological structures of both non-infected and infected eels were not differences (Plate 8 continued).

Comparison of histological observations of swim bladder in non-infected and infected *Anguilla bicolor bicolor*

The normal swim bladder wall consists of the mucosa, simple cuboidal epithelial cells and lamina propria, the muscularis mucosa, the sub mucosa, serosa and normal blood vessels. The swim bladder wall was caused mainly by the substantially increased volume of the submucosa; however, in these eels the mucosa and the serosa are also much thicker than usual. The mucous membrane had deep folds, covered by an epithelium. The folds contained dilated vessels of thickened walls. The propria shows branch-like ramifications and is covered by a single epithelia layer composed of markedly elongated columnar epithelial cells. The submucosa has widened and its fibers loosened.

In the present study, the histological observation of swim bladder of healthy and disease symptom structures of are the same. There is no difference in both non-infected and infected eels (Plate 8 continued).

Comparison of histological observations of spleen in non-infected and infected *Anguilla bicolor bicolor*

The spleen of *Anguilla bicolor bicolor* is a dark red organ, located in the peritoneal cavity, adjacent to one of the liver lobes. The spleen consists of red pulp and white pulp within a meshwork of reticular fibers enclosed by a dense connective tissue capsule. The red pulp contained too many sinusoids filled with red blood cells. White pulp mainly contains lymphocytes cells around arteries. The spleen has a capsule and small trabeculae. The capsule composed of one layer including an epithelium of squamous to cuboidal cells. Trabecula is a supporting or anchoring strand of connective tissue. Melanomacrophage of the spleen contain brown and yellow pigments. Venous sinuses can be found throughout the red pulp.

In the present study, the histological structures of spleen were not differences in both non-infected and infected eels (Plate 8 continued).

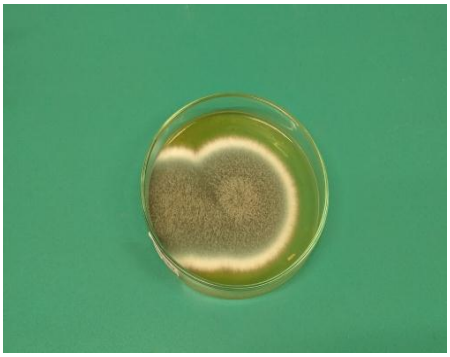
Comparison of histological observations of gallbladder in non-infected and infected *Anguilla bicolor bicolor*

The gall bladder is a sac that is lined with a simple columnar epithelium and smooth muscle. The gallbladder has a mucosal layer, thrown into folds, but not true villi. This mucosal layer is the epithelium, and the connective tissue underneath the epithelium, called the lamina propria. The gall bladder also has no submucosa. It has a thin muscularis externa with interlacing, non-distinct layers. There generally are no goblet cells, as the lining cells secrete small amounts of mucus. When the gall bladder is empty, this layer is extremely folded. When full, this layer is smoother but still has some short folds. Lamina propria is composed of loose connective tissue.

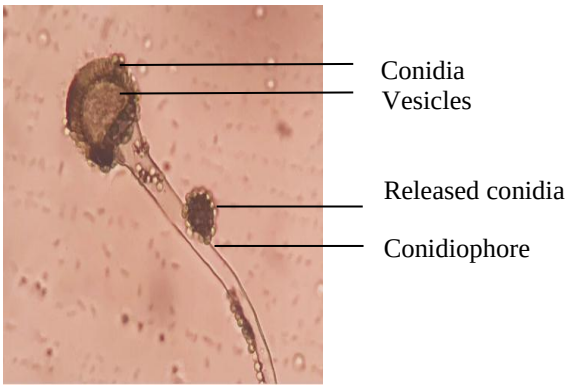
In the present study, the histological structures of gall bladder were not differences in non-infected and infected eels (Plate 8).

