The aquatic environmental quality of koi fish (*Cyprinus carpio*) pond infected by *Myxobolus* sp. based on the biological status of the phytoplankton

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Abstract. The purpose of this study was to observe the biology of phytoplankton found in the waters pond of *C. carpio* and the *Myxobolus* sp. infect *C. carpio* in Blitar, Indonesia. Analysis of the sample of phytoplankton in water pond, identification of phytoplankton in the targeted fish organs infected with *Myxobolus* sp. *C. carpio* samples have a length of 1-3 cm and weight of 5-8 grams. Observation of both phytoplankton and myxobolus samples was done using the Olympus CX31 microscopes and Olympus E330-ADU1.2X cameras. The identification results showed that the phytoplankton was from the Charophyta division, genus Mougeotia, Division of Chlorophyta, genus Pediastrum, and Ulothrix, Division of Cyanobacteria, genus Mycrocystis. In *Myxobolus* sp. infected koi, the gills, the stomach, and the intestine indicated phytoplankton. The phytoplankton found in water cultured pond of koi fish allegedly biologically became an intermediary for infection in koi fish. The conclusion is the biological phytoplankton from the genus Mougeotia, Pediastrum, Ulothrix, and Mycrocystis, found both in the waters pond and in the target organs of infected fish such as in the gill, the stomach, and the intestines. This finding used to make a decision to treat the waters pond for successfully fish culture.

1. Introduction
The disease is one of the obstacles to the development of farmed koi fish and can cause economic losses for fish farmers [1]. The attacks diseases on koi fishes were caused by unbalanced interactions between fish as hosts, water as an environment and disease agents (pathogens). Unbalanced interactions cause stress on the fish, so the body's defence mechanism decreases and is susceptible to disease [2]. One of the parasites often attacks koi fish is *Myxobolus*. *Myxobolus* is a dangerous parasite and can result in deaths up to 80%. *Myxobolus* is recognized by spore morphology, number, and location of polar filaments. Fish that are attacked to show clinical symptoms of the form of reddish nodules. If this nodule rupture, the spores will spread to the water that often swallowed by fish because of relatively small spores [3].
Koi fish infected with Myxobolus sp. usually will interfere with the respiratory process, besides the presence of nodules on the gills will make losing balance and cause fish to swim in a spiral from the bottom to the surface of the water. Large infestations that occur to the gills cause tissue death (necrosis) and respiratory dysfunction. Infection that occurs in the intestine, will cause monolithic on the intestinal wall [4]. The smaller the size of the koi fish, the more susceptible to Myxobolus infection because at the size of the seeds, all organs of the body have not functioned perfectly and are susceptible to disease [5].

Base on the area of Fish Pest Disease Monitoring (HPI) / Pests of Quarantine Fish Diseases (HPIK) at the Fish Quarantine Center, Class I Surabaya Fishery Product Quality and Safety Control I Myxobolus attacks from 2012 to 2014 was reported Myxobolus koi infecting koi fish seeds with 3–16 cm in size, located in Kemloko Village, Nglegok District, Blitar Regency, with 90% of deaths. In addition to causing death, Myxozoa parasites can also reduce the economic value of freshwater ornamental fish. The spread of these parasites occurs because of the transfer of parasites from infected fish to healthy fish, both directly and through the host between certain phases of the life cycle of the parasite. The prevalence of attacks varies from low in severe with chronic patterned mortality. Myxobolus diagnosis can be done by making a visual observation of the behavior and clinical symptoms. Further observations can be made microscopically on the gills [6]. In aquaculture activities there are three important components that are interconnected and influence one another, these components are the environment, host, and pathogen. If the water quality conditions decrease, it is very possible that seeds that are not good will be easily infected with the disease [7].

