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Short Communication: Diversity and prevalence of ectoparasites associated with cultured fish from coal ponds in East Kalimantan, Indonesia

GINA SAPTIANI^{1*}, CATUR AGUS PEBRIANTO¹, AGUSTINA¹, ESTI HANDAYANI HARDI¹, FIKRI ARDHANI²

¹Laboratory of Aquatic Microbiology, Faculty of Fisheries and Marine Sciences, Universitas Mulawarman, Jl. Gunung Tabur, Kampus Gunung Kelua, Samarinda 75124, East Kalimantan, Indonesia. Tel./Fax: +62-541-749482, *email: gina_saptiani@yahoo.com.

²Program of Animal Husbandry, Faculty of Agriculture, Universitas Mulawarman, Jl. Paser Balengkong, Kampus Gunung Kelua, Samarinda 75124, East Kalimantan, Indonesia

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Abstract. Saptiani G, Pebrianto CA, Agustina, Hardi EH, Ardhani F. 2017. Short Communication: Diversity and prevalence of ectoparasites associated with cultured fish from coal ponds in East Kalimantan, Indonesia. *Biodiversitas* 18: 666-670. In East Kalimantan, coal pits are used for fish culture. The most fish species cultured in the coal ponds were carp and tilapia. The study aimed to identify ectoparasites types, diversity, and prevalence of fish cultured in coal ponds. Sampling was carried out between January and March 2016 on coal ponds in Kutai Kartanegara District, East Kalimantan. Samples were taken from two different coal ponds, but both coal ponds cultivated the same species of fish samples (carp and tilapia). The number of fish samples taken from coal ponds was 40 samples for each fish types in each location of coal ponds with the body weight of the fish samples between, 20-30 g. The preparation of parasite identification was conducted by taking every fish's external organ which was then scraped for microscopical preparation and observation. The identification of parasites was determined based on the morphological characteristics including the shape performances, specific spots and also the type of environment and habitat. The results showed that ectoparasites found in carp and tilapia cultured in coal ponds were *Ichthyophthirius multifiliis*, *Trichodina*, *Oodinium*, *Dactylogyrus*, and *Gyrodactylus*, while *Epistylis* was only found in carp. The average percentage of ectoparasites prevalence in carp was from 30.00 to 59.17% with the attacked intensity was between 2.47 to 4.43 parasites/fish, while in tilapia was from 27.5 to 40.83% with the attacked intensity between 1.04 to 2.83 parasites/fish. The highest prevalence and intensity of parasite attacks was *Trichodina* in carp from a pond which has been used for a long time for fish culture. The prevalence and attacked intensity of ectoparasites on fish were higher in the longer use of coal ponds rather than those in new ponds.

Keywords: Coal pond, ectoparasite, fish, prevalence

INTRODUCTION

East Kalimantan province is well known as one of the biggest coal-mining regions in Indonesia. This mining activity has contributed to the economical development of the province and Indonesia in general. However, this mining activity has also some negative impacts, especially on the environment. One of the biggest issues was the emerging of large coal ponds as a result of the mining excavation causing further problems for the environment and local society. To solve the problems, the use of the coal ponds for fish culture has been intensively conducted. The fish culture in the coal ponds has been conducted for a decade (6-11 years). However, in the last three years, another new problem arise from the fish culture in this coal ponds such as the high number of fish death, the slow growth of the freshwater fish and the worse fish condition. Generally, fishes will be dying in the large number after the hard rain due to the changes of the water quality leading to the stress fish-condition. Unfavorable environmental conditions, such as changes in water temperature, water quality, dissolved oxygen, pH, and the density of fish, have

effects on the fish growth and condition. Choresca et al (2010) has reported that stress is one of the causes of catfish death in the displayed aquarium. Furthermore, the high density of fish will cause the unsuitable environment condition for fish itself but it will lead to the overwhelmed growth of certain parasites (Omeji et al. 2011). In the fish culture, most parasites cause serious disease outbreaks.

Parasites are organisms that live on other organisms for food purposes. Parasites can reduce the sale value of the fish, because it can damage the skin, flesh, and the condition of the fish. Parasitic infections and diseases are some of the factors that hamper the productivity of fish culture (Kayis et al. 2009). Some parasites live inside the host's body and some other parasites living outside the host body. Generally, ectoparasites that live in the body of fish are a class of *Protozoa* and flatworms *Monogenea*. This parasite attached to the host for life and take nutrients from the host. Based on the location of ectoparasites, certain species such as *Ciliate* and *Monogenea* class can only live on the gills, while other parasites species prefer to live on the skin and fins of fish (Bruno et al. 2006). Parasites can be a limiting factor for the development of fish farming. It

happens due to fish parasites affect the fish growth by inducing diseases causing to the low number of fish production. These also lead to the high production cost especially for disease control so that it will reduce the profits of fish farmers (Pantoja et al. 2012).