Table 1 Mean intensity and prevalence of parasites

Infected eel (n=3)			
Parasites	**Detected fish**	**Prevalence %**	**Mean intensity**
Myxobolus	2	60	1
Myxidium	2	60	3
Trichodina	3	100	1

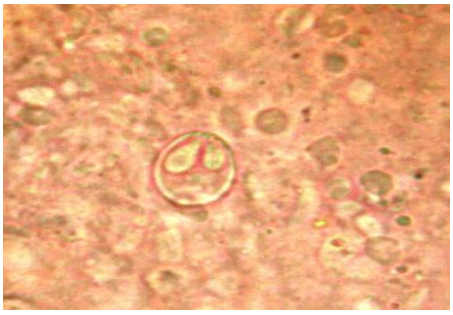


A. Fungal colony

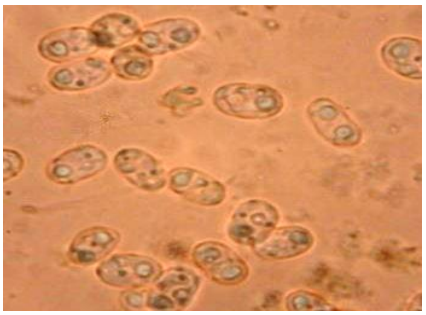


B. *Aspergillus* sp.

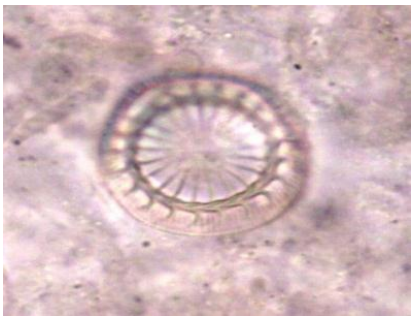
Plate 6 Morphology of fungal colony and *Aspergillus* sp.



A. *Myxobolus* sp. (1000X)

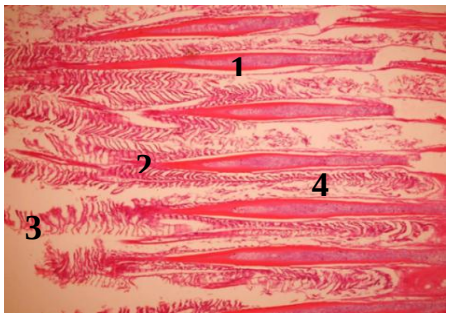


B. *Myxidium* sp. (1000X)

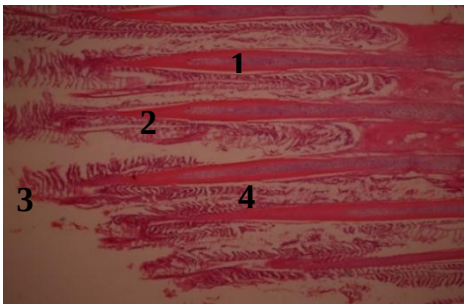


C. *Trichodina* sp. (1000X)

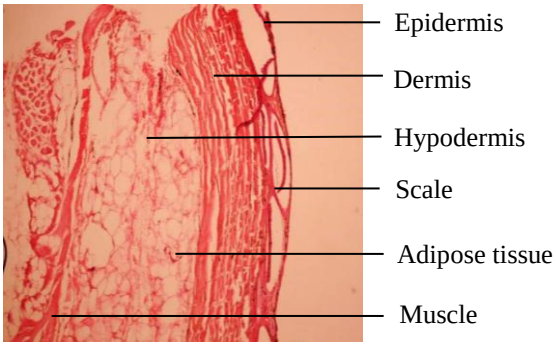
Plate 7 Parasites infection in *Anguilla bicolor bicolor*



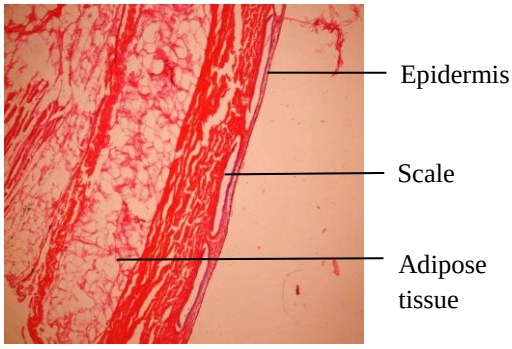
A. Non-infected gills (100X)
1. Cartilage
3. Secondary lamellae



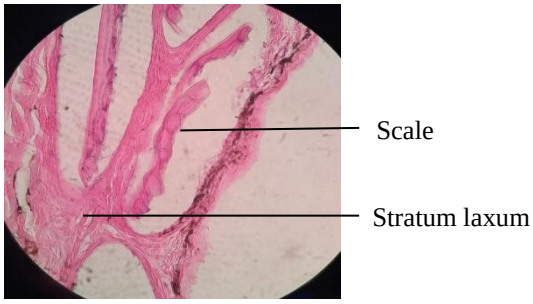
B. Infected gills (100X)
2. Primary lamellae
4. Inter lamellae



