The profile of myxobolus infection in the gill tissue of common carp (Cyprinus carpio L.) strain punten from concrete ponds

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Abstract. One of the successes in cultivation activities is by maintaining the quality of fish and their environment. Common carp (Cyprinus carpio L.) cultivation is one of the leading commodities in various regions, especially East Java. In Indonesia, there are several types of common carp strains, one of the seventh strains in Indonesia is the Punten strain from the Punten, Batu area of Malang. However, several cases show incidents of disease attacks, one of those diseases that often infect fish in Punten is the parasite Myxobolus sp. The observation of tissue lesion was used gills from common carp (C. carpio L.) taken from The Unit of Freshwater Cultivation Management (UPBAT) Punten, Batu Regency. Based on the results of observations, the visible lesion in the gill tissue, among, oedema, haemorrhage, the fusion of lamellae and vacuoles with scores and proportions of damage per field of view (%) in infected fish, respectively are, score 2 moderate (52%), 1 minor (25%), 1 minor (12%) and 2 moderate (18%). Based on the assessment, the scoring results show mild to moderate damage, so it is still classified as early-stage damage or it can also show the fish's ability to recover in a good environment. However, the presence of infectious infections also allows the management of waste and water quality management in each pond, to maintain the environmental health of the common carp.

1. Introduction

Carp (Cyprinus carpio L.) has many varieties, with different characteristics: some are classified as edible fish, while some are ornamental. The edible ones are Punten, Majalaya, Sinyonya, Red, and Taiwan carp, while the ornamental carps are Kancra-domas, Glass, Kum-pay (long-fin), and Koi [1]. One of its several strains is the Punten carp, which originated and was first developed in Punten, Batu, East Java. The good quality and healthy fish show the success of carp cultivation. Even though it is easy to cultivate, carp handling must be done properly, especially the seeds as they are vulnerable to diseases, such as parasites, bacteria, and viruses. The disease can be caused by an unbalanced interaction between fish as the host, water (as the environment), and disease-causing agents (pathogens). Unbalanced interactions lead to stress and make it easier for pathogens to enter the fish body [2]. The optimum quality of water is an absolute requirement for the success of intensive cultivation. Bad water quality can result in a high water acidity (pH) and ammonia, which might come from fish faeces and food remain; other organisms, such as bacteria, mushrooms, and infusoria, can also produce water acidity (pH) and ammonia in the aquarium [3].

Almost extensively, freshwater fish from the Cypriniformes order are hosts for Myxobolus parasites [4]. Myxobolus sp. is included in the Myxosporea class, causing Myxobolusis disease (Myxosporeasis) and leading to death up to 80%. Myxobolus sp. is identified by the spore morphology, number, and location of polar filaments. Fish suffering from the parasite will show the following clinical symptoms:
reddish nodules on the gills and incompletely-closed operculum [5,6]. Therefore, the pathological examination of Myxobolusis is needed to determine the damage to the affected tissue. Histopathological analysis of fish can be used as a biomarker to monitor fish's health conditions, including those suffering from a disease. The observations can be done to organs with essential functions in metabolism for early diagnosis of fish's health problems [7]. In the previous study by Yanuhar et al. [2] and Yanuhar et al.[8] showed that there were histological changes in gill tissue in koi fish infected with Myxobolus by using probiotics and diflubenzuron treatments. In this study, it was conducted to see the histological changes in the Carp strains of Punten infected with Myxobolus and not infected with Myxobolus. This study aimed to study and determine the infection profile of Myxobolus parasites through histological observations of damage to the gill tissue of Punten carp (C. carpio) in a concrete pond.

2. Material and Methods

2.1. Research Methods
This research was conducted in the Fish Biological Resources Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. The research method was descriptive exploratory using histopathological observations to determine carp gill tissue's infection profile. The researchers observed the clinical symptoms and conducted a histological analysis of carp gill tissue. The water quality parameters were directly measured in carp ponds, including temperature, pH, DO, and CO2; they were used as supporting data.

2.2. Analysis Data
Punten carp samples were obtained from a concrete pond in the Freshwater Management Unit (Indonesian: UPBAT - Unit Pengelola Budidaya Air Tawar) of Punten, Batu, Malang, East Java. A sampling of fish consisted of 10 healthy punten carp and 10 punten carp infected by Myxobolus, each of which had a juvenile size of 7-12 cm. Healthy fish seeds were selected based on fish's morphological observations: the fish had (1) a healthy body shape, (2) normal gill cover (not thick and no white spots when opened), (3) clear eye-lens, (4) normal scales (bright), (5) strong, normal and healthy tail's base, (6) seeds with similar length and weight, and (7) agile movements [3]. Meanwhile, the infected fish were selected based on observations of external clinical signs showing infection characteristics caused by the Myxobolus, as seen in the study [2]. The fish samples were taken to the laboratory for surgery and fish gill tissue preparation using the method of Raskovic et al. [9].

