Histopathological study of hepatopancreas and kidney of butini fish (*Glossogobius matanensis*) in Matano Lake, South Sulawesi, Indonesia, caused by metal contamination

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Abstract. Butini fish (*Glossogobius matanensis*) is one of the most endemic species of freshwater fish that lives in Matano Lake. The purpose of this study was to determine the histopathology of hepatopancreas and kidney from butini fish. The samples used were six Butini fishes with a size of approximately 18.5–20.5 cm. Organ preparations (hepatopancreas and kidneys) were fixed using 10% neutral buffered formalin (NBF), dehydrated using multilevel alcohol, embedded using paraffin, cut with a thickness of 5 µm, stained using Haematoxylin-Eosin (HE) then covered for microscopic observation. Data analysis used was qualitative descriptive. Measurement of metal content of Matano Lake water showed nickel content of <0.0184 mg/L, iron 0.0238 mg/L, zinc 0.0491 mg/L, and copper <0.0136 mg/L. Histopathological study on hepatopancreas showed fat degeneration and cell necrotic, while kidney showed renal tubular lysis, basal membrane detached, and cell necrotic. Thus, the high concentration of metal was thought to be related to the abnormal tissue of fish organs observed in this study.

1. Introduction
Matano Lake is located in East Luwu Regency, South Sulawesi, with 595 m depth and has many endemic species. The endemic species, consists of freshwater and marine water biota, are commodities that can be developed [1]. Among them are butini fish (*Glossogobius matanensis*) [2]. Many local and foreigner researchers were interested in taxonomy, anatomy, and other subjects of this species. The population of butini fish is decreasing by years, due to human activities which cause environmental damage and also due to the presence of other predator alien species. Human activities caused habitat degradation and extinction, pollution, and commercial exploitation. The climate change also threatens fish survival [3].

The extinction of freshwater fish is about 78%, due to habitat changes, loss of habitat, places for feeding, breeding and shelter causing fish unable to adapt to environmental changes. Of the 86.2% endangered freshwater fish, is estimated as endemic species [4]. This study aimed to determine the histopathological changes in the organs of butini fish (*Glossogobius matanensis*) collected from Matano Lake.
2. Materials and methods

2.1. Measurement of metal content
Measurement of metal content in Matano Lake was determined by water quality test and grab sampling method. The sample types were water from Matano Lake, and the grab samples were wastewater from a particular location.

2.2. Animals sampling
Butini fishes were collected by local fishermen using fishing rods at a depth of ±25–50 meters. Six butini fishes with a size of approximately 18.5–20.5 cm and unsexed, were collected. The gills, hepatopancreas, and kidney were sampled for histopathological study.

2.3. Histology preparations
Tissue samples were fixed with Buffered Neutral Formalin (BNF) with a volume of 10 times of the samples. The fixation time was 48 hours. Further, the specimens selected for examination were cut to a thickness of 0.5–1 cm then inserted to processing basket. The embedding cassettes filled with tissue specimens then inserted into tissue processor with fixation time set as follow, gradual dehydration 70% for 1 hour, 90% for 1 hour, 100% for 1 hour, 100% for 2 hours, and 100% for 2 hours. Further was clearing process using toluene three times, each was for 1 hour and 1.5 hours, followed by impregnation two times, each was 2 and 3 hours. The embedding cassettes then removed from the tissue processor. The specimens then blocked by paraffin, cut by microtome to a thickness of 5–6 µm. The specimens stained using Haematoxylin-eosin, dehydrated, then covered for microscopic observation.

2.4. Microscopic observation
Observations were carried out using a light microscope equipped with Optilab® and magnification of 10×40.

3. Results and discussion
Measurement of metal content in Matano Lake water (table 1) showed that zinc and copper content approach the maximum content limit refers to South Sulawesi Governor Regulation No. 69 of 2010 concerning Standard Quality and Criteria for Environmental Damage Appendix I Criteria for Water Quality (Class III).

| No. | Metals | Unit | Result | Maximum content limit |
|-----|--------|------|--------|-----------------------|
| 1.  | Nickel | mg/L | <0.0184| -                     |
| 2.  | Iron   | mg/L | 0.0238 | -                     |
| 3.  | Zinc   | mg/L | 0.0491 | 0.05                  |
| 4.  | Copper | mg/L | <0.0136| 0.02                  |

Nickel and iron’s maximum content limit is not regulated in the South Sulawesi Governor Regulation Number 69 of 2010 concerning Standard Quality and Criteria for Environmental Damage Appendix I Criteria for Water Quality (Class III), but both are dissolved heavy metals which are not allowed in clean water or fisheries ecosystem. Those metals were thought to originate from the mining industry and also from community waste around the Matano Lake.

Nickel is heavy metal, toxic to the living organism. This metal poisoned the blood, affected the respiratory system, caused tissue and mucosal membrane damage, changed cell system, chromosome damage. In the year 2006, the European Union (EU) community proposed World Trade Organization (WTO) to categorized nickel as a dangerous substance. Fish mortality due to heavy metals is caused
by the reaction of cations with oxygen and certain fractions of lenders and disturbs the biochemical processes that occur in the blood [5].

Two of six butini fish samples showed liver enlargement (hepatomegaly). Gross anatomical observations showed the enlargement in the liver (figure 1B), characterized by a larger liver size than other fish (figure 1A) and showed fat deposition. The liver enlargement was associated with the malfunction of this organ, caused by overactivity. The fat deposition caused a more yellowish color of the hepatopancreas compared to other fishes.

Figure 1. Gross anatomical observations of butini fish. Normal liver (A) and hepatomegaly (B)

The histopathological observation of hepatopancreas showed fat degeneration and necrotic cells (figure 2). While the kidney showed renal tubular lysis, basal membrane detached, and cell necrotic. (figure 3). Fat degeneration occurred because of the accumulation of fat (neutral fat) with the damage of the cell nucleus and shrinking liver cell tissue. Research conducted by [6] showed that milkfish (Chanos chanos Forsskall) exposed to Pb metal resulted in liver degeneration.

Figure 2. Cross-section of the butini fish hepatopancreas. Vacuoles (black arrow), necrotic cells (blue arrow). HE staining. Magnification 400×.

Fat degeneration is characterized by the presence of vacuoles in histopathological features. Figure 3 showed the lysed tubules. In addition, the expanding intertubular space is accompanied by a detachment of the tubular basement membrane. In accordance with the study of the effect of mercury chloride on the growth and histopathology features of Nile tilapia (Oreochromis niloticus Linn) kidney conducted by [7], the administration of mercury chloride with the concentration of 0.04 ppm showed lysis and necrosis of tubules epithelium.
Figure 3. Cross-section of the butini fish kidney. Lysis of tubules (grey arrow), detach of the basal membrane (orange arrow), and necrotic cells (arrowhead) of the kidney. HE staining. Magnification 400×.

Damage to the cell wall or inhibition of cell wall synthesis due to certain compounds will result in cell lysis. The longer exposure of toxic compounds to the kidney will increase the number of kidney cells necrosis [8]. Necrosis is described as the condition of a decrease in tissue activity characterized by the loss of several parts of the cell, gradually, until the cell died. The death of cells or tissues following cell degeneration in a living organism is an irreversible stage [8]. The tissue damages that occur were allegedly caused by the exposure of dissolved heavy metals to fishes in the ecosystem.

4. Conclusion
The detection of dissolved heavy metals in Matano Lake water was suspected to cause tissue damage in butini fish. Histopathological study on hepatopancreas showed fat degeneration and cell necrotic, while kidney showed renal tubular lysis, basal membrane detached, and cell necrotic.

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