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Histopathological Assessment in Liver and Kidney of *Boleophthalmus pectinirostris* Collected from The Kuala Tambangan Estuaryof South Kalimantan, Indonesia

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ABSTRACT

Kuala Tambangan, being an estuary area with high anthropogenic activities, is prone to water quality issues. To assess its health, bioindicator species play a crucial role. Histological alterations in fish organs can indicate environmental pollutants. *Boleophthalmus pectinirostris*, commonly found in Kuala Tambangan estuary, is a potential bioindicator species.The study aimed to evaluate Kuala Tambangan estuary waters' quality using physical and chemical parameters through the pollution index (PI) method. Additionally, histopathological assessments of *B. pectinirostris*' liver and kidney were conducted. The PI method followed the Government Regulation of the Republic of Indonesia No. 22 of 2021 Annex VIII concerning seawater quality standards. Histopathological lesions were identified using the paraffin method microtechnique, which is considered the gold standard of toxicity testing. Lesions were scored and compared descriptively. Results determined PI values of 2.20 and 2.24 for water quality in two observations during July 2022 and February 2023, respectively. Mild liver and kidney alterations were characterized by necrosis, fatty degeneration, inflammation, and congestion. Based on physical and chemical data, the Kuala Tambangan estuary was classified as slightly polluted, affirming the potential of *B. pectinirostris* as a bioindicator species.

INTRODUCTION

Globally, pollution of aquatic biota is a major problem, which is usually caused by increasing industrial, transport, agricultural, domestic, and human activity effluents **(Mustafa, 2020)**. Various types of chemicals are being discharged into the waters, ultimately settling in the estuary. This discharge has a detrimental impact on the biota residing in the area, particularly fish. To comprehensively assess the adverse effects of these intricate chemical mixtures on fish, pollution monitoring was carried out. This monitoring employed bio-indicator species, complementing the analysis of chemical and physical parameters of the waters. Fish are an important tool to prove the transfer of contaminants to human populations and can indicate potential hazards from pollutant exposure. Fish are generally considered to be an important bio-indicator of aquatic environments. Fish are the inhabitants that cannot escape from the harmful effects of these pollutants. Furthermore, due to their position in top of the food chains in estuary ecosystems, fish are suitable to determine the degree of chemical pollution in aquatic ecosystems. Fish are widely applied for the evaluation of the health of aquatic ecosystems since pollutants build up in the food chain and are responsible for adverse effects and deaths in the aquatic system. Fish are more sensitive to many toxicants than most other freshwater animals, and are a convenient test subject for indication of the ecological health of an estuary **(Solgi &Galangashi, 2018; Aytekin *et al*., 2019)**.

Kuala Tambangan estuary, situated along the coast of the Java Sea in South Kalimantan, Indonesia, is characterized by intense anthropogenic activities. These activities include the anchorage of fishing boats, residential settlements, transportation of fishing vessels, and coal-related operations. The pollutants generated as a result of these anthropogenic activities have the potential to significantly impact the water quality of the estuary. To address this concern, there is a crucial need for monitoring efforts, utilizing fish as bio-indicator species. Surprisingly, despite the evident risks, there has been no prior evaluation involving the analysis of the physical and chemical parameters of the waters, coupled with the use of bio-indicator species. This comprehensive assessment is essential to understand the full extent of the impact of anthropogenic activities on the ecological health of the Kuala Tambangan estuary. It is important to assess the quality of estuary health using wild fish such as mudskipper fish *Boleophthalmus pectinirostris* as a bio-indicator species. These fish are permanent residents and spend most of their time in estuaries, making them suitable as bio-indicators of pollution to mitigate the adverse effects of pollutants on the biodiversity and health of estuary waters. Histopathological studies, both in the laboratory and field, have proven to be a sensitive tool for detecting direct toxic effects of chemical compounds within the target organs of fish **(Abdel-Moneim *et al*., 2012)**.

