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Analysis of Kidney Tissue Keting *Mystus gulio* (Hamilton, 1822) in Gembong and Rejoso River, Pasuruan, Indonesia

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Abstract: *Mystus gulio* is a local fish that can open up opportunities for the community. Pollution causes water quality to decrease. Fish are an important indicator in the aquatic environment, Histological analysis can be said to have a function as a natural biological sign to determine the health condition of fish which can be seen from changes in tissue structure in organs exposed to pollutants. Organs that's vulnerable to water pollution is kidneys. Kidney tissue is susceptible to damage because kidneys function as a means of filtration and excretion, The method used in this research is descriptive method. Fish were caught from river than dissected to remove the kidneys for histopathological analysis using the HE method, analyzed using the scoring method. The results obtained in both rivers were that fish kidneys caught in the Gembong River were categorized as moderately damaged, while fish kidneys caught from the Rejoso River were categorized as lightly damaged. This is in line with the results of the heavy metal lead from both rivers. Where the yield of lead in fish kidneys caught from the Gembong is on average higher than the yield from fish kidneys caught from the Rejoso.

Keywords: Histopatology; Kidney; Lead; *Mystus gulio*

Introduction

Keting *Mystus gulio* is a type of catfish with a long mustache, *Mystus gulio* belongs to the order Siluriformes of the Bagridae family. The origin of this genus is from the coastal waters of Bangladesh and several countries in Southeast Asia, from India, Indonesia, Vietnam and Pakistan (Rahman et al., 2020). *Mystus* gulio is a fish that can be used as a bioindicator of ecology contaminated with heavy metals (Lestari et al., 2021; Paujiah et al., 2023).

Pollution causes the quality of water to decrease, which can cause damage to the organs of aquatic organism. Fish are an important indicator in the aquatic environment, because fish are directly affected by pollution. Histological analysis can be said to have a function as a natural biological sign to determine the state of fish health which can be seen from changes in tissue structure in organs exposed to pollutants. Histopathological examination has the function of diagnosing infectious diseases, namely by knowing the possible causes of infection (Manan et al., 2015). Pollutants have detrimental effects on the physiological, reproductive and immune functions of fish. Pollutants can also cause pathological abnormalities in fish at various levels. starting from the cell, tissue and organ level (Karim et al., 2022). Fish tissue is easily exposed to heavy metals. If you have been exposed to heavy metals for a long period of time, they will accumulate in the

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organism's body which can cause biomagnification (Bawuro et al., 2018; Lestari et al., 2021).

One of the organs that is vulnerable to water pollution is the kidneys. Kidney tissue in fish is susceptible to damage due to the kidney's function as a tool for filtration and excretion, so if the kidneys cannot function properly it will disrupt the body's metabolism in fish (Billah et al., 2023). According to Mandia et al. (2013), one of the organs most sensitive to pollution is the kidneys. The kidneys perform important functions related to electrolyte and water balance and maintaining a stable internal environment (*Osmoregulation*). The fish kidneys can be used as an indicator of water pollution. The aim of this research was to determine kidney damage in fish caught in the Gembong River and Rejoso River.

Method

The fish samples used in this research from Gembong and Rejoso River which were collected between Octobers to December 2023. To collect *Mystus gulio* samples using fishing gear, namely hand fishing. Then tissue collection was carried out in the pathology laboratory, Faculty of Medicine, Brawijaya University, Malang.

Rules of hystologi preparation Samples from fish kidneys Figure 1 were first fixed using 10% NBF for 24 hours. Next stage is based on Oktafa et al. (2017).

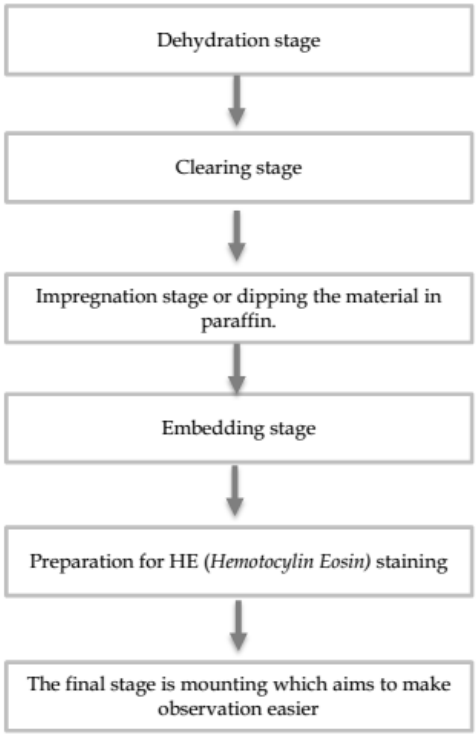

Figure 1. Rules of histology preparation sample

Data analysis used in this histology is using descriptive methods which are linked to the literature. The tissue samples that had been stained were then observed using a microscope with 400x magnification. Next, the histology preparations were photographed using an Optilab digital microscope camera with Optilab Viewer software. Next, cell damage is calculated and analyzed to determine the level of damage using a scoring system. The percentage of damage is calculated using the following formula:

$$\text{Percentage} = \frac{\text{number off damage cells}}{\text{number of cells analized}} \text{ x } 100\% \quad (1)$$