Biological parameters, in this case, are organisms that are often used as one of the water quality parameters as a marker for environmental conditions. Plankton has unique properties because of plankton position on the bottom of the food pyramid, that knowledge of the typical conditions and plankton density can be the basis of the analysis of resource abundance. The presence of plankton in water can provide information about the condition of the waters, that plankton is a biological parameter that can be used as an indicator to evaluate the quality and productivity of the waters. The presence of plankton can be used as an indicator of water quality, the description of total number species of plankton lives in water and the dominance species of plankton [8]. The aim of this study to observe the biology of phytoplankton in the waters of koi fishes (C. carpio) pond and koi fish infected with Myxobolus sp. in Blitar, East Java.

2. Materials and Methods
The research method was carried out descriptively, including the analysis of the research sample is phytoplankton samples in pond water. This research was conducted in June - August 2018 at Nglegok District, Blitar Regency, East Java.

2.1. Phytoplankton Sampling
Phytoplankton sampling was conducted on maintenance pond of koi fish (Cyprinus carpio) infected with Myxobolus sp. Phytoplankton was filtered using plankton net with mesh size 25 (25 microns) to filter phytoplankton [9]. Samples were collected in the film bottle are then preserved by adding 3 drops of Lugol. Furthermore, the samples have been obtained are taken to the laboratory to continue the identification process.

2.2. Phytoplankton Identification
Phytoplankton identification was conducted in the Hydrobiology Laboratory of the Faculty of Fisheries and Marine Sciences, Brawijaya University. Phytoplankton samples were observed using an Olympus CX31 type microscope and Olympus E330-ADU1.2X camera and assisted by a plankton identification book. Identification of phytoplankton species was used identification keys book[10] and [11] to determine the types of phytoplankton obtained.

2.3. The Abundance of Phytoplankton
The abundance of phytoplankton was conducted using Lackey Drop Methods. The abundance of phytoplankton was calculated with formula (1) [12]:

\[
N = \frac{T \times V \times P \times n}{L \times v \times p \times W}
\]

\[\text{Where:}\]
\[N: \text{The abundance of phytoplankton per liters}\]
\[T: \text{Wide cover glass (mm}^2\text{)}\]
\[L: \text{Wide field of observed (mm}^2\text{)}\]
\[P: \text{The total number of phytoplankton in the field of observing}\]
\[p: \text{The total number of wide field of observed}\]
\[V: \text{Volume of phytoplankton samples filtered (ml)}\]
\[v: \text{Volume of phytoplankton under cover glass (ml)}\]
\[W: \text{Volume of water samples filtered (liter)}\]
\[n: \text{The number of cells/individuals per ml}\]

\[2.4. \text{The Relative Abundance of Phytoplankton (KR)}\]

The Relative Abundance of Phytoplankton was calculated with formula (2) [13]:

\[
KR = \frac{a}{a + b + c} \times 100\%
\]

\[\text{Where:}\]
\[a: \text{Total number individual of each species observed}\]
\[a, b, c: \text{Total number of all species observed}\]

\[2.5. \text{Dominance Index}\]

Species dominance was calculated using Index Dominance, formula (3), [14]:

\[
D = \left(\frac{N_i}{N}\right)^2
\]

\[\text{Where:}\]
\[D = \text{Simpson index dominance}\]
\[N_i = \text{Number of each species}\]
\[N = \text{Number of all of species}\]

With definitions, Dominance Index (C):
- \(C < 0 - 0.50\) showed low dominance,
- \(C > 0.50 - 0.75\) showed medium dominance,
- \(C > 0.75 - 1.00\) showed high dominance.

\[3. \text{Results and Discussion}\]