Histopathology in fish is ideally used as a bio-indicator to show the adverse effects of pollution on waters as it is a definitive biological endpoint of pollutant exposure. **(Stentiford *et al*., 2014)**. Histopathology in fish is considered an ideal indicator of environmental health to determine anthropogenic influences and risks posed on the estuary environment. As an indicator of pollutant exposure, histological examination is a relevant method to evaluate the degree of pollution. In this context, histological examinations performed on fish to determine the degree of pollution in estuary waters can provide useful information on the health of the estuary ecosystem **(Hussain *et al*., 2019)**. In many cases, pollution causes morphological and cytological changes in the liver and kidney. Various environmental pollutants may be detected on the basis of histological alterations in the fish organs.The fish liver is the organ responsible for detoxification and excretion. Therefore, it is the target for several water contaminants including heavy metals. Fish kidneys may serve as valuable indicators of environmental pollution **(Abdel Rahman *et al*., 2019)**.

Mudskipper fish *Boleophthalmus pectinirostris* is a fish species of the Gobiidae family distributed in Africa, Madagascar, India, South Asia, Northern Australia, Southern China, Japan and Indonesia **(Polgar *et al*., 2017)**. This fish species is found in estuaries in both shallow water and mud around river mouths and coastal areas. The mudskipper is an amphibious fish individual since it spends most of its time out of the water doing air breathing **(Velayutham, 2007)**. Mudskippers have many morphological and physiological features to adapt to their habitat **(Kumaraguru *et al*., 2020)**.

Boleophthalmus pectinirostris is considered a potential bioindicator organism due to its characteristics: abundance, easy to provide large samples, resistance to stress, and accumulation of pollutant concentrations in its tissues **(Salvat-leal *et al*., 2020)**. **Santoso *et al.* (2021)** reported that mudskipper fish *Periophthalmodon schlosseri* has a wide tolerance to polluted environments and is able to accumulate heavy metals in its tissues as indicated by histological changes in organs. Ni, Pb and Cd have been reported to be accumulated by mudskipper fish liver **(Ansari *et al*., 2014)**. The aim of this study was to assess the health quality of Kuala Tambangan estuary waters utilizing the pollution index method. Additionally, it seeked to evaluate the occurrence of histopathological changes in the liver and kidney of *B. pectinirostris* captured in *situ*. These histopathological alterations serve as evidence of the adverse effects stemming from anthropogenic activities in the Kuala Tambangan estuary area of South Kalimantan.

MATERIALS AND METHODS

Study area

The study area is located in Kuala Tambangan estuary, South Kalimantan, Indonesia (Fig. 1). The estuary of Kuala Tambangan, Takisung District, Tanah Laut, South Kalimantan, Indonesia, is an estuary region directly adjacent to the Java Sea. Kuala Tambangan has an administrative area of 5.92km^2 and 6.95km of shoreline **(Central Agency on Statistics, CAS, 2021)**. Geographically, Kuala Tambangan lies between 114°30'20" E to 155°23'3" E and 3°30'33" S to 4°11'38" S. The Kuala Tambangan estuary area is a container for the Kuala Tambangan watershed, measuring 1,315.9km^2 in length and 50m in breadth **(Hidayat, 2018)**. The sampling locations are placed at 3°56'56.14836" S, 114°38'9.39912" E (Location 1/river estuary) and 3°58'6.81996" S, 114°37'46.49664" E (Location 2/coastal) (Fig. 1).