The parasitic infection can cause the irritation and sores on the fish organ which further triggers the easier introduction of microbes into the body of fish. Therefore, objectives of this study were to identify the types and prevalence of ectoparasites in the fish cultured in the coal ponds. The results of this study are expected to be useful as a reference and additional information for preventing parasitic infection in cultured fishes from coal ponds in East Kalimantan.

MATERIALS AND METHODS

Fish samples

The fish material used in this study were carp and tilapia with the body's weight around 20 to 30 grams. These samples were taken in two different location of coal ponds in Kutai District, East Kalimantan Province. Fish sampling was conducted from January to March 2016. In the first pond's location, both carp and tilapia have been intensively cultured for eight years while the second ponds have cultivated those fish types for four years. The total number of fish samples were 160 individuals consisting of 40 individuals in each fish types in each different pond location.

The preparation of ectoparasites observation in fish samples

Before ectoparasites observation, performances of fish samples (carp and tilapia) were intensively observed such as the movement activity and the swim pattern one by one. Afterward, fish samples were immediately killed by entering the probe into the nostril to destruct its brain. In the death condition, the condition and the anatomical pathology of the outside organ of fish samples were observed. Anatomical pathology observations were conducted by observing the changes in shape, color, and organ consistency in all outside organs of the fish. Furthermore, the preparation of parasites of the fish was done by scrapping each external organ of the fish, such as the mouth, eyes, gill cover, or operculum, fins, tail, skin and gills. Each result of scrapped organs has peered on the glass preparations, which were directly added a physiological NaCl 0.85% or stained with Giemza 7% and were then dried at the room temperature. Afterward, prepared samples were dropped by Giemza for 30 minutes and rinsed with aquadest followed by drying samples at the room temperature. The preparations were then observed under a microscope. The identification of parasites in carp and tilapia were determined by the shape characteristics, morphological performances, and the type of habitat based on the published description by Lom and Dykova (1992) and Klinger and Floyd (2016).

RESULTS AND DISCUSSION

The type of ectoparasites

Results of this study showed that ectoparasites found from fishes cultured in the coal ponds were *Protozoa* and worm *Monogenea*. Group protozoan infecting carp and tilapia was *Trichodina*, *Ichtyophthirus multifilis*, and *Oodinium*, while *Epistylis* was only found in carp. *Monogenea* parasites found in the ectoparasite observation were *Dactylogyrus* and *Gyrodactylus*. Both parasites were found in carp and tilapia (Table 1). The other found parasites such as *Trichodina*, *I. multifilis*, and *Epistylis* included in the phylum ciliates (Klinger and Floyd 2016) or *Ciliophora* (Lom and Dykova 1992). *Oodinium*, or also known as *Dinoflagellata*, including the phylum of *Sarcomastigophora* as part of *Zoomastigophora* class (Lom and Dykova 1992). *Dactylogyrus* and *Gyrodactylus* were categorized as monogenic trematode or monogenea worms (Klinger and Floyd 2016). A specific host of the fish correlated with parasites, habitat (freshwater, brackish and marine), along with the water temperature and other environmental conditions, can also be used as an additional indicator in the identification of parasites (Bruno et al. 2006). The level of prevalence and intensity of attacks from a parasitic infection related to the changes of seasons and fish species as the host (Koyun 2011).

