Diversity of *Trichodina* spp. on The Larvae of Freshwater Fish in Banyumas and Surrounding Areas of Central Java

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Abstract. *Trichodina* sp. has a fast -splitting reproduction ability, and it is widespread, infect more than one species of fish. Freshwater fish cultivation increases every year, but high mortality caused by this parasite occurs. Research to determine *Trichodina* sp. on freshwater fish larvae was conducted in the district of Exs. Banyumas Residency, Central Java. We used a survey method to collect the fish larvae samples consisting of tawes, mujaher, nilem, and bawal of hatchery centers in Purbalingga, Banyumas, and Banjarnegara. Isolation and identification of parasites were conducted at the Parasitology-Entomology Laboratory, Faculty of Biology. The results showed that the prevalence of Trichodiniasis in freshwater fish from Banyumas, Banjarnegara, and Purbalingga was lower than Trichodiniasis in gourami larvae. Trichodiniasis intensity in freshwater fish was moderate.

1. Introduction

*Trichodina* sp. is a pathogenic ectoparasite protozoon of the ciliate group that commonly attacks freshwater and marine fish. It has a fast-splitting reproductive ability, with a widespread, and can infect more than one fish species. It has a rounded body with the lateral side of a bowl or bell shape, with cilia around and on the ventral part of its body. The species has a denticle (a tooth-like structure) as a sticking apparatus that acts as an adhesive disc to attach to its host's surface [1]. These ectoparasites are known to be the cause of death of mas fish larvae (*Cyprinus carpio*), mujair (*Oreochromis mossambicus*), tawes (*Puntius javanius*), nila (*Oreochromis niloticus*), betutu (*Oxyeleotris marmorata*), rainbow trout (*Salmo gairdneri*), salmon (*Salmo salar*), and gurami fish (*Osphronemus gouramy*). These advantages of the ectoparasite infection are not massive, but the infection may be a predisposing factor for the infection of more dangerous pathogenic organisms [2]. These organisms live by eating bacterial cells that die in the waters or attached to their host surface and can survive for two days without a host [1, 3].

There are 300 species of *Trichodina* sp., which infect the fish in the world [4], and its life is ubiquitous or as an obligate parasite of aquatic environments, both in aquaculture ponds and in river waters. *Trichodina* sp. is easily and freely move circling like a circular motion wheel with cilia. Its reproduction is to escape from the host, splitting into two, with each part multiplying [5]. *Trichodina* sp. infections lower the immune system of fish characterized by a low immune system and can lead to a secondary infection. These ectoparasitic's pathogenic ability to attack fish generally decreases with increasing the fish age and body size. The larger the size and the older the fish, the smaller the ectoparasitic attack due to the improved fish resilience system [6]. Ciliated protozoan of Trichodinidae is among the main etiological agents of mortality in farmed fish [7]. They can be considered opportunistic ectoparasites and are present in both marine and continental fish species. Because of their
direct transmission, trichodinid ciliates can invade their hosts within a short period, especially fish that are under optimum conditions.

The organs of the host attacked by *Trichodina* sp. include gills, fins, scales, and operculum. Fish affected by *Trichodina* sp. become weak with dull body color. The fish appetite drops so that fish become thin, move slowly, often rub their bodies against pool walls, irritated, and fish larvae often result in faulty or falling fins. The fish body is also shiny due to excessive mucus production. *Trichodina* sp. infects the epithelial layer of fish with the help of a sharp membrane tip. Furthermore, the parasites circle to damage the cells around attachment, destroy, and consume the epithelial cells and cause irritation. This condition is more dangerous if the parasite population is present in an environment with high organic content [8].

Fish infected by *Trichodina* sp. shows body color changes, rubs its body against the pool wall, resulting in skin irritation, hyperplasia, degeneration and cell/tissue death (necrosis) in epithelial cells and proliferation of mucus cells. The most influential factor in the level of pathogenicity is the ability of *Trichodina* sp. to infect its prevalence and intensity [5]. The type and rate of infection of *Trichodina* sp. in larvae among locations of ponds of cultivation will be different. Parasite infection is influenced by the difference of feed given, fish age, fish size, water condition, and cultivation activity [9](Handayani et al., 2014). The incidence or prevalence of *Trichodina* sp. on the gourami larvae in Bantul, Jogjakarta, is 100% [10]. The prevalence of *Trichodina* sp. on the goramy of farmers of Luwung Village, District of Raft Banjarnegara, is 100% [11]. *Trichodina* sp. has a disc shape similar to a bowl with teeth in the middle. It has a flat-dorsoventral but looks like a concave on one of its edges, and it sticks to the parasite-host to suck the host’s blood but has a convex shape on another edge. This type of parasite is equipped with two nuclei, the big and small ones. The small nucleus has a round shape like a vacuole, and the big nucleus is a horseshoe shape. *Trichodina* sp. belongs to a mobile parasite group, which is completed with cilia, and attacks the freshwater fish [12]. *Trichodina* sp. breeds fast since they belong to a specific bacteria that live in aquarium or fish ponds and utilize all available nutrition [13]. So, the attack on the fish takes place there. Trichodiniasis is a fish disease caused by *Trichodina* sp., and the infection starts on fish larvae, fish, or even small fish [10], and the most common host is Cyprinidae [8]. The abundance and variance of types of *Trichodina* sp., which infect the fish from different ponds, might also be different as their presences are related to the fish’s feed, age, size, and pond’s condition even to cultivation activity [9].