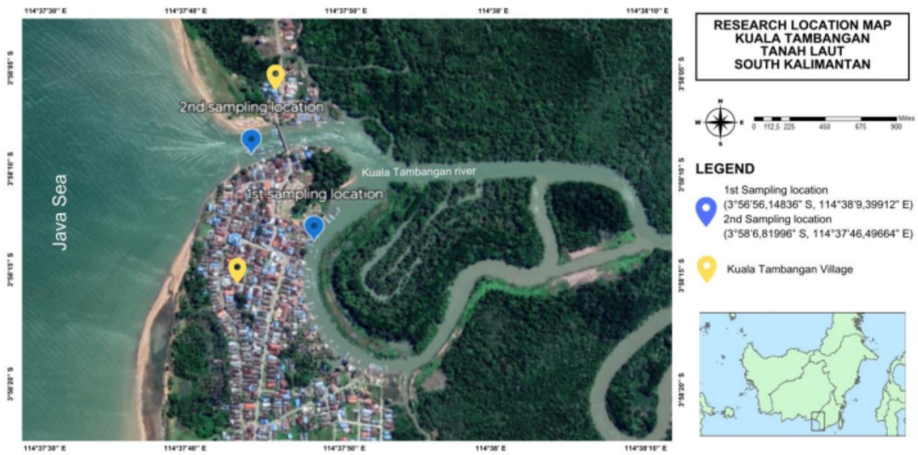

Fig. 1. Research location map Kuala Tambanganestuary

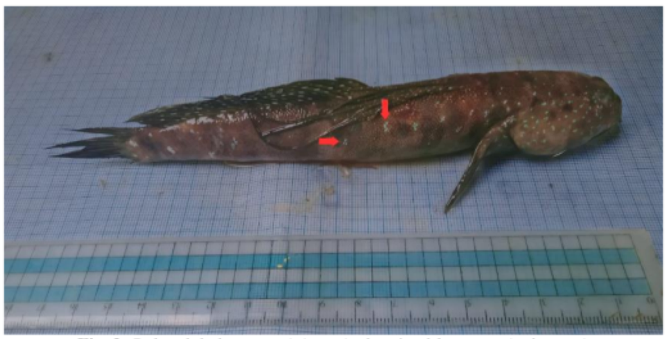

Fig. 2. *Boleophthalmus pectinirostris* showing blue spots (red arrow)

Sample collection

The method employed for fish and water sampling is "purposive sampling", which involved selecting samples based on specific research requirements. The sampling methodology for river water followed the guidelines outlined in the Indonesian National Standard (SNI) 6964.8:2015. The sample location of fish at the research station is determined by the site ordinate at a salinity value of 0.5-5‰ (the optimal salinity value for coastal swamp fish life). From July 2022 and February 2023, samples of *Boleophthalmus pectinirostris* (body weight: 150-200g; total length: 19–22cm; N = 10 per site) (Fig. 2) were collected using a bottom trap net. All were anesthetized with benzocaine. Euthanasia was then performed by medullary section, and the liver and posterior kidney were collected. Fish were dissected and samples of liver and kidney were fixed for histopathological studies. Concurrently with fish collection, 2L of water samples were gathered from each site to analyze physical and chemical water parameters. These samples were carefully transported to the laboratory at 4℃ in clean plastic bottles. Subsequently, the analyses were conducted following standard procedures outlined by the Indonesian National Standard (SNI).

Physical and chemical examination

Water quality tests were carried out on water pollution parameters, which include physical parameters (temperature and Total Suspended Solid) and chemical parameters (dissolved oxygen, pH, salinity, biological oxygen demand, and chemical oxygen demand). Measurement of water quality parameters followed the guidelines outlined in the Indonesian National Standard (SNI) and digital instrument shown in Table (1).

Table 1.Water quality parameter analysis

No	Parameter	Unit	Method specification
	Field		
1.	Temperature	°C	Thermometer
2.	pH	-	pH indicator
	Laboratory		
3.	BOD	mg/L	SNI 6989.72:2009
4.	TSS	mg/L	SNI 06-6989.3:2019
5.	COD	mg/L	SNI 6989.2:2019
6.	DO	mg/L	digital DO meter

Water quality analysis

Water quality measurements were compared to the water quality standards outlined in the Government Regulation number 22 of 2021, which pertains to the Implementation of Environmental Protection and Management. Moreover, the analysis was conducted utilizing the pollution index (PI) methodology stipulated in the State Ministry of Environment No. 115 of 2003, issued by the State Minister of Environment of the Republic of Indonesia. The status of the waters is classified as good criteria if the PI value is at a score of $0 \leq Pij \leq 1.0$; slightly polluted if the PI value is at a score of $1.0 < Pij \leq 5.0$; fairly polluted if the PI value is at a score of $5.0 < Pij \leq 10$, and heavily polluted if the PI value is at a score of $Pij > 10$.