Ichtyophthirus multifilis possesses a very dark in body's color because it has thick cilia covering the entire cell body and has an active movement known as *amoeboid*. Based on the shape of macronucleus (a great shape of the nucleus), it is identified that the macronucleus of *I. multifilis* has the same shape like a horseshoe. Another identified parasites infecting the fish samples, *Trichodina*, is one of the most common ciliates. *Trichodina* usually has a discoidal body shape like a hat although the shape can be varied (Bruno et al. 2006). *Ciliate Epistylis* is attached to the skin or fins of the host. *Epistylis* becomes the greatest concern of all other Ciliate because it can secrete proteolytic enzymes causing worse injury of the host. Furthermore, it can easily facilitate the invasion of bacteria at the site of attachment (Klinger and Floyd 2016). Another type of parasites, *Oodinium* have flagella which are found on the skin, gills, and eyes. *Oodinium* is *Dinoflagellata* with two flagella. Interestingly, one of flagellum extends transversely around the cell and generally attacks the gills and skin (Lom and Dykova 1992). *Monogenea trematodes*, also called flatworms or flukes, generally attacks the gills, skin and fins. *Monogenea* has a direct life cycle (without an intermediary host). Interestingly, they had the specific site of attachment to hosts. Specifically, some of the adult worms could be permanently attached to one site of the host. While another type of *Monogenea trematodes*, *Gyrodactylus* do not have eye spots (eyespot), but having two pairs of hooks anchor (anchor hooks) which is commonly found on the skin and fins of fish. *Dactylogyrus* has eye spots between 2 and 4, a pair of large anchor hooks, and a layer of eggs (Klinger and Floyd 2016). Commonly, *Monogenea* is parasites which cause a big economical problem due to it can injure the skin or gills of cultured fish (Borji et al. 2012).

The location of parasites in the fish

Most parasites which infected most carp samples were *Trichodina* and *I. multifilis*, especially found in the 1st coal pond. Parasitic infections in the fish were likely to increase when water conditions changed. The anatomical pathology of fish retained in the 1st pond apparently showed in worse performances compared to those in the 2nd pond. The anatomical pathology of fish from the 2nd pond was likely in good condition even though some fish showed in poor condition (Table 1). Parasites infecting on other organisms for food purposes would actively interfere the host, which lead to the injured organ of the host. The fish showing severe performances of anatomical pathology had the high number of parasite infection especially from protozoa class such as *I. multifilis*, *Trichodina*, and *Oodonium*. The obvious impact of these parasite infections in the fish was the outer part damage, such as gills, eyes, fins and skin. The parasitic flatworms found in the gills were *Dactylogyrus* and *Gyrodactylus*. Of those parasitic flatworm infections, it shows that the condition of the fish was not in good performances, such as scales or skin dull or missing, fins and gills reddish and torn, as well as gills reddish and dirty. These become favorable conditions for the bacteria as a way to enter the body of the fish. *I. multifilis* infection on tilapia, for example, showed the high number of the death rate (60.40%) after the bacteria (*F. columnare*) was exposed in the infected fish compared to that in unexposed parasite infection of the fish (control) (29.10%). The high expenses of fish attacked and infected by *F. columnare* and *I. multifilis* were 13-17 times higher than the fish that was not infected with parasites (Xu et al. 2014).

Based on obtained results, ectoparasites was mostly found on the skin, the entire surface of the body, as well as on the fins and gills of fish (Table 1). *I. multifilis* parasites were found on the gills and skin of fish, while *Trichodina* sp. was found on the skin and fins (Omeji et al. 2011). *Epistylis* was found on the skin and fins of fish (Lom and Dykova 1992). Some epithelial tissue damage found in the gills and skin of fish was caused by the development of the parasite through the skin and gills of fish during the infection (Raissy and Ansari 2012). Parasites can infect the gills, eye damage, and internal organs, hunger, the swim bladder inflammation, as well as the inhibition of the oxygen exchange at the gill lamella (Pantoja et al. 2012). *Oodonium* infection can injure skin tissues, which commonly found on the outer surface erosion, dislocation of muscle fibers, as well as shrinkage and atrophy due to the development of parasites, required a large space between muscle fibers (Khalil et al. 2012). The location of parasitic infection in or on a host is also important, because some parasites are only found in organs or specific tissues (Bruno et al. 2006). Parasites can be an obstacle in the fish culture, because it can inhibit the growth of fish. Moreover, it can also cause disease, and cause the abnormal performance of the fish's body, lead to the harmful risk of the fish. The protozoan parasite commonly found in the fish culture will cause the economical losses of fish culture.

The prevalence and intensity of parasite attacks

Results showed that *Trichodina* found in the carp has the highest prevalence amongst parasites obtained in the

1st ponds which gave 100% with the attacked intensity around 5.5 parasites/fish, followed by tilapia from the 1st pond, carp from the 2nd ponds, and tilapia from the 2nd ponds. The prevalence of parasites *I. multifilis* found in carp from the 1st pond was 55%, while tilapia was 50%. Both parasite prevalence is quite high because more than 50% of the fish was infected by the parasite. Thus it will increase the risk of contagion. The average prevalence of parasites in carp or tilapia from the 1st pond is high compared to the second pond fish which in line with the intensity of attacks (Table 2, Figures 1-2). The existence of parasites in fish should be concerned. If the water quality is low, it can affect the health conditions of fish, so that the fish was infected with parasites or bacteria that was already presented in the fish. Therefore, it would easily occur parasitic or bacterial infection. Parasites in fish may cause health problems and if a number of parasites was in abundance, it will cause the organ damage in the gills and cause anemia.

