Study on the impact of environmental pollution: Parasitic infestation and conditions factor of fish living in amalgamation ponds

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Abstract. Artisanal and small-scale gold mining activities often leave pools. During rainy seasons, they form lakes where various species of fish live. Fish that live in amalgamation lakes or near streams may obtain contaminated water from mercury. Therefore, the aims of this research was to investigate the species of parasites as well as the condition of tilapia that live in the former pool of gold mining in Bombana Regency, Southeast Sulawesi, Indonesia. The samples that were used were tilapia fish (Oreochromis niloticus) with a total sample size of 40 fish. Fish condition factor was evaluated on the basis of body weight and length data. Examination of ectoparasites and endoparasites were performed on fish for prevalence and intensity parameters, while the measurements of heavy metals Hg were carried out on water and sediment and sampled four times. Based on the research results, three species of parasites attacking on external body of fish (ectoparasites) were found, namely Dactylogyrus sp., Ergasilus sp., and Microsporidia (Glugea sp.). Prevalence of those three parasites ranged from 10-90%, while intensity ranged from 1.7-3.5 parasite/fish. Condition factor of tilapia was between 2.70-3.81. Moreover, an average of Hg concentration was higher in sediment (0.167 ppm) than in water (0.039 ppm). Although it seems that the presence of parasites in fish is still relatively low and fish shows normal growth, people should not consume fish live in ex-mining ponds often for the health of the local community.

Keywords: mercury, pollution, fish condition, fish parasites

1. Introduction

Gold mining activities in various locations cause mercury (Hg) pollution problems. The impact caused by the presence of heavy metals in waters depends on the presence of metals in water and sediments, their toxicities, and their concentration in the environment. Heavy metals, if entered into the body of living things, will experience bioconcentration, bioaccumulation and biomagnification. Bioconcentration is the direct entry of pollutants from water by living things through tissues such as gills or skin [1]. While bioaccumulation is the entry of pollutants by living things from an environment through a mechanism or trajectory. While biomagnification is the process by which pollutants increase in concentration with the increasing position of living things in a food chain. According to [2] states that bioaccumulation of chemicals in waters is an important criterion in evaluating the ecology and level of pollution of an environment. To measure the level of water pollution is to measure the bioconcentration of the biota that lives in those waters.

There has also been an increasing interest in the role of parasites as bioindicators of heavy metal pollution in aquatic habitats, and in particular, in the interrelationship between parasitism and pollution [3][4][5]. This relationship is not simple and essentially involves a phenomenon in which
parasitization may increase the vulnerability of the host to toxic pollutants or in which pollutants may result in an increase or decrease in the infestation of some parasites.

Knowledge of parasitic infections in fish is not only related to fish health in particular, but also to the understanding of ecological problems [6-7]. Parasites may therefore be considered as a substitute for chemical analysis or conventional biological monitoring (number of bacteria and invertebrates) as an indicator of ecosystem dysfunction.

Mining activities in various regions have left various environmental impacts, one of which is the dredging of the land left behind, making big holes. The same cases happened at the gold mining site in Bombana Regency, Southeast Sulawesi, Indonesia. The large holes are inundated by water-formed ponds during the rainy season. Various fish live in the pond, even consumed by local communities by fishing.

One of the organisms that is mostly obtained from the former gold mining excavation is tilapia (*Oreochromis niloticus*). Fish as one of the aquatic biota that can be used as an indicator of the level of pollution that occurs in the waters. If in the body of the fish has been contained high levels of heavy metals and exceeds the predetermined normal limits can indicate the level of pollution that occurs in the environment. The content of heavy metals in fish is often associated with the disposal of industrial waste in the vicinity of the fish. The amount of heavy metal that is absorbed by the fish's body depends on the type of the compound and the concentration of pollutants, the activity of microorganisms, the texture of the sediment, and the types of fish that live in the environment [8].

One of the pollutants found in the former mining quarry is mercury. Not many people know that the area of the former gold mining waters may have been contaminated by Hg metal, as well as the dangers and impacts that will result from metals on aquatic organisms that live in these waters, even adversely affecting humans themselves because they consume fish from the area. Mercury is the only metal that is in liquid form at normal temperatures. Mercury is found in nature in the form of metals, inorganic salts and organic salts. In the form of inorganic salts, mercury can cause liver and kidney damage, because the highest Hg deposits in human internal organs such as in the liver and kidneys.