Histopathological examination

Paraffin method microtechnique with slice preparations was used to identify histopathological lesions as the gold standard of toxicity testing. The initial stage of the microtechnical process is to dissect and remove the liver and kidneys. Subsequently, the liver and kidneys of the *B. pectinirostris* were subjected to fixation in a 10% buffered neutral formalin solution (BNF), followed by a series of dehydration steps using alcohol concentrations of 70%, 80%, 90%, and absolute alcohol. Furthermore, xylol was employed for two cycles, followed by two cycles of infiltration lasting 1.5h each (Thermologic oven, melting point 60°C). The specimen was then embedded in a paraffin block in preparation for sectioning. The tissue bands transverse sections were sectioned using a microtome of 5µm thickness made in a MicroTec rotary microtome (Germany). Subsequently, the Hematoxylin-Eosin staining technique was employed to introduce chromaticity. According to **Abdel-Moneim *et al.* (2012)**, paraffin microtechniques employing split preparations are utilized to identify histopathological lesions. Histopathological examination of liver and kidney tissues was conducted using an Olympus CX 41 (Japan) light microscope and an Olympus DP 20 camera (Japan). The examination commenced by employing a 4x objective lens to acquire a comprehensive tissue perspective, followed by objective lenses with higher magnification power, specifically 10x, 20x, 40x, and 100x to discern and identify any lesions present.

Histopathological analysis

The presence of histological alterations for each organ was evaluated semi-quantitatively by scoring which is based on the severity of the lesions. The analysis involved the microscopic examination of histopathological images in liver and kidney specimens. The images were subsequently juxtaposed with the histological images sourced from the Atlas of Fish Histology journal **(Genten *et al*., 2009; Chinnah *et al.*, 2021; Dorostghoal, 2022)**. The percentage of damage was calculated from the weight average of histopathological change scores from 5 different fields, viewing each organ with the Manja Roenigk Histopathology Scoring model. Scoring results were obtained by calculating the percentage of cells that are damaged by expanding the view of the observation in a light microscope. Percentage of damage found was recorded and totaled. The quantified extent of damage is presented in Table (2).

Table 2. Determination of the histopathological scoring value **(Roenigk, 2009)**

Alternation %	Score	Category
< 25%	0	Normal
25% - 50%	1	Light
50% - 75%	2	Fair
> 75%	3	Heavy

Data analysis

A descriptive statistical analysis was performed expressing data as mean ± SE. The homogeneity of variance (Levene's test) and data distribution normality (Kolmogorov-Smirnov test) were performed to verify these assumptions. Data that are not normally distributed were subjected to non-parametric statistical tests using the Kruskal-Wallis test, which is used as a test of influence between variables using the probability (*P*) or Sig (significance) value. The value of Sig <0.05 is used to determine the significant changes that occur between each variable, if Sig. <0.05 then the influence between variables is significant, while if the Sig value > 0.05, it means that the influence between variables is not significant (**Corredor-Santamaría, 2019**). The statistical procedures were performed using the IBM SPSS software for Windows version 25.

RESULTS

1. Physical and chemical analysis

Tables (3) shows the physical and chemical parameters of the water in the examined areas of Kuala Tambangan estuary in July 2022 and February 2023.

Table 3. Water's physical and chemical parameters from studied areas of estuary Kuala Tambangan in July 2022 and February 2023

Parameter	Sampling[1)]	Measurement result	Seawater quality standard [2)]
pH	I	6.7	7−8.5
	II	6	
Temperature (°C)	I	29.75	28-30
	II	28.15	
Salinity (‰)	I	4	34
	II	3.5	
DO (mg/L)	I	3.1	5
	II	4.7	
BOD_5 (mg/L)	I	1.05	20
	II	0.25	
COD (mg/L)	I	19.5	40
	II	60.65	
TSS (mg/L)	I	49	20
	II	49	

Note: [1)] I = July 2022; II = February 2023
[2)] Government Regulation number 22 of 2021 concerning the Implementation of Environmental Protection and Management of Seawater Quality Standards for Marine Biota

2. Pollution index analysis

Table (4) displays the result of water quality analysis by pollution index in the examined areas of Kuala Tambangan estuary in July 2022 and February 2023.