All those factors affect the fish’s activity differently and lead to a different intensity of the parasite attack. For example, the gourami fish has a low survival rate since they prefer to live in the middle to the bottom of the pond. This phenomenon is different from other fish like tawes, mujahir, and nilem, which are actively mobile and swim to all areas of the pond leading to different severity of the parasite-attack. [14] predicted that fish mortality is due to this disease, and better handling of the fish as soon as possible is the best way to do. To have good handling, information about the morphology, types, habitat, similarity, and differences of the parasite, and their abundance become important factors [9,15].

The previous statements lead to the formulation of the research’s problem on knowing the parasite attack intensities on particular fishes, namely gourami, tawes, mujahir, and nilem cultivated in a polyculture system. This research was then intended to measure the intensity of *Trichodina* sp. on those particular fishes. This research gives some benefits to early good handling practices, to measure the effect of each type of *Trichodina* sp. attack on the fish.

The diversity study of *Trichodina* sp. in goramy larvae has been so many with several different sample locations by knowing its prevalence, intensity, and species. Gourami fish with other freshwater fish have some morphological differences in the body. Its behavior (way of swimming/moving, how to eat and breathe) allegedly can affect the pathogenicity of *Trichodina* sp. found. The formulation of the problem is there any influence on characteristic differences of each fish body morphology on *Trichodina* sp. pathogenicity level on each of the freshwater fish. The benefit of this research is to map the extent of these protozoan incidents in some freshwater fish and primary data for early control of this disease.
2. Methods

This research used a survey method with a random sampling technique. *Trichodina* sp. was isolated at the age of the larvae of some freshwater fish (bawal, tawes, nilem, mujaher) kept in fish larvae centers in Banyumas, Purbalingga, and Banjarneega districts of Central Java Province.

Isolation-identification of *Trichodina* sp. was done from various freshwater fish of larvae centers in Banyumas, Purbalingga, and Banjarneega regencies of Central Java Province. Preparation of *Trichodina* sp. was done by tweezers, then the pectoral fins, dorsal fins, anal fins, caudal fins were taken by cutting. They were placed longitudinally above the glass object. Skin mucus was obtained by scraping the surfaces of the right and left sides of the scales using a scalpel. Mucus was wiped on top of the glass object, then air-dried. The prepared body parts of fish larvae in object-glass are checked using a 40 to 100 magnification microscope. If it has been seen, then count the number of infected fish larvae and the number of individuals *Trichodina* sp., which are found in any organs of fish larvae like a pectoral fin, dorsal fin, anal fin, tail fin, and skin surface. Then calculate the prevalence and intensity of *Trichodina* sp.

*Trichodina* sp. preparation is soaked in a chamber bottle that has been filled with 2% AgNO3 solution for 10 minutes, lifted then wait until half dry and then rinsed with water with a small discharge. The object-glass is then dried for 15-20 minutes in the sun at noon.

3. Results

Prevalence and Intensity of *Trichodina* sp. in some fish larvae from each sampling site are varied (Table 1). It ranged from 10 to 73,3% and 2 to 62.8 for prevalence and intensity, respectively.

| Sample Number | Fish larvae | Prevalence (%) | Intensity |
|---------------|-------------|----------------|-----------|
| Banyumas      | Tawes       | 22,7           | 3,1       |
|               | Mujaher     | 63,3           | 32,4      |
|               | Nilem       | 73,3           | 48,1      |
|               | Bawal       | 56,7           | 62,8      |
| Banjarneega   | Tawes       | 61,3           | 5,0       |
|               | Mujaher     | 48,0           | 4,8       |
|               | Nilem       | 66,6           | 4,7       |
|               | Bawal       | 54,0           | 4,0       |
| Purbalingga   | Tawes       | 10,7           | 2,0       |
|               | Mujaher     | 38,0           | 20,5      |
|               | Nilem       | 46,7           | 10,0      |
|               | Bawal       | 10,0           | 3,6       |

Figures 1 and 2 represent both histogram parameters to provide a dynamic illustration for the *Trichodina* prevalence and intensity. In Banyumas, it showed a relatively higher prevalence compared to that in Banjarneega and Purbalingga. The intensity also demonstrated the most elevated in Banyumas.
4. Discussion