The results of this study indicate koi fish attacked by Myxobolus parasite will experience swelling by nodules on the gills, furthermore, the koi operculum cannot close completely. In addition, these nodules can cause fish to have difficulty breathing and impact on death. Based on the identification results was indicated that koi fish has clinical symptoms of the form of open gill cover caused by the presence of Myxobolus nodules on the gills of koi fish. Myxobolus koi infects the gills of common carp and goldfish fish with characteristics of white or slightly reddish nodules or red on gill tissue. This parasite forms a cyst on the gill sheet of the fish, thus blocking the process of oxygen absorption.
Fish attacked by *Myxobolus sp.* show symptoms of the appearance of a reddish nodule, the nodule is a collection of spores and causes the gill cover to open. This type of parasitic infection can cause respiratory problems to decrease respiratory function [16]. Spores came out of the fish's body or gills caused by broken nodules and feces that pollute the waters. Spores spread in the waters as plankton and are eaten by Oligochaeta worms. Spores enter the digestive tract of the intestine, after which spores develop into sporoplasm. Parasites that develop in the intestinal tissue will produce actinospore (an infective stage for fish [17]. If the nodule breaks then the spores inside will spread liquid like plankton that swallowed by fish caused its relatively small size [3]. Spores swallowed by fish will break into two parts and transform into two flagella that are able to penetrate the walls of fish intestinal cells [18].

### 3.1. Phytoplankton Identification

Identification of plankton using identification keys book [10-11]. Phytoplankton identification in the first pond has founded the division of Chlorophyta (Genus Mougeotia, Genus Pediastrum), and the division of Cyanophyta (Genus Mycrocystis). However, in the second pond obtained results from the Chlorophyta (Genus Mougeotia, Genus Pediastrum, and Genus Ulothrix) and the Cyanobacteria division (Genus Mycrocystis).

| Division         | Genus     | N  | KR%   | D         |
|------------------|-----------|----|-------|-----------|
| Chlorophyta      | Mougeotia | 29 | 0.928 | 0.00008613|
|                  | Pediastrum| 57 | 1.856 | 0.0003445 |
|                  | Ulothrix  | 496| 16.009| 0.02563   |
| Cyanobacteria    | Mycrocystis| 2263| 73.086| 0.5342    |
| **Total**        |           | 2845| 91.879| 0.560     |

### 3.2. The Abundance of Phytoplankton

The high abundance of phytoplankton shows high waters that can support the life of organisms. Otherwise, if the abundance is low, the waters are classified as in low enrichment [19]. Increasing the number of phytoplankton in the waters will also cause eutrophication. The presence of algae or phytoplankton blooms will reduce the function of the waters and disrupt the ecosystem in them, including affecting plankton abundance [20]. Based on the calculation of phytoplankton abundance (Figure 1) was shown that the highest abundance of phytoplankton is found in the Mycrocystis species with a number of 2263 cells/ml. Then the abundance of Ulothrix species is 496 cells/ml and Pediastrum as much as 57 cells/ml and the abundance of Mougeotia 29 cells/ml.

### 3.3. The Relative Abundance of Phytoplankton

The Relative Abundance of Phytoplankton was shown in Figure 2. The relative abundance result of each genus of phytoplankton is Mougeotia 0.928%, Pediastrum 1.856%, Ulothrix 16.009% and Microcystis 73.086%. The increase in the number of plankton both phytoplankton and zooplankton in the goldfish rearing pond caused by nutrients such as nitrates and phosphates. The flow of pond waters which is the flow of agricultural areas and settlements allows the addition of nutrients dissolved in water so that it has a direct impact on plankton. Based on research [21], the existence of agricultural activities (fertilization) has an impact on the input of nutrients into the waters, especially phosphorus which is a nutrient source for plankton to grow. In addition, the decrease in plankton in the pond is
also influenced by biotic factors such as producers, which is a food source of plankton and species interactions and life cycle patterns in each species in the community [22].

**Figure 1.** The phytoplankton abundance in koi pond

**Figure 2.** The Graph of Phytoplankton Relative Abundance Result

### 3.4 Dominance Index

Dominance is a number that shows the composition of species of organisms in a community. The greater the dominance value means the greater the tendency of certain species to dominate their
abundance. Based on the Simpson dominance index value, if the dominance index is close to 1, then there are certain species that dominate the waters, however, if the dominance index is close to 0, then no species dominates in these waters [23]. The dominance range of 0-0.5 indicates that the area is of low dominance. The range of 0.5 - 0.75 indicates that the area is of moderate dominance and for the dominance value of 0.75-1 indicates the state of an area with high dominance [24]. Based on the calculation results of the phytoplankton domination index, the highest results were found in Microcystis at 0.5342 then followed by Ulothrix at 0.02563, Pediastrum 0.0003445 and Mougeotia 0.00008613.