Damages were then matched to a Table 1 according to Wolf et al. (2015), as follows:

Table 1. Scoring Hystopatologi

Percentage of damage	Damage category
P < 25%	Normal
25 ≤ P < 50%	Slightly damage
50 ≤ P <75%	Moderate damage
P > 75%	Badly damage

Water quality measurements are carried out in situ and ex situ for in situ water quality such as temperature (using an Hg thermometer), pH (using a pH meter), dissolved oxygen (using a DO meter), while the other parameters were carried out ex situ for the TSS parameters (using the gravimetric method), COD (using spectrophotometry) and lead (using the Atomic Absorption Spectroscopy method).

Result and Discussion

Based on the results of histopathological observations of the kidneys in *Mystus gulio* caught in the Gembong and Rejoso, it was found that there were 3 types of damage to the kidneys, these damages were Necrosis, Congestion and Cell Degeneration. Among the three damages, the highest average percentage of damage was necrosis.

Necrosis is cell death that occurs due to pathological conditions such as infection or inflammation. Necrosis is characterized by changes in the nucleus that cause the nucleus to lyse and also rupture or fracture (Purwaningsih, 2017; Wyllie et al., 2000). This is in line with the opinion of Jamin et al. (2016), Necrosis is cell death and death of body tissue. Signs of necrosis can be recognized because cells or tissues show certain changes. Damage in the form of necrosis in kidney tissue caught in the two rivers can be seen at Figure 2.

Necrosis was found in both rivers. This necrosis has the characteristics of a pale color compared to the others.

This necrosis has a poor consistency or is deformed. Kidney damage in fish is most often found as a result of changes in water quality. One of the damages is necrosis and degeneration. Necrosis is a decrease in tissue activity which is characterized by the loss of several parts of cells so that over a relatively long period of time it can cause death. According to Yolanda et al. (2017), necrosis is an advanced stage of degeneration due to too many substances which must be reabsorbed by hepatocyte cells so that cell death occurs. Necrosis is the occurrence of cell death.