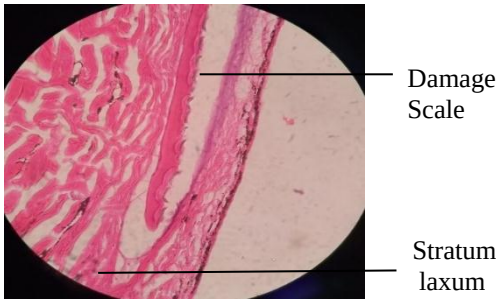
C. L.S non-infected skin layers (40X)



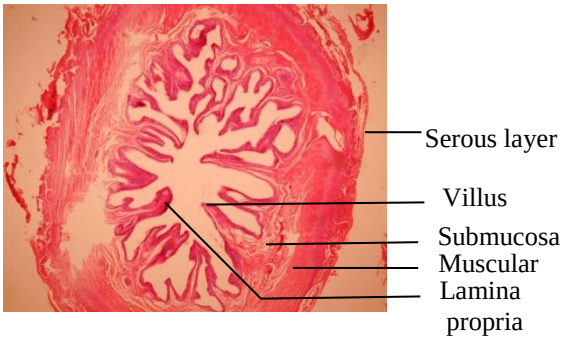
D. L.S infected skin layers (40X)



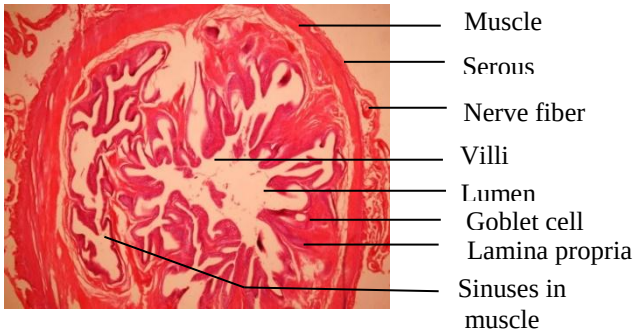
E. Scale embedded in skin (non) (400X)



F. Scale embedded in skin (infect)(400X)

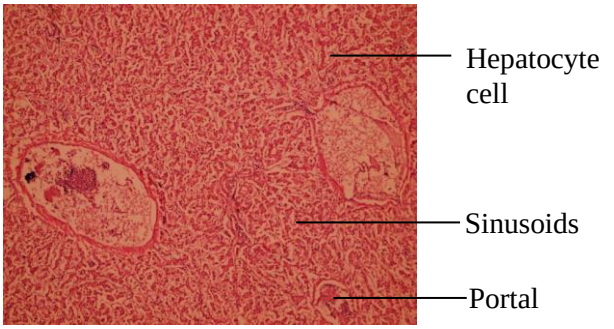


G. T.S Intestine of non-infected eel (100X)

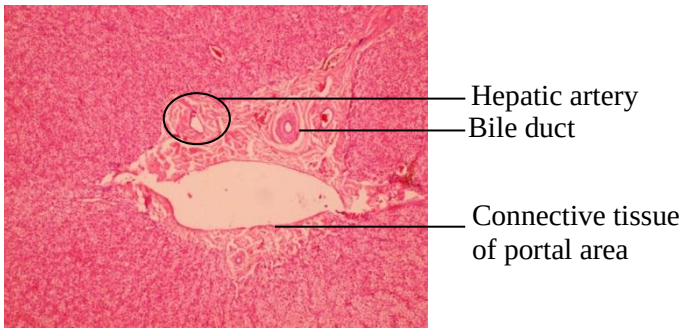


H. L.S Intestine of infected eel (100X)

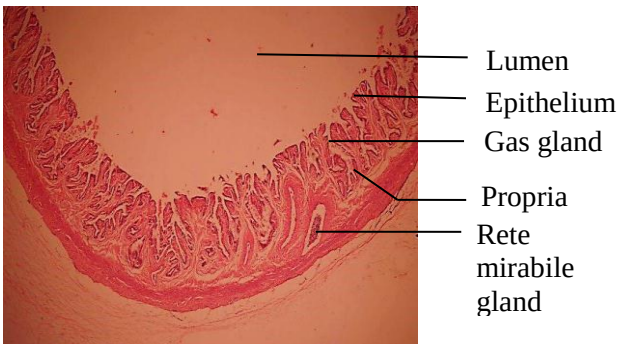
Plate 8 Histopathological changes of gills and internal organs