The pathogens cause disease or pathogenicity. The infection mechanism is from disease progression to invasion of the host by microbes that multiply and associate with host tissue. Prevalence and intensity of Trichodina sp. in some fish larvae from each sampling site were varied (Table 1). The samples were different from the scales on the gourami fish based on each fish's type of scales and fins. This means that in the gourami prevalence level of Trichodina sp. can be obtained up to 100%. The maximum prevalence rate was 73.3% in other fresh fish, at nilem, and pomfret fish was very low 10%. One of the markers to determine Trichodina sp.'s pathogenicity is the high-intensity value of Trichodina sp. which infects gourami fish larvae. The intensity of Trichodina sp. attack in fish will decrease along with the increasing age and body size of fish. The larger the size of the fish, the fish's endurance system will be better. The resilience of larvae size fish is weak and highly susceptible to environmental changes making it more susceptible to parasites [6]. The high prevalence and intensity of this protozoan to fish are affected by the fish's age or size. In larvae fish, the incidence or prevalence and intensity will be high, and with increasing age, the incidence or prevalence and intensity/abundance will be low [10].

In the fish larvae body, Trichodina sp. can adhere to adhesion (pressure from the outside) and consume cell fluid in the mucus in the epidermis. Trichodina sp. also takes organic particles and bacteria
when attached to the host body. The sticking of *Trichodina* sp. is very strong in its host because there are disc hooks [17]. The high intensity of *Trichodina* sp. will cause damage, scar, some scales are peeled off, and some scales turn in reddish in some surface part of the offspring fish body. *Trichodina* sp. moves from one to another body part inside its host with cilia until the left body parts are damaged and peeled off. In this condition, bacteria will enter and develop as a secondary infection. These bacteria will be a food resource for *Trichodina* sp. to divide and breed. This *Trichodina* sp. breeding will affect offspring fish intensity.

Damage caused by *Trichodina* sp. does not cause injuries, only some scales are visibly released, and there are red spots in some areas on the surface of the fish larvae body. *Trichodina* sp. moves from one body part to another body part of its host by utilizing cilia causing abnormal body parts to be peeled off. In such circumstances, bacteria will enter and develop as secondary infections. These bacteria will then become food for *Trichodina* sp. to perform division and reproduction. The reproduction of *Trichodina* sp. will affect its intensity.

Variations in body morphology and the behavior of some freshwater fish are different. Nilem fish is slightly elongated and flattened, the tip of the mouth is pointed with the muzzle (rostral) folded, and has a large black bintim on its tail. In addition, nilem belongs to the omnivorous group, which consumes food in attachment algae. Nilem fish has a mouth with two pairs of touch tentacles; the dorsal fin is supported by three hard fingers and 12-18 soft fingers. The caudal tail fins, symmetrical in shape, anal fins are supported by three delicate fingers and five delicate fingers. The abdominal fins are supported by one hard finger, 13-15 soft fingers, and several scratchy scales of 33-36 pieces. Tawes has a long, flat body morphology with a raised back, a small mouth resting on the nose's tip, and a tiny tentacle. The ribs are 5½ pieces and 3-3½ pieces. The ribs are entirely 29-31 pieces. The body is silvery and somewhat dark on the back; on the snout, there are small bulges, dorsal fins, and caudal fins are grey or yellowish, the caudal tail fin is. Mujaher fish are easy to maintain, multiply, and grow quickly. The foods are such as plankton or algae, which are easy to grow in ponds and rivers. The dorsal or dorsal fins have 15-17 sharp spines, 10-13 soft-toed ends, and anal fins with three spines and 9 to 12 fingers. The body color of tilapia fish is generally greenish gray, brownish, or blackish. Mujahir fish and Tilapia fish are a close family, the family of Cichlidae. Freshwater pomfret has a slightly round body, flat body shape, small scales, almost round head, rather large nostrils. Pectoral fins are beneath the gill cover. Stomach fins and anal fins are separated, the back is dark gray back, and the belly is white grey and red. Freshwater pomfret (*Colossoma macropomum*) has two dorsal fins that are slightly shifted to the back. The abdominal fins and the anal fins are separated, while the tail fins are homocercal. The different fish whit pathogenicity is *Trichodina* sp.

Measurement of morphometric variation for species determination *Trichodina* sp. found in each fish was no different [11]. *Trichodina* species sp., which were found in the cultivated bawal, tawes, nilem and mujaher were the same, namely *Trichodina nigra*, *Trichodina acuta*, *Trichodina nobilis* and *Trichodina heterodentata*. *Trichodina* sp. found in gourami larvae were *Trichodina acuta*, *Trichodina heterodentata*, and *Trichodina magna*, and *Trichodina nobilis*, *Trichodina reticulatus*, and *Trichodina pediculus*, and *Trichodina nigra* [18].

5. Conclusion

The average prevalence of Trichodiniasis in freshwater fish from samples of Banyumas, Banjarnegara, and Purbalingga was lower than in gourami larvae. Trichodiniasis intensity in freshwater fish is moderate. Thus, this research recommends that there should be a follow-up research measurement of morphometric variation for species determination *Trichodina* sp., and phenotype of *Trichodina* sp. to early control disease.

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