4. Conclusion
Dominance phytoplankton was founded in koi fish pond infected with *Myxobolus sp.* come from the type of Microcystis. Spread of *Myxobolus sp.* in the waters can be through phytoplankton, caused by myxobolus spores that come out of the fish’s body through the gills caused by broken nodules and feces that pollute the waters then spores spread in the waters as plankton and eaten by koi fish.

References

[1] Alifuddin M, Hadiroseyni Y and Ohoiulun I 2003 *J. Akuakultur Indonesia* 2 (2) pp. 93–100.
[2] Suwarsito A and Mustafidah M 2011 *JUITA: Jurnal Informatika* 1 (4) 131–140
[3] Mahasri G and Kismiyati 2011 *Parasites and Fish Disease* I (Surabaya: Airlangga University) p 3–4. In Indonesian.
[4] Sugianti B 2005 *Personal Paper Science Philosophy* 1–37. In Indonesian.
[5] Abowei J F N and Ezekiel E N 2011 *J. Pharmacol. Toxicol.* 2 (5) pp. 236–250.
[6] Noga E J 2010 *Fish Disease: Diagnosis and Treatment, Second Edition*.
[7] Maftuch and Dalimunthe S 2013 *Aquaculture Animal Disease*. UB Press. pp 11.
[8] Fachrul M F 2005 *Proceeding Semin. Nas. MIPA* 17–23. In Indonesia.
[9] Sari A, Hutabarat S, and Soedarsono 2014 *J. Maquares (Management Aquat. Resour)* 3 (2) 82–91.
[10] Prescott G W 1970 *How To Know Freshwater Algae*. (Dubuque, Iowa: W.M.C. Brown Company Publishers). pp 348.

[11] Davis C C 1955 *Marine and Fresh Water plankton*. Michigan State Univ pr; First Edition.

[12] APHA 2005 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association.

[13] Dahuri R 2003 *Marine Biodiversity: Indonesia’s Sustainable Development Assets*. (Jakarta: PT Gramedia Pustaka Utama) 412. In Indonesia

[14] Barus 2002 *Introduction to Limnology*. (Medan: Universitas Sumatra Utara). In Indonesia.

[15] Paperna I 1991 *Annu. Rev. Fish Dis.* 1 155–194.

[16] Handayani R, Adiputra Y T, and Wardiyanto. 2012. *Aquasains: J. Ilmu Perikan. dan Sumberd. Perair*. 1 149–155.

[17] Yokoyama H, Grabner D, and Shirakashi S 2012 *Health and environment in aquaculture* (London: Intechopen Press) 1-42.

[18] Ruidisch S, El-Matbouli M, and Hoffmann R W 1991 *Parasitol. Res.* 77 (8). pp. 663–667.

[19] Erdina L and Ajizah A 2010 *J. Wahana Bio*. 3 (2)72–91.

[20] Samudra S R, Soeprobowati T R, and Izzati M 2013 *Bioma* 15 (1) 6–13.

[21] Wijaya T S and Haryati R 2005 L. Ekologi, J. Biologi, and F. M. Undip 55–61.

[22] Oktavia N, Purnomo T, and Lisdiana L 2009. *J. Biologi, F. Matematika, and P. Alam*. 4 (1) 103-107.

[23] Kusmeri, Lusi, and Rosanti D 2015 *Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam* 12 (1) 8-20.

[24] Adithya R, Raza’i T S and Zulfikar A 2015 *Jurnal Umrah* 12 (7) 1-9.