Table 4. Water quality analysis by pollution index

Sampling[1)]	Pollution index value	Range[2)]	Categories
I	2.20	$1.0 < Pij \leq 5.0$	slightly polluted
II	2.24	$1.0 < Pij \leq 5.0$	slightly polluted

Note: [1)] I = July 2022; II = February 2023
[2)] State Ministry of EnvironmentNo. 115 of 2003

3. Histopathological examination

3.1 Examination of the liver

The results showed that the liver of normal fish referenced a structure consisting hepatocytes, sinusoids, and central vein (Fig.3.1). *B. pectinirostris*liver structure obtained during July and February had a normal fish liver structure, consisting of polyhedral hepatocytes organized in cords around a central vein and divided by sinusoids (Fig. 3.2), while liver showed necrosis (Fig. 3.3, 3.4). Additionally, it showed hepatocytes with fatty degeneration (Fig. 3.5, 3.6) and blood vessel congestion (Figs. 3.7, 3.8).

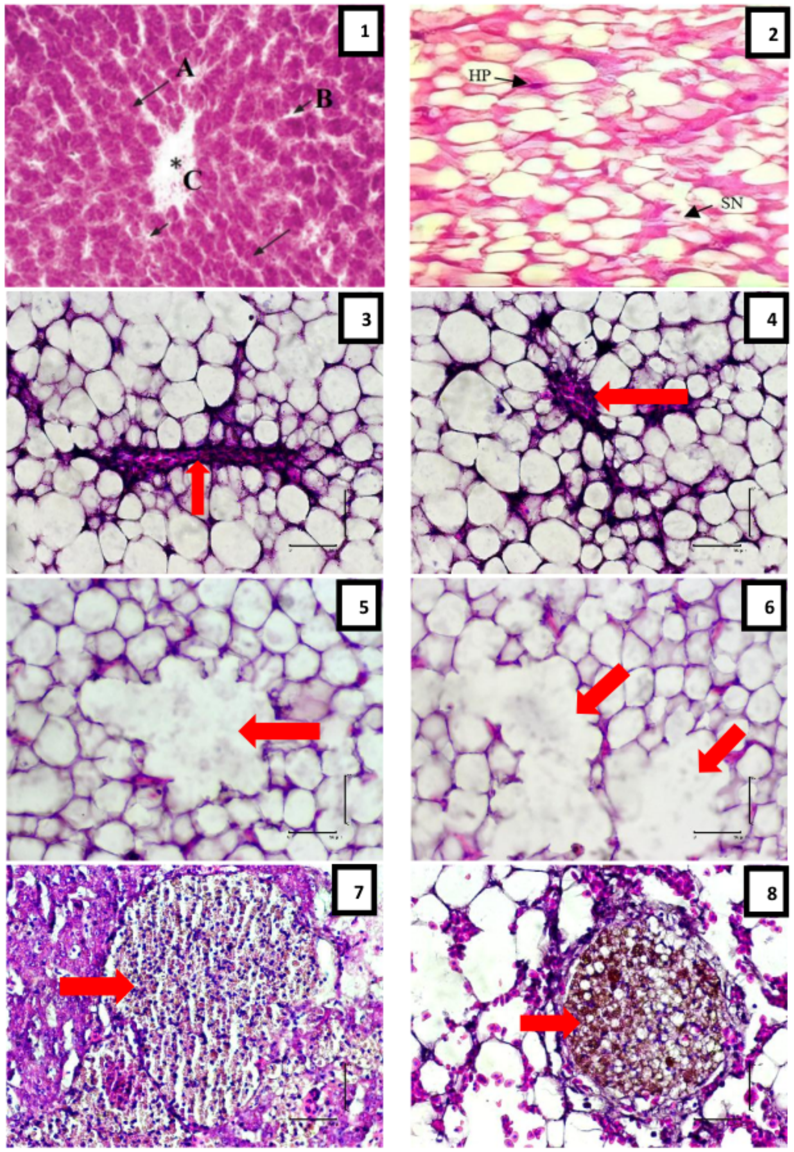

Fig. 3. The liver of *Boleophthalmus pectinirostris* stained with H&E showing: (1) normal fish liver with hepatocytes (A), sinusoids (B), and central vein (C) **(Genten *et al*., 2009),** x10; (2): normal *Boleophthalmus pectinirostris* liver with hepatocytes (HP), and sinusoids (SS) **(Chinnah *et al*., 2021),** x 10; Sampling in July 2022 and February 2023 (3,4): liver showing necrosis (red arrow), x 40; (5,6) liver showing fatty degeneration (yellow arrow), x 40; (7,8) liver showing blood vessel congestion (blue arrow), x40, scale bar 50μm