Parasites are organisms whose lives can adapt and harm other organisms that they occupy (host) and cause disease. The interactions that make up parasitic host relationships are complex. When a parasite tries to cause an infection, the host responds with a defense mechanism. The ability to prevent disease that will enter the defense mechanism is called resistance (immunity). The parasite harms the host because it takes nutrients from the host which can cause death. Fish parasites will choose the best attachment location in the body of the fish. Based on the location of the attachment, parasites can be divided into ectoparasites, endoparasites and mesoparasites [9]. According to [10], ectoparasites are parasites that live on the skin, gills, and parts of the outer surface of the body and endoparasites are parasites that live inside organ cells. Parasites interact with the host in various ways, they show both behavioral and physiological interactions with their hosts [11].

Given that pathogens are generally more proliferating in bad environments and with high susceptibility, there may be a relationship between parasitic attack and Hg pollution [12]. In this effort, it is necessary to conduct research that aims to determine the type of parasites that infect tilapia that live in the ponds of former gold mining in the Watu-watu Village, Lantari Jaya District, Bombana District.
2. Materials and Methods

2.1 Time and Research Location

This research was conducted from October 2018 to January 2019, which consisted of two main stages of activities, namely field and laboratory activities. Field activities include water sampling in the pool of the former community gold mining in Watu-Watu Village, Lantari Jaya District, Bombana Regency, SE-Sulawesi, Indonesia. Laboratory activities were parasitic examinations carried out at the Fisheries Laboratory, Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari, Indonesia.

2.2 Research Procedures

2.2.1 Field Sampling

Field activities include sampling which was taken from the location of the former gold mining ponds in the village of Watu-watu district, Bombana. Samples used were tilapia fish (*O. niloticus*) obtained from the bait. Sampling was carried out 4 times with the number of samples per collection being 10 fish in interval of one month per sampling (for four months).
2.2.2 Laboratory Examination

Ectoparasite examination procedure refers to the procedure proposed by [13], which observes the outside of the organism's body, then records if there was bleeding, injury or swelling and pays attention to the type of organism attached to the body of tilapia (O. niloticus). Scraping was done on certain parts of outside of the body of tilapia (O. niloticus) such as scales, tails, and gills. Taking parasites with tweezers then put on the glass object that has been provided and observed under a microscope with a magnification of 100-400x. The parasites found were identified books [13-14], and comparative journals [15-16].

The endoparasitic examination procedure refers to the procedure recommended by [17]. Dissecting fish was done carefully by cutting the upper abdominal cavity, starting from the anus to the pectoral fin. Abdominal cavity was opened and examined carefully, whether there was bleeding or attachment of disease organisms to internal organs. Then the organs and the parasite observes were removed using tweezers and the organs were moved into separate petri dishes. The internal organs were examined then observed, namely the stomach, intestine and liver. The organ was cut and placed in a petri dish. The contents in the stomach were scraped using a scalpel. Furthermore, the scraping results were inserted into the petri dish and stirred with aquadest. The mucus from the scraping was then observed under a microscope.

2.3 Observed Variables

2.3.1 Prevalence and Intensity of Parasites

The analysis of parasitic infestation for finding the incidence of infection (prevalence) and intensity were carried out by following equation [18]:

![Figure 2. Map of Research Locations in Watu-watu Village, Lantarijaya District, Bombana Regency, SE-Sulawesi, Indonesia](image-url)
2.3.2 Fulton's Condition Factor

Data in the form of total length and weight of fish are used to determine the Fulton’s condition factor (FCF), calculated using the formula [19]:

$$FCF = \frac{W}{L^3} \times 100$$

W = total weight of fish, and L = total length of fish

2.3.3 Hg concentration

Detection and measurement of mercury (Hg) heavy metals. Determination step of heavy metal was divided into several stages, namely destruction, reading absorbance of samples, and calculation of heavy metal content. Parts of the fish's body (muscles) and other internal organs (such as liver, intestines, and gills) were separated surgically and washed with tap water followed by stratified distilled water. After being weighed the fish was dried in an oven at 101°C for 3 hours. Samples of dried fish organs were then crushed to powder with a mortal device and stored in polyethylene at 30°C until analysis. Subtle samples were then destructed based on the AOAC (2012) method, that was, the heavy metal elements in a sample were degraded by wet ashing using a mixture of concentrated acid HNO3 and HClO4. For this stage, 2.5 ± 0.5 g for each organ uses microwave digest. After being destructed, the residue was then dissolved into 25 ml of 2.5% HNO3. About 1 ml of HNO3 was added to the sample water to prevent the utilization of heavy metals by microorganisms. Samples of fish, sediment and water that have been destructed were then measured with Atomic absorption spectrometry (AAS) (Variant) for heavy metals Hg.