The low intensity of parasitic infection has no harmful effect on the fish. However, it will turn to serious damage to the fish if there were a high-stress environment and the worse quality of water. It happens because in that conditions, parasites will multiply rapidly leading to the serious damage of fish (Hoai and Van 2014). The intensity of *D. spincirrus* infection was influenced by seasons. The prevalence and intensity of parasites attacks were higher when the season fluctuation which can be seen from the increased level of water temperature (Mbokane et al. 2015). Prevention and treatment of parasitic infections in the fish will reduce the direct damage of the fish due to the reduced number of parasites will decrease the number of mortality due to the secondary bacterial infection (Xu et al. 2015). Based on the percentage of prevalence, it shows that the percentage of parasites in the first coal pond was higher than those in the 2nd pond, due to the longer use of the first pond for fish culture. It might happen due to the water used for fish farming in the 1st pond had no good water circulation so that the water become stagnant and increase during the rainfall. The same water conditions commonly caused parasites being life persistence as long as its host still survived. Moreover, most of the fishes cultivated in the coal ponds could not be yielded due to some fishes could pass through the trapping cage in the coal ponds. These conditions might lead the increased number of parasites if the coal ponds were used for a long time as fish farming. The parasite population will significantly increase again if the fish as a host of parasites experienced the stress condition especially the changes of water quality in the rain season.

Based on the obtained results in this study, it can be concluded that the types of parasites which infected the fish samples (carp and tilapia) cultivated in the coal ponds was a class of *Protozoa* and worms monogenea. Protozoa found in the tilapia was *Trichodina*, *I. multifilis*, and *Oodonium*, while *Epistylis* was only found in carp. *Monogenea* found in the fish samples were *Dactylogyrus* and *Gyrodactylus*. The prevalence and intensity of parasite attacks were highest in fish which was cultivated in coal ponds used as a fish culture for eight years, compared to those new pond used as a fish culture for four years.

Table 1. The type of parasites and the pathological condition of fish cultivated in the coal ponds in East Kalimantan, Indonesia

Ectoparasites	The attacking site on the fish	The total number of parasites				The pathological anatomy			
		1 st pond		2 nd pond		1 st pond		2 nd pond	
		Carp	Tilapia	Carp	Tilapia	Carp	Tilapia	Carp	Tilapia
<i>Trichodina</i>	Skin, fins, gills	220	90	152	30	Loose scales, fins, and gills damaged	Torn fins, scales off	Pale gills, fins tom and bloody	Pale gills
<i>Ichthyophthirius multifiliis</i>	Gills, fins	50	44	26	8	Gills and tail fin white speckled	Pale gills, fins mottled white	Normal	Normal
<i>Oodonium</i>	Skin, gills, eyes	24	12	10	4	Gills bleeding and red eyes	Red eye	Red eye	Normal
<i>Epistylis</i>	Skin, fins	180	0	12	0	Scales loose, damaged fins	Normal	Normal	Normal
<i>Dactylogyrus</i>	Gills	50	100	40	28	Bleeding gills and gill cover damaged	Pale gills	Pale gills	Normal
<i>Gyrodactylus</i>	Skin, fins, gills	110	80	28	14	Scales loose, damaged fins, gills bleeding	Pale gills	Normal	Normal

Table 2. The prevalence and attacked intensity of parasites in the fish cultivated in the coal ponds in East Kalimantan, Indonesia

Type of parasites	The location of parasites	The percentage of prevalence (%)				The number of attacked intensity (parasite individual/ fish number)			
		1 st pond 1		2 nd pond		1 st pond 1		2 nd pond	
		Carp	Tilapia	Carp	Tilapia	Carp	Tilapia	Carp	Tilapia
<i>Trichodina</i>	Skin, fins, gills	100	85	75	60	5.50	2.65	5.07	1.25
<i>Ichthyophthirius multifiliis</i>	Fins, gills	55	50	30	30	2.27	2.20	2.17	0.67
<i>Oodonium</i>	Skin, fins, gills	40	20	20	10	1.50	1.50	1.25	1.00
<i>Epistylis</i>	Skin, fins	40	0	20	0	3.75	0	1.50	0
<i>Dactylogyrus</i>	Gills	40	50	40	45	3.13	2.50	2.44	1.56
<i>Gyrodactylus</i>	Skin, fins, gills	30	40	30	20	9.17	2.33	1.75	1.75
Average		59.17	40.83	30.00	27.50	4.22	2.83	2.47	1.04