Table 5. Average histological damage score for *Boleophthalmus pectinirostris* liver in Kuala Tambangan estuary (mean± S.D)

Sampling	Histopathological alternations		
	Necrosis	**Fatty degeneration**	**Blood vessel congestion**
July 2022	1.40±0.20[a]	1.20± 0.20[a]	1.13 ± 0.12[a]
February 2023	1.53±0.12[a]	1.33±0.31[a]	1.20 + 0.18[a]

Values with different letters indicate statistical significance ($P< 0.05$).

3.2 Examination of the kidney

The results revealed that the kidney of normal fish referenced a structure consisting of glomerulus, lumens, tubules, and blood vessels (Fig. 4.1, 4.2). *B. pectinirostris* kidney structure obtained during July 2022 and February 2023 showed dilation of the tubular lumen (Fig. 4.3, 4.4), kidney showing inflammatory response melanomacrophages center (Fig. 4.5, 4.6), and kidney showing necrosis (Fig. 4.7, 4.8).

Table 6. Average histological damage score for *Boleophthalmus pectinrostris* kidney in Kuala Tambangan estuary (mean ± S.D)

Sampling	Histopathological alternations		
	Lumen tubular dilation	**Inflammation**	**Necrosis**
July 2022	1.42 ± 0.2[a]	1.27± 0.27[a]	1.06 ± 0.14[a]
February 2023	1.47 ± 0.18[a]	1.41± 0.15[a]	1.27 ± 0.15[a]

Values with different letters indicate statistical significance ($P< 0.05$).