The concentration for each heavy metal element was expressed in milligrams (mg) per gram of fish weight. The mean value and variation from a minimum of two replications were used to interpret and analyze the data. To guarantee the accuracy of the data, Certified Reference Material (CRM) was used. The method used was validated first and then the measurement detection limit was determined.

3. Result

A total of 40 samples of nile fish were examined during the study period, resulting in the detection of three different species of parasites (three ectoparasites and none endoparasites). Ectoparasites were found mainly in the skin (the entire body surface of the hosts as well as the fins) and gills of the host fish. Based on observations of parasites from different sampling periods, varying values of intensity and prevalence have been obtained (Figure 3 and 4).
Figure 3. Level infestation of parasites (intensity) on nile fish among sampling period.

The picture above shows that the intensity of the parasitic infestation of parasites from October to January in rainy season. The overall highest mean intensity value was 3.50 (recorded in October by Dactylogyrus sp.), while the lowest was 1.00 (recorded in December by Ergasilus sp). Dactylogyrus sp. decreased from sampling I to sampling IV with the acquisition of values of 3.5; 2.3; 2.0; and 2.6 individuals/fish, respectively. The intensity of the parasitic infestation of Ergasilus sp. obtain different results for each sampling, namely in sampling I reached 1.7 individuals/fish, sampling II reached 2.2 individuals/fish and sampling III reached 1 individuals/fish. The intensity of parasitic infestation of Microsporidia (Glugea sp.) has increased from 0 to 3 individuals/fish in sampling I to sampling II, while in sampling III and IV had decreased to 1.8 individuals/fish.

Figure 4. Level infestation of parasites (prevalency) on nile fish among sampling period.
Monthly fluctuations in the overall prevalence of total parasites are shown in the Fig. 4. The highest parasite prevalence (90.0%) of Glugea sp was observed in December, while none (0%) was observed in October. Parasites Dactylogyrus sp. always had a relatively high prevalence in each sampling. While parasites Ergasilus sp. often had a fluctuation in prevalence, but tend to be the lowest infestation.

Figure 5. Mercury concentration of water and sediment in nile habitat among sampling period.

Figure 5 shows the results of measurements of heavy metal Hg concentration in water and sediments or subgrade in the pits of the former gold mining excavation. Based on the above data, it shows that at each sampling the concentration of heavy metal Hg in the pond tend to decreases, especially in water. From the beginning of sampling to the end, the concentration of Hg in water decreased from 0.059 ppm to 0.044 ppm. Slightly different in sediment, the change in Hg concentration was not noticeable at each sampling time. However, the lowest concentration was found in the last sampling of the study period (0.148 ppm), which was (0.163 ppm) at the beginning of sampling. The data on concentrations of Hg in both organs that play an important role in the accumulation process have been shown in Figure 6 below. Nile tilapia that live in ponds suspected of being contaminated with Hg have been considered to be studied. Data on average Hg concentration in muscle showed about 2.5 times higher than that accumulated in gills.
Figure 6. Mercury concentration of muscle and gill in nile fish living in the pond.

The condition factor is one that is used to describe the plumpness of a fish which is stated based on the measured length and weight of body. Based on the results of the study the average value of the condition factors in tilapia can be seen in Figure 7.

4. Discussion

4.1 Parasitic infestation in tilapia lived in ponds

During this research three species of parasites recovered from 40 fish. The ectoparasites mainly found on gill and skin of fish, namely monogenean *Dactylogyrus* sp., crustacean *Ergasilus* sp. and protozoan *Glugea* sp. While internal part of the the body, parasites were not found classified as endoparasites.

The dominant nile fish found was the species of parasite *Ergasilus* sp. which infected the gills. That is because the gills are breathing apparatus in fish that are directly related to the waters in their environment. This finding is consistent with the statement of [20] that parasites often concentrate in...
the gill. Due to their structure, location and mechanism, gills are in direct contact with the environment, which makes them very susceptible to changes in environmental conditions and they are the right place to survive infections by pathogenic organisms that cause diseases such as parasites. According to [21], that the Ergasilus sp. parasites may extend over large areas of the gills, causing fusion and embedding of lamellae, and thus impacts the function of the gill in osmoregulation, as well as respiratory gas exchange. This effect is enhanced by high temperature in the tropics, which causes a reduction of the oxygen binding ability of water, creating additional stress to the host fish.