2.3. Histopathological Observation of Gill Tissues
Histopathological observations on gill tissue can be performed as an early diagnosis of infection in fish. The gill rigging made was then observed under an Olympus light microscope with a 400x magnification. Following Pantung et al. [10], tissue damage for each organ was assessed using a semi-quantitative scoring method.

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\text{Damage Percentage} \% = \frac{\text{Damaged tissue cells}}{\text{Total cell tissue observed}} \times 100\%
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The assessment requirement was based on the percentage of damage per visual field (%) consisting the following scores: 0 (zero) meant no damage (0%), 2 (two) meant mild damage (less than 30%), 3 (three) meant moderate damage (30-70%), and 4 (four) meant severe (more than 70%). Researchers observed the specified field of view three times.

2.4. Water Sampling and Measurement of Water Quality Parameters
The measurement of water quality parameters as supporting data included temperature, pH, DO, and CO2. Water sampling was done at the inlet and outlet of the concrete pool on the same day. PH and DO temperature were measured directly in the fishpond, while CO2 was measured in the laboratory. The temperature measurement used a thermometer, and the salinity was measured using an Atago refractometer. The water's DO value was obtained using a DO meter, and the pH value was obtained using a pH meter. CO2 measurement used the method asserted by Prasetyawan et al. [11].
3. Results and Discussion

3.1. Clinical Symptoms of Koi Fish (Cyprinus carpio) Infected with Myxobolus
Observing clinical symptoms was an initial prediction of pathogenic infection in fish. One of the most apparent signs was a nodule-like ulcer on the gills' surface, resulting in an open operculum with limited circulation. The fish breathed difficulty, its limb produced much mucus, and it had pale skin colour. It is in line with the symptoms found by Yanuhar et al. [2] and Atkinson et al. [12]. Common clinical symptoms in fish infected with Myxobolus showed the following behaviours: that the fish swam slowly, they stayed in the water's bottom most of the time, and they showed a low feeding response; eventually, they died. Also, the operculum was open or incompletely-closed due to the swelling caused by Myxobolus sp., leading to a difficult respiration process. The fish's health status could be seen from the body and their behaviour. Healthy fish did not have wounds on their bodies, had bright colours, were actively moving, and did not swim on the water's surface.

3.2. Observation of Koi Fish Gill Tissue Histology
Fish gills are the main respiratory organ working by the surface diffusion mechanism of respiratory gases (oxygen and carbon dioxide) between blood and water [8]. The gills consist of structured filaments and have a large surface area. The very thin filaments and gill lamellae (respiratory epithelium) function as a place to exchange respiratory gases [13]. Based on histological observations' results using a microscope, carps not infected with Myxobolus showed less damage in their body than infected ones. Gill tissue damage was assessed using Pantung's [10] scoring method, based on observations per field of view (%). The results of the histopathological analysis of gill tissue are presented in Figure 1.

The observation showed damage to the gill lamella tissue, such as oedema, congestion, hyperplasia, and lamella fusion. Although no nodules were found in healthy fish gills, some damages were found in fish not infected with Myxobolus, such as oedema (3%) and congestion (2%), indicating stress in fish. In this study, damage found in uninfected fish was not severe (<5%). In comparison, the infected fish showed damage by oedema as much as 55%, congestion as much as 32%, hyperplasia as much as 48%, and lamella fusion as much as 30%. The percentage of tissue damage in uninfected and infected fish was significantly different. The damage to infected fish was moderate; it might be because the infection had not spread widely.