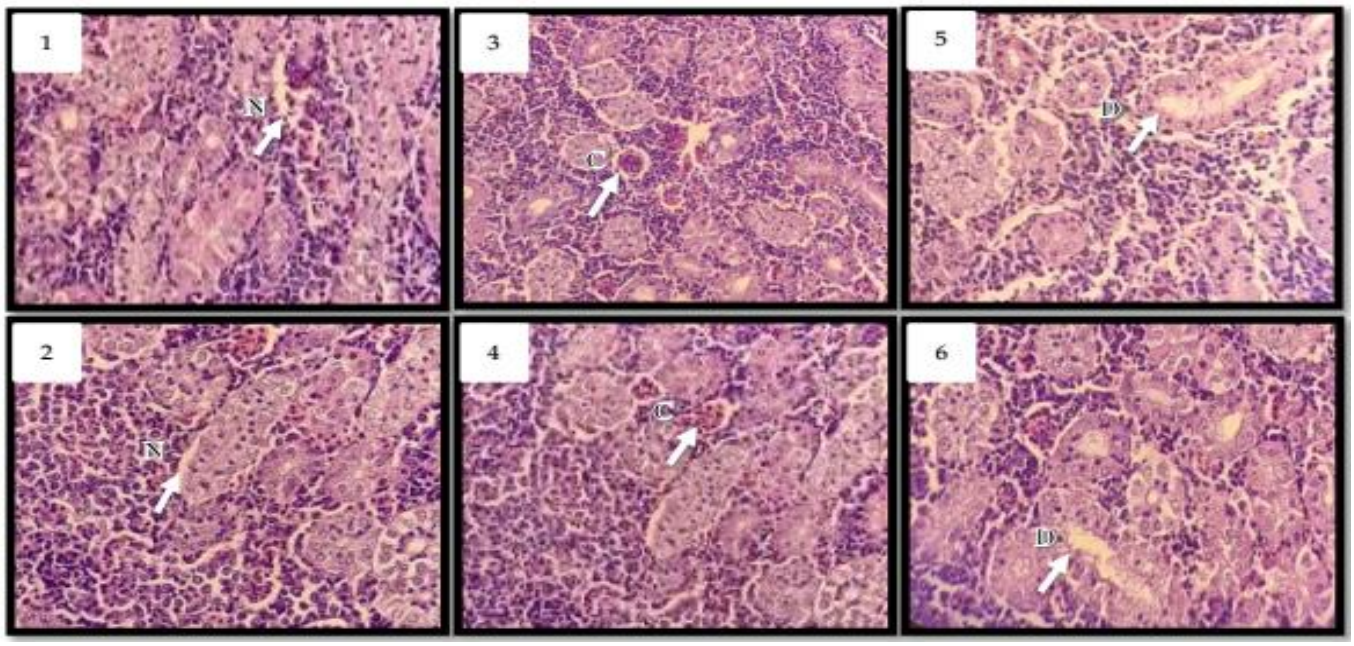

Figure 2. *Mystus gulio* kidney histology (400x magnification). (1) *Mystus gulio* kidneys from Gembong which is necrosis; (2) *Mystus gulio* kidneys from Rejoso which is necrosis; (3) *Mystus gulio* kidneys from Gembong which is congestion; (4) *Mystus gulio* kidneys from Rejoso which is congestion; (5) *Mystus gulio* kidneys from Gembong which is degeneration; (6) *Mystus gulio* kidneys from Rejoso which is degeneration. Information: (N) Necrosis; (C) Congestion; (D) Degeneration

Damage in the form of congestion in kidney tissue caught in the two rivers can be seen in Figure 2. Congestion was found in the fish tissue in both rivers. This congestion is characterized by the presence of blood droplets and a buildup of blood in one particular area. The continuous entry of toxic substances results in fluid accumulation and results in injury to the blood vessels, so that other blood components enter the injured bloodstream and result in widening and accumulation of blood vessels. This is in line with the statement from Mayasari (2017), which states that there is dilation of blood vessels and inside. The vessels are full of blood (exceeds normal capacity).

Damage in the form of cell degeneration in kidney tissue caught in the two rivers can be seen in Figure 2. The results of the observations found cell damage in the type of cell degeneration which was characterized by the presence of empty space and an increase in cell size. This is caused by cell instability caused by the presence of toxic substances, which triggers an increase in fluid from outside the cell entering the cell, causing swelling.

Table 2. Percentage of Tissue Damage in the Kidneys of Fish Caught in Gembong and Rejoso

River	Monts	Damage type kidney histopatology			Average (%)	Type of damage
		Necrosis (%)	Congestion (%)	Degeneration (%)		
Gembong	October	78	44	44	56	Moderate
	November	56	44	56	52	Moderate
	December	89	67	33	63	Moderate
Rejoso	October	56	22	56	44	Slinghtly
	November	78	11	33	41	Slinghtly
	December	56	44	44	48	Slinghtly

The average percentage of Mystus gulio kidney tissue damage can be seen in Table 2. Based on Table 2, damage to the kidney tissue of *Mystus gulio* in the Gembong River is around 52-63% with moderate

damage status, in the Rejoso River it is around 41-48% with slightly damage status. In both rivers, the order of most damage is necrosis, congestion and cell degeneration. The percentage of necrosis type damage is higher compared to other types of damage. The damage caused by necrosis generally occurs as a result of toxic materials such as heavy metals.

Necrosis is a condition of cell death that occurs as a result of acute cell damage. Cell death that occurs is uncontrolled. What causes necrosis is generally a pathological stimulus. Apart from that, it also happens because it is programmed, where after the cell reaches a certain life span, the cell will die. This mechanism is called apoptosis or cells destroying themselves (Indriana et al., 2020). Meanwhile, congestion according to Sandra et al. (2021), congestion in the kidneys generally occurs due to an inflammatory reaction due to the presence of toxic substances in the kidneys. When toxic materials enter the body, the main response that occurs is the accumulation of fluid from the blood vessel system and the migration of lymphocytes, neutrophils, macrophages and other blood components towards the area where the wound occurred. Based on the opinion of Wagiman et al. (2014), cell degeneration occurs because the electrolyte charge outside and inside the cell is in an unbalanced condition. The instability of the cell in pumping sodium ions out of the cell can cause an increase in the influx of extracellular fluid into the cell so that it is unable to pump enough sodium ions. This causes the cells to swell and causes the cells to release cell material which results in cell death or necrosis. According to Fahrimal et al. (2016), and Jannah et al. (2018), cell degeneration is a condition where cells experience loss of normal cell structure as a result of external influences. Cell degeneration causes accumulation of materials intracellularly and extracellularly which is the beginning of damage to a cell in the opinion of Abubakar et al. (2019), any damage to the kidneys will generally cause big problems. For example anemia and reduced formation of new blood cells.Damage to the kidney tissue of *Mystus gulio* caught in the Gembong River and Rejoso River is generally caused by water quality. The results of water quality parameters in both rivers can be seen in Table 3.