The parasite species Ergasilus sp. is a parasite with an oval body shape, has 4 pairs of swimming legs and in the anterior region there are claw-shaped legs that are used to attach themselves to the host. The parasite Ergasilus sp. females have egg sacs in the posterior part, reproduce by laying eggs and can infect the skin. Fish infected by the parasite Ergasilus sp. will have difficulty absorbing oxygen (O2) and can cause stunted fish growth [21].

Besides Ergasilus sp. another parasite that infects Nile fish was Dactylogyrus sp. found in the gills and skin. It is a parasitic organism that attacks fish on the outside of the body or so-called ectoparasites. The parasite Dactylogyrus sp. often found in freshwater fish and sea water fish. Dactylogyrus is a monogenean attached in gills, contrary Gyrodactylus usually on skin, both worms can be collected from gills and skin in the same fish [22]. The presence of parasitic organisms can inhibit the growth of fish and even death, so that its presence can cause harm to the cultivation business. According to [23], the fish that are infected by this parasite will generally become thin and the skin does not look clear anymore. The mechanism of respiration and osmoregulation is interrupted (fish seem to cough for lack of oxygen), unnecessary white blood cells, often seen as fish rubbing their bodies against the bottom or the pond. The mechanism of respiration and osmoregulation is interrupted (fish seem to cough for lack of oxygen), unnecessarily white blood cells, often seen as fish rubbing their bodies against the bottom or the pond.

The fish culture activity almost always meet problems of fish diseases such as ectoparasites infection. The monogenean infection can have an effect on immune responses of parasitized fish [24]. Nevertheless, the losses due to ectoparasitic infections are not as high as the losses caused by infections of other phagogenous species, such as viruses and bacteria. Various factors affecting parasite assemblies in host fish, including seasonality and other environmental factors that may promote the establishment and proliferation of parasites in host populations [25] Fish are cultivated in poor environmental conditions (crowding, poor water quality) and various pollutions can be accelerated the rate of reproduction of parasitic. The impact of parasites on their fish hosts may be exacerbated by various toxins including heavy metals and hydrocarbons, organic contamination of domestic sewage sediments, and others such as life cycles of parasites and the size of the fish population [26].

Intensitas value of parasites that infect Nile fish ranges from 1-3.5 individuals/fish. The highest intensity is found in parasites of Ergasilus sp. in sampling I and lowest found in parasites of Dactylogyrus sp. in sampling III (Figure 4). This shows that the level of parasitic attacks in Nile fish was still classified as mild parasites and has not shown clinical symptoms that can indicate the presence of diseases caused by ectoparasites. This is in accordance with the statement of [27] that the intensity of parasitic attacks of 1-5 individual/fish has not been harmful to the life and growth of aquatic organisms in ponds. Low intensity of Dactylogyrus sp. allegedly due to a joint infection between two or more parasitic species. According to [28] that joint infections between species will inhibit the development or even harm other parasites. In addition, joint infections can also be synergistic or mutually supporting the life of each parasite.

The highest prevalence was found in parasites of Glugea sp. those who infect Nile fish obtained a prevalence value of 90% in sampling III. Based on the category of [27] suggested that the prevalence is included in the category of "very often" which means the level of parasitic infection type Glugea sp. very often infects Nile fish. While the lowest was found in the parasite Ergasilus sp. the prevalence value was 10% at sampling IV. According to [27] suggested that the prevalence is
included in the category of "almost never" which means the level of parasitic infection has never been.

The difference in intensity and prevalence of each sample was suspected due to several factors, one of which was host size. According to [28], the prevalence and intensity of each parasite species is not always the same due to the many factors that influence the size of the host. In some fish species, the larger the size of the fish, the higher number the parasite infection.

4.2 Mercury Accumulation (Hg) in Fish and Their Habitat

The results of the water analysis found that the concentration of mercury metal in the pool in the former pit mining area of the Bombana Regency reached 0.059 ppm (Figure 7), this has exceeded the threshold several times from the established standard of the content of mercury. The World Health Organization has set the maximum consumption level for foodstuffs is 0.5 ppm total Hg [29]. This is also supported by the decision of Minister of Health Republic Indonesia No. 907/Menkes/ VII/2002 that the allowable mercury content in water is not more than 0.001 mg/l.