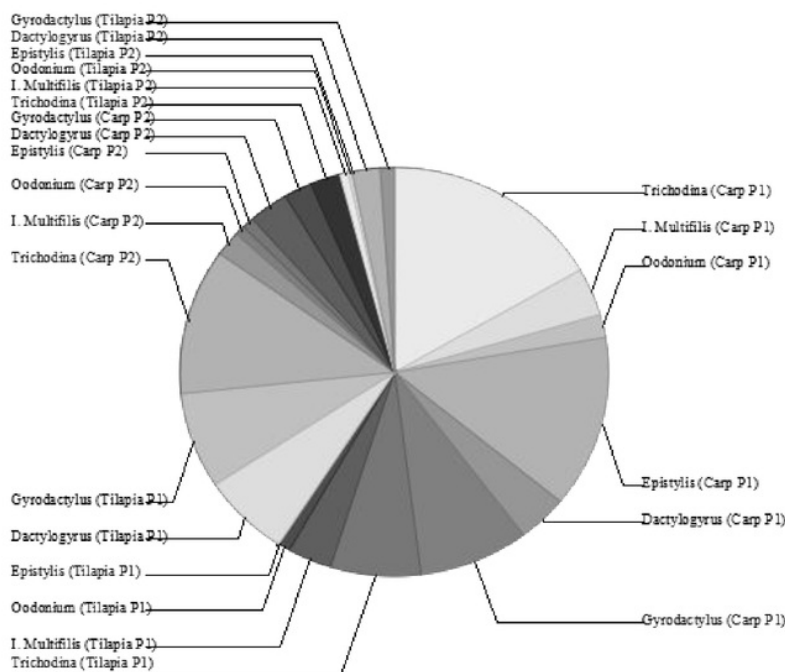


Figure 1. The type of parasites on the carp and tilapia found in the 1st pond (P1) and 2nd pond (P2)

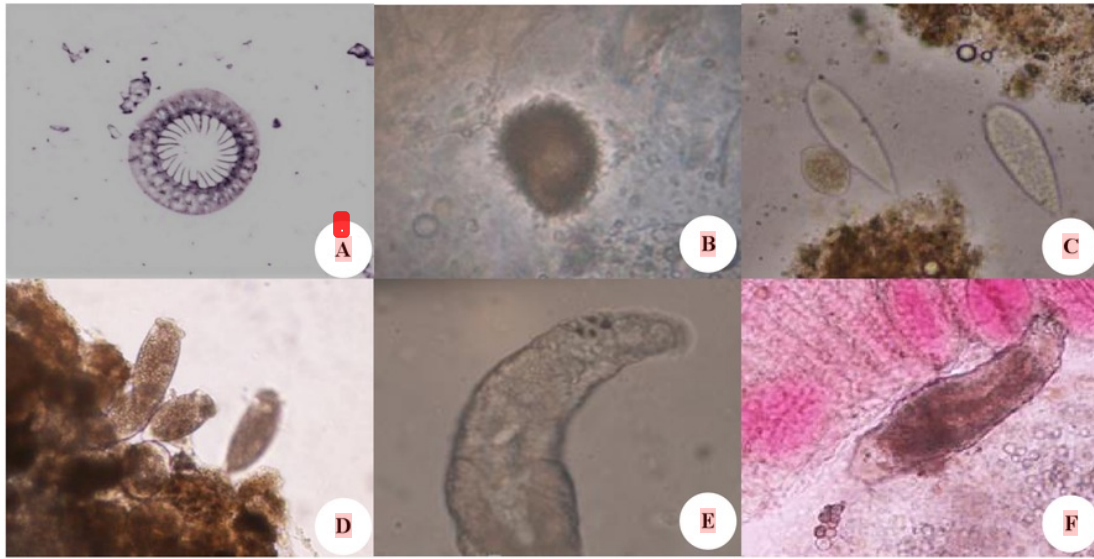


Figure 2. The type of parasites on the carp and tilapia found in the 1st pond (P1) and 2nd pond (P2) observed under a microscope. A. *Trichodina*, B. *Ichthyophthirius multifiliis*, C. *Oodinium*, D. *Epistylis*, E. *Dactylogyrus*, F. *Gyrodactylus*.

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