Table 3. Average Water Quality in the Gembong and Rejoso River. (G) Gembong; (R) Rejoso

Monts	Water Quality Parameter											
	Temperatute (°C)		TSS (mg/l)		pH		Dissolved Oxygen (mg/l)		Chemical Oxygen Demand (mg/l)		Lead (mg/l)	
	G	R	G	R	G	R	G	R	G	R	G	R
October	28.6	27.9	133	105	6.9	7.24	6.05	5.9	37.4	29.5	0.9	1.02
November	29	27.6	128	99	7.18	7.1	5.17	6.08	32.8	33.2	1.14	1.3
December	29.6	29.1	159	104	7.12	7.2	4.7	5.4	32.7	27.9	1.54	1.5

From the results shown in Table 3, it can be seen that the water quality that exceeds the quality standards is TSS and Lead. Lead is a heavy metal that is toxic if it enters the body of aquatic biota. According to Wahyuni et al. (2017), the kidney is one of the organs that is easily exposed to toxic materials that enter the fish's body, so that if toxic materials enter the fish's body, it will show changes or damage to the fish's kidney tissue. Kidneys are important body organs in fish. The kidneys have a role as water regulators in the fish's body (osmoregulation). Another water quality that exceeds the threshold is TSS. The other water quality parameters are still in the optimum category, all for the class III water quality standard category Table 4.

Water quality standards based on Government Regulation Number 22 of 2021 can be seen in Table 4. The body of aquatic biota doesn't need the lead, so if there is heavy metal in the body of biota, the body will excrete some of it. And the rest accumulates in certain parts of organs, such as the kidneys and results in damage to kidney tissue. The content of heavy metals in aquatic biota is influenced by the levels of heavy metals in the water (Dharmadewi et al., 2019). In the lead test carried out on the kidney organs of *Mystus gulio* caught in these two rivers, the results obtained can be seen in Table 5.

Table 4. Water Quality Standards

Parameter	Category III
Temperatute (°C)	25-29
TSS (mg/l)	100
pH	6-9
Dissolved Oxygen (mg/l)	≥3
Chemical Oxygen Demand (mg/l	≤40
Lead (mg/l)	≤0.03

Table 5. Average Lead in Kidney

Monts	Lead in Kidney (mg/kg)	
	Gembong	Rejoso
October	1.11	0.95
November	1.16	1.13
December	1.71	1.49

Lead result from the kidneys is in the same direction as the lead result in the water. The average lead value in the kidney organs of fish caught in the Gembong River is higher compared to the average lead

value in the kidney organs of fish caught in the Rejoso. This is also in line with the results of scoring damage to the fish's kidney organs, where in the kidneys of fish caught in the Gembong, the scoring results showed that they were in the moderately damaged category. This is in line with the results of the average lead value in kidney organs, which tends to be higher than that of fish caught in the Rejoso. In the scoring results, the kidneys of fish caught in the Rejoso were slightly damaged.

The high and low levels of the heavy metal Pb in the fish's body are because the heavy metal Pb in fish is non-essential, which means that the heavy metal Pb is not needed by the fish's body. However, the concentration of the heavy metal Pb in the fish's body will continue to increase along with the increase in heavy metals in the waters (Selpiani et al., 2015). In general, fish live in limited waters such as rivers and lakes. Therefore it will be difficult for fish to avoid the effects of pollution. Then pollution elements such as heavy metals will enter the fish's body (Mardani et al., 2018). Kidneys in teleost fish are generally affected by contaminants in the water (Hadi et al., 2012). Many factors influence the ability of fish to accumulate heavy metal. Body measurements such as length and weight (Ruelas-Inzunza et al., 2013), gender, age, behavior and excretory ability will provide variations accumulation of heavy metals in the body of fish, even within the same species (Corredor-Santamaría et al., 2021; Luoma et al., 2009). The result of pollution can result in structural and functional damage to organs. Organs that are sensitive due to contamination, one of which is the kidneys. The kidneys have important functions related to electrolyte and water balance as well as maintaining the stability of the internal environment (osmoregulation). Kidneys can be used as an indicator of pollution in water (Paramita et al., 2021).

Conclusion

The histopathology of fish caught in the Gembong River is in the moderately damaged category, while those caught in the Rejoso River are in the slightly polluted category. The most common tissue damage found is necrosis, apart from that the damage is congestion and cell degeneration. The average water quality parameters are still considered optimal except for TSS and lead, the value is above the quality standards that have been set. The kidney organs in fish caught in both rivers all contain lead.

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