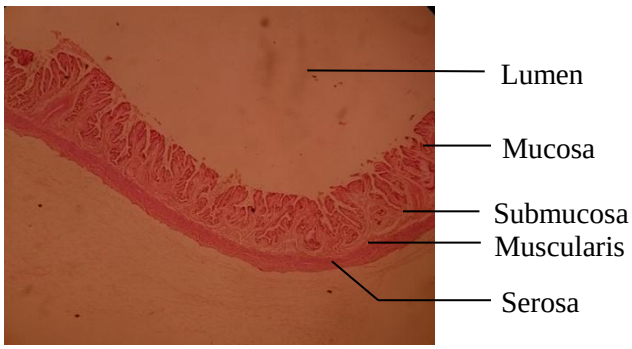
I. Liver of Healthy eel (40X)



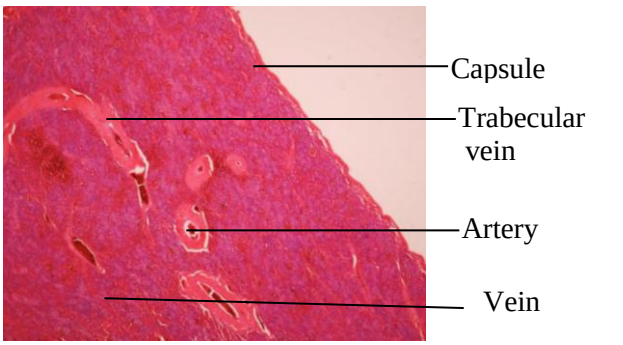
J. Liver of infected eel (40X)



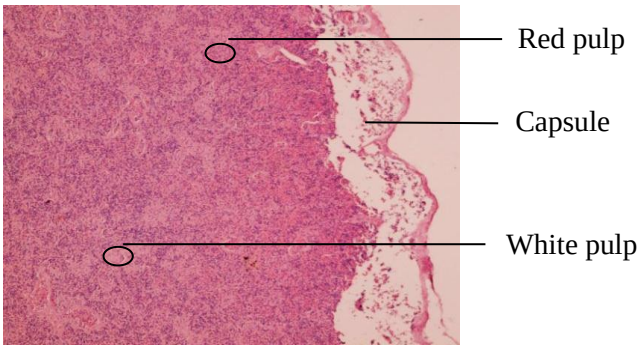
K.T.S Swim bladder of non-infected eel (40X)



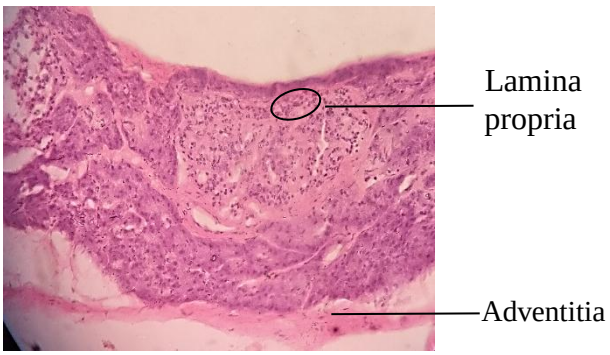
L. T.S Swim bladder of infected eel (40X)



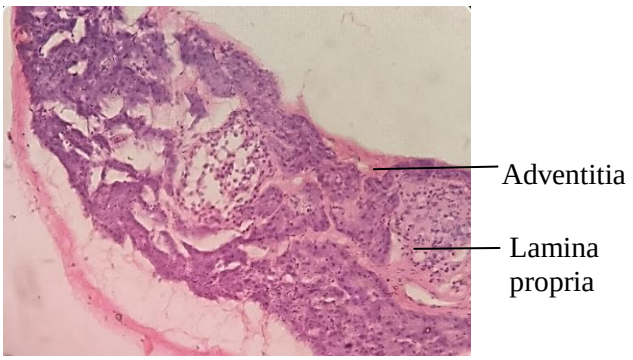
M. T.S Spleen of infected eel (100X)



N. T.S Spleen of non-infected eel (100X)



O. Gall bladder of non-infected eel (400X)



P. Gall bladder of infected eel (400X)

Plate 8 Continued Histopathological changes of internal organs

Discussion

During the study period, a total number of six *Anguilla bicolor bicolor*; three non-infected eel and three infected eel, were examined for fungal and parasitic infections.