The presence of Hg in water and sediment is thought to be due to gold mining activities carried out at these sites. Heavy metal content in fish habitats at high concentrations can cause harm. They were initially required for various metabolic processes to become poisons for aquatic organisms. Increasing rainfall in December and January led to the increase the concentration of Hg in water, but this is not the pattern seen in sediment (Figure 7). The decrease in the concentration of heavy metals in waters is also influenced by the rainy season. [30], explains that from the pre- to post-rainy season, the content of heavy metals in water tends to be lower due to dissolution, whereas in the dry season it tends to be higher because the metal becomes concentrated.

Fish is an aquatic biota that can usually be used as a bioindicator of water pollution. If fish that live in waters that have been contaminated with heavy metals such as mercury (Hg), there will be accumulation in the body of the organism. According to [31-32], heavy metals accumulation in fish occurs directly as it is exposed to contaminated water and accumulates in the body through the skin, gill and indirectly by feeding contaminated aquatic organisms through the digestive tract, resulting in tissue and organ damage. Heavy metals can accumulate in the body of the fish through several ways including breathing (respiration), food channels (biomagnification) and through the skin (diffusion). These heavy metals can not be degraded and are therefore permanently added [32].

The results of the comparative observations, Hg concentrations in water was higher than gills and muscle. The high content of heavy metals in water was influenced by the sedimentary water environment being absorbed by organisms and accumulation. This is in accordance with the research [33], that heavy metals entering the aquatic environment will experience precipitation, dilution and dispersion, then absorbed by organisms that live in these waters. Precipitation of heavy metals in a waters occurs because of the presence of carbonate and hydroxyl anions and chlorides.

The results of observations on the gills reached 0.014 ppm in Figure 6. Gills are organs that are in direct contact with toxic substances in the waters. This is explained by [34], that the gills are the first organs that are directly related to toxic substances in the water, with a wide and open surface, thus causing this section to become the main target for the toxic substances in the waters.

Muscle is the result of the body's metabolism entering through the bloodstream, including metal deposits. The results of the analysis of mercury content in fish muscle organs can be seen in Figure 6 showing the average higher mercury content compared to gill organs and water. Levels of mercury in muscle was 0.036 ppm. There is a possibility of higher Hg levels when using larger fish samples. In this work, the size of the fish used varies from 5-25 grams, indicating that the age of the fish was still young, leaving low accumulation of heavy metal Hg in the muscle. This is in accordance with the research of [35], that the difference in Hg content in the fish body is caused by the ability of biota uptake to different metals and is very dependent on the size and feeding behavior of the organism. Tilapia muscle that lives in a former pit of a gold mine excavated in Watu-watu Village, Lantarijaya District, Bombana Regency was still below the threshold (0.5 ppm), licensing by the Republic of Indonesia Drug and Food Control Agency (BPOM-RI 2009).
Hg accumulation in the body of fish is assumed to influence the health of fish, which is usually indicated by non-ideal growth. According to [36], condition factors indirectly indicate the physiological conditions of fish that receive the influence of intrinsic factors such as gonad development and fat reserves, while the extrinsic factors, such as food availability and environmental stress.

The average value of condition factors in tilapia obtained during research period was ranging from 2.7-3.81, this was indicated that the fish are in good condition. This result is consistent with [37], suggesting that the state of the fish was higher than average. Initially we suspected that Hg pollutants in water would affect fish growth. However, based on the results of the study, condition factor of tilapia was ideal. According to [38], condition factors are influenced by biotic and abiotic factors, and whether or not the waters are managed, including the number of organisms that exist, the condition of the organism, food availability and environmental conditions of the waters. In line with [39-40] that condition factor very closely related to parasite infections, physiological factors and environment stress. Although the Hg concentration data in the waters found was 0.030 - 0.059 ppm, the values of condition factor recorded in the present study were 2.7-3.81, indicating they were in good fitness. Condition factor of greater than one showed the well-being of fishes. The appreciable growth rate exhibited by the fish condition factor indicated that the prevailing environmental conditions were within the tolerance range for the species [41].

5. Conclusion
The present study describes the intensity of parasitic infestation of nile tilapia (O. niloticus). During the study period we identified three parasite species. The intensity and prevalence of parasites indicate that the presence appears at every monthly sampling. The highest peak of parasite intensity and prevalence was recorded in December, while the lowest was recorded in September. This might be due to their physico-chemical parameters. Hg pollutants that are detected in sediments at each monthly sampling in water and sediments can show an association of parasites and Hg pollutants. However, more in-depth research is needed to explore the parasitic infestation of in that habitat. The presence of parasites and Hg pollutants found in the environment and even accumulating on the body has not yet had an effect on fish condition factor. The authors hope that this study will promote further parasitological research on fish that live in metal polluted areas.

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