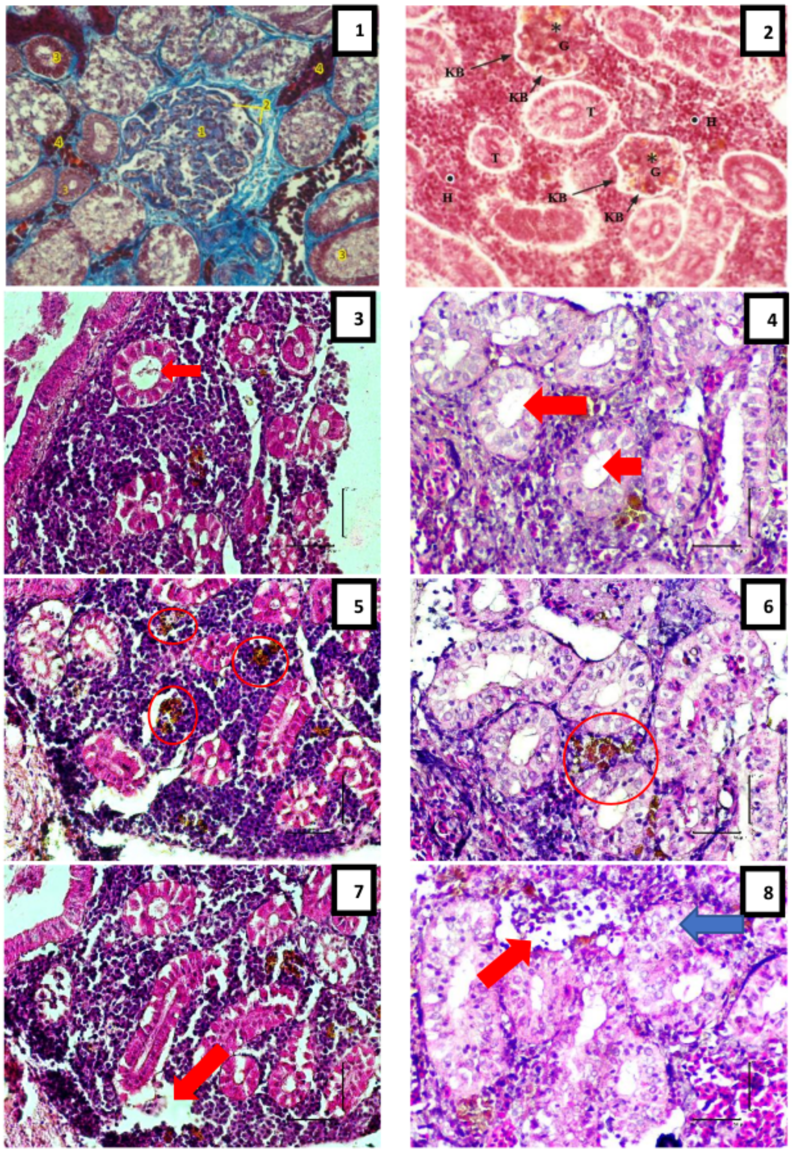

Fig 4. The kidney of *Boleophthalmus pectinirostris* stained with H&E showing: (1) normal fish kidney with glomerulus (1), lumens (2), tubules (3), and blood vessels (4) **(Genten *et al*., 2009)**, x 100; (2): normal fish kidney with glomerulus (G), Bowman's capsule (KB), tubules (T),and Hemapoetik (H) **(Genten *et al.,* 2009)**, x 10; Sampling in July 2022 and February 2023 (3,4): kidney showing dilation of the tubular lumen (red arrow), x 40; (5,6) kidney showing inflammatory response melanomacrophages center (circle), x 40; (7,8) kidney showing necrosis (red arrow) and tubules necrosis (blue arrow), x40, scale bar 50 μm

DISCUSSION

Pollution index is one of the methods used in assessing the physicochemical quality of waters. The pollution index can be calculated based on the measured water physicochemical parameters and used to determine the level of pollution at a water location. The results of this assessment can provide important information for the management of water resources and protection of the aquatic environment. The physicochemical analysis of Kuala Tambangan estuary waters using the pollution index (IP) method showed that the pollution index values in July 2022 and February 2023 were 2.20 and 2.24, respectively, which are categorized as lightly polluted. It is suspected that the TSS parameter that exceeds the quality standard contributed to the increase in the pollution index in the Kuala Tambangan estuary, while temperature, pH, salinity, DO, BOD & COD were in the normal range. The value of the pollution index in the second observation in February 2023 was higher than that recorded in July 2022.

Total suspended solid (TSS) value is an important water quality parameter and is closely related to the level of pollution. High TSS values block the penetration of sunlight, hindering photosynthesis and reducing dissolved oxygen levels. TSS values are influenced by the tides that occur in estuary waters. Tides are an important factor in the circulation of water flow in estuaries, affecting the suspended sediments that move along the estuary. Suspended sediments will move upstream at high tide and move downstream at low tide. Tides cause differences in TSS values due to turbidity shifts. The tidal wave will spread into the estuary to a considerable distance from the estuary, which is accompanied by a very large amount of sea water mass transport. Apart from the seawater flow, the estuary also receives upstream river discharge, a volume influenced by seasonal variations and the hydrological traits of the river. Consequently, both the seawater and river water masses accumulate significantly in the estuary.TSS values in estuaries or coastal areas are higher during low tide conditions because water masses moves the estuary back to the sea in large volumes with large flow velocities and depths. Total suspended solids (TSS) are suspended materials that create turbidity in water, such as mud, fine sand, and minute particles, formed mainly by soil erosion or erosion transported by water bodies **(Effendi, 2003)**. The average TSS concentration in the estuary of Kuala Tambangan in July 2022 and February 2023 is 49mg/ L. The findings of this measurement indicate that the TSS level exceeds the 20mg/ L of marine water quality standard stipulated by the Government Regulation No. 22 of 2021. Based on TSS standardization, there are three groups: Group 1 with TSS < 20mg/L is clear, Group 2 with TSS 40 – 80mg/L is murky, and Group 3 with TSS >150mg/L is muddy. High concentrations of TSS in water can decrease the availability of dissolved oxygen. According to **Tarigan and Rozak (2003)**, high total suspended solids (TSS) levels can lead to particle accumulation in the gills, hindering respiration and potentially causing mortality in aquatic organisms.TSS values that exceed quality standards are thought to be caused by anthropogenic activities in the Kuala Tambangan estuary.The estuary of Kuala

Tambangan, situated in South Kalimantan, is positioned along the coast of the Java Sea. This area experiences significant anthropogenic activities, serving as a berth for fishing boats, residential areas, and transportation routes for both fishing and coal boats. The influence of human activities produces contaminants that can influence the quality of aquatic health, the ecological balance of the aquatic environment, and aquatic biota **(Carpenter *et al*., 2011)**.