Fish can suffer from various fungal diseases. Most fungal infections invaded on external tissues and only few fungal infections affect the internal organs of fish (Verma, 2008).

In white patches one fungal infection *Aspergillus* species detected in the present study. Kumari and Kumar (2015) studied the analysis of fungal infection in some economically important freshwater fishes including freshwater eels, *Anguilla* sp. They found that two species of fungal infection, *Aspergillus fumigatus* and *Aspergillus niger* in the skin of *Catala catala* and *Labeo rohita*.

Podeti and Benarjee (2017) described that the studies on histopathological mycosis variation of *Channa* species found infected with *Aspergillus* sp. Amrullah *et al.,* 2019 examined the parasites and fungi characteristics on short finned eel *Anguilla marmorata* in Indonesia. They have been studied parasites and fungi that infected eel (*Anguilla* sp.). According to the present study, eels with white pout were disease symptom of *Aspergillus* infection.

No infection is recorded in non-infected eels while 3 parasitic infections were found in infected eels. The fish infected with primary infection are susceptible to secondary infection (Ogawa *et al*., 2015). Therefore, it might be assumed that fish infected with fungal infection has been introduced easily by parasitic infection.

Ecto-parasites are parasites that can live outside the body like on the surface of the body, fins and gills (Faisal and Gunanti, 2015). Ectoparasitic protozoan diseases are the most important parasitic diseases of cultured fishes (Woo, 1995).

Three parasites, *Myxobolus*, *Myxidium* and *Trichodina* were recorded in *Anguilla bicolor bicolor* in present work. This is first report in Myanmar for parasitic infections of *Anguilla bicolor bicolor*.

Nagasawa, *et al.,* 2017 recorded *Myxobolus* sp. was found in only skin of eel. Kristmundsson and Sigurður, 2007 studied the *Myxobolus* was detected from fins. However, Ahmed and El-Ashram, 2007 stated that the *Myxobolus* infection was noticed in the skin and whitish nodules of eel fins. In the present study, *Myxobolus* sp. was observed in skin of *Anguilla bicolor bicolor*. Site of skin infection are similar with previous authors but fin was not similar present study.

The *Myxidium* species was originally described from the kidney of the European eel, *Anguilla anguilla* (Cepede, 1906). This species is a widely distributed parasite, infecting numerous species of eels, in multiple organs (Mark and Arni, 2018). Myxidiosis is a one of the most important expanding protozoan diseases of cultured eels caused by *Myxidium* species (Le Breton and Marques, 1995).

Globally, numerous species of anguillid eels have been reported to have similar *Myxidium* spp. infections, many of which have been distinguished using morphological features such as spore size and number or lack of valvular striations (Fujita, 1927 and Hine, 1975). Site of infections in eels are skin, gill, kidney and stomach (Ishii, 1915). In present study, *Myxidium sp.* is found in the gall bladder of infected eel. Not many authors reported that this species can be found in many fish, especially in their gall bladder but also (Mark and Arni, 2018) findings are not similar with site of infection.

Trichodiniasis is one of the major protozoan diseases found in fish Worldwide (Faruk, 1974). *Trichodina* species is regularly encountered in wild and cultured eels (Buchmann and Bresciani, 1997). *Trichodina* species was detected in gills and skin scraping from wild and culture eels (Ahmed and El-Ashram, 2007). Kristmundsson and Sigurður, 2007 reported that *Trichodina* species was frequently on the gills of eels. Nagasawa, *et al*., 2017 stated that this species found on the gills. The previous authors and present finding are similar with infection of *Trichodina* sp. on the gills.

Eel culture using the present study is Recirculation Aquaculture system where water is filtrated with filter floss. RAS reduced the transmission of diseases in aquaculture. Therefore, intensity of infection of parasites in present study is very low infection intensity in eels. Due to the low intensity of infection histopathology changes of internal organs of non-infected and infected eel are not differ.

However in skin, the histological structures of non-infected and infected eel *Anguilla bicolor bicolor* was markedly differences. The fat cells separate by muscle layer. Fungal infection recorded in the skin of eels might become critical issue in eels farming in Myanmar. Control management for fungal infection in intensive eel farms should be conducted as further study.

Infected eel and non-infected *Anguilla bicolor bicolor* were examined for fungal and parasitic infections. Although three parasites were recorded from infected eel, intensity of infection was very low. Skin lesions in infected eel *Anguilla bicolor bicolor* are mainly caused by fungal infection.

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