Figure 1. Gill histology (400x) in carp not infected with Myxobolus (A) and fish infected with Myxobolus (B); Edema (E), Congestion (C), Hyperplasia (HP), and Lamella Fusion (FL)
Gill lamellae, oedema, and congestion were found in infected and uninfected fish. Oedema is inflammation of the cells due to the accumulation of excess fluid in a tissue so that the tissue enlarges and cannot function properly. Oedema will reduce gas diffusion efficiency because the secondary lamella absorption surface area is much narrower. Increased hydrostatic pressure tends to force fluids into the body, causing oedema [8]. Oedema will generally be followed by epithelium releasing in the secondary lamellae and epithelial cell death, disrupting the epithelium's function as a dissolved gas trapper [14]. The gills' swelling or oedema can be caused by direct contact between the myxosporean and the epithelial cells attached to the gills' surface, irritating it. Parasites such as Trichodina, Trematoda, and Myxosporidia are common in carp culture, and they can contribute to certain levels of primary epithelial hyperplasia. However, the degree of hyperplasia caused by parasites is never massive [9]. Hyperplastic cell tissue is thought to proliferate from the primary epithelium to produce differentiated cells needed to adapt to environmental changes, especially in injury or inflammation. Damage to epithelial hyperplasia and lamellar fusion is often found adjacent to the damaged gill filament cartilage, as was found in the study conducted by Lovy et al. [15].

### Table 1. Scoring and damage percentage of uninfected fish’s gill tissue

| Type of Damage found | Average (%) | Score* | Damage Level |
|-----------------------|-------------|--------|--------------|
| Oedema                | 3           | 1      | Mild         |
| Congestion            | 2           | 1      | Mild         |
| Hyperplasia           | 0           | 0      | No damage    |
| Lamella Fusion        | 0           | 0      | No damage    |

*(Pantung et al. [10])

The feeding activity of the fish can lead to gill damage caused by protozoan parasites. The parasite is filtered into the gills; it attaches, fixes, and spreads, injuring the respiratory epithelial cells. Infected fish gills will carry out a defence process against parasitic infections, which can cause bleeding. Congestion in the gills means damaged efferent vessels caused by pathogens; low blood pressure causes extensive bleeding and blood clots leading to rapid blockage of blood vessels, ischemia, and necrosis in some areas [16]. The fusion of two secondary lamellae can result from the injury caused by parasites in the secondary lamella, forcing the organ to release a lot of fluid to cover the wound, causing the secondary lamellae to stick with one another. Fusion as a more severe form of proliferation of the filament-coated epithelium is of a mild degree [17]. In this study, no hyperplasia or lamella fusion was found; it was estimated that the signs of oedema or congestion had not significantly affected the condition.

### Table 2. Scoring and damage percentage of fish gill tissue infected with *Myxobolus*

| Type of Damage found | Average (%) | Score* | Damage Level |
|----------------------|-------------|--------|--------------|
| Edema                | 55          | 2      | Moderate     |
| Congestion           | 32          | 2      | Moderate     |
| Hyperplasia          | 48          | 2      | Moderate     |
| Lamella Fusion       | 30          | 2      | Moderate     |

### 3.3. Water Quality Measurement Results

Increased stocking density of fish in ponds can increase fish feces excretion and uneaten food waste, reducing water quality. The use of intensive aquaculture must be handled with proper water quality management because a bad water environment can lead to fish's stress and make microbes or parasites easily enter the fish's body [18]. The results of water quality measurements, including temperature, pH, DO (Dissolved Oxygen), and CO2, showed relatively good results (based on quality standard values).

Temperature can affect metabolism, respiration, growth, and reproduction in fish [18]. Also, Mahasri et al. [5] showed that there was an effect of temperature treatment on *Myxobolus* spore damage. The optimum temperature for carp growth is between 25ºC - 30ºC [19]. The pH value good for carp's growth is 6-9, and 5-7 mg/l of dissolved oxygen [20,21]. Meanwhile, fish can live well at CO2 concentrations
below 10 mg/l. At a concentration of 20 mg/l, CO2 can affect fish metabolism. It can also be toxic at this level, causing death for domestic fish' thus, the recommended minimum concentration is 4 mg/l [22].

| Table 3. Water Quality Data |
|-----------------------------|
| Water Quality Parameters    | Unit | Inlet | Outlet |
| Temperature                 | °C   | 27    | 25     |
| pH                          | -    | 6     | 7      |
| DO                          | mg/L | 5.3   | 6.2    |
| CO2                         | mg/L | 4.8   | 5.3    |

4. Conclusion
Edema, congestion, hyperplasia, and lamellar fusion were found in fish infected with *Myxobolus*. The average scoring result and the damage percentage of the infected fish were mild to severe. The results of observations of water quality parameters, temperature, pH, DO, and CO2 as supporting parameters in water quality management were still tolerable and were not the main cause of the infection. It is also possible that the infection was transmitted by other organisms hosting *Myxobolus* sp. and infiltration or flow from other water sources.

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