The degree of acidity (pH) is crucial in evaluating water quality. In July 2022 and February 2023, the average pH of Kuala Tambangan's estuaries was 6.8 and 6, respectively. This number is typically near neutral pH, but it is still below the range of seawater quality standards outlined in the Government Regulation No. 22 of 2021, which is 7 – 8.5. A pH values of 6.0 – 8.0, 6.4 – 7.0, and 6.5 – 8.5 are required for everyday fish life, breeding, and optimal living, respectively (**Kristanto, 2002**). Fish die at pH levels of 4 and 11, respectively **(Siswanto *et al*., 2021)**.

Temperature is one of the external criteria used to evaluate the healthfulness of water. **Hamuna *et al*. (2018)** stated that temperature can influence aquatic organisms' metabolic rate and dispersion. In July 2022, the average temperature of the estuary water of Kuala Tambangan was 29.75°C, while in February 2023, it was 28.15°C. This value meets the water quality guidelines for marine life established by the Government Regulation No. 22 of 2021, which ranges between 28 and 32°C. In addition, this figure is included in the ideal temperature range for tropical fish life, which extends from 25 to 32°C (**Undap *et al*., 2018**). Fish metabolism is influenced by temperature. Temperatures that are too low can cause fish to lose their appetite and inhibit the optimal functioning of enzymes (**Ridwantara *et al*., 2019**).

Salinity, which refers to the concentration of ions in a body of water, is a crucial factor in determining the health and ecological balance of estuarine environments (**Syahrul & Romadhon, 2020**). The salinity of water influences its osmotic pressure; the higher the salinity, the higher the osmotic pressure **(Gemilang *et al*., 2017)**. In July 2022, the average salinity of Kuala Tambangan's estuary was 4‰; whereas, in February 2023, the average salinity was 3.5‰%. This number meets the Government Regulation No. 22 of 2021 for water quality criterion for marine life, which is 34%. The osmoregulation process is affected by the equilibrium between water concentration and ions in a fish's body, which is influenced by salinity **(Francisca & Muhsoni, 2021)**.

Dissolved oxygen (DO) is the total amount of dissolved oxygen in water. Fish require DO for respiration, metabolic activities, and the exchange of chemicals, which provide energy for development and growth. Fish require varying amounts of dissolved oxygen depending on species, life stage, and activity level **(Gemilang *et al*., 2017)**. In July 2022, the DO value of Kuala Tambangan estuary measured an average of 3.1mg/ L; while in February 2023, it measured an average of 4.7mg/ L. This value is below the 5mg/L seawater quality level established by Government Regulation 22 of 2021.

According to **Kadim *et al.* (2017)**, the optimal oxygen level for fish is between 3 and 7mg/ L.

The biochemical oxygen demand (BOD) represents the dissolved oxygen that microorganisms require to break down organic materials under aerobic circumstances. In July 2022, the average BOD_5 concentration in Kuala Tambangan's estuary was 1.05mg/ L; whereas in February 2023, it was 0.25mg/L. The results of this measurement indicate that this water meets the 20mg/ L BOD quality criteria established by the Government Regulation No. 22 of 2021. BOD values between 0 – 10mg/L indicate low pollution levels, whereas values between 10 – 20 mg/L indicate moderate pollution **(Daroini & Arisandi, 2020)**. Chemical oxygen demand (COD) is the amount of oxygen needed to break down all organic compounds in water **(Boyd, 1990)**. COD is the total quantity of oxygen required to oxidize organic materials chemically **(Soukotta *et al.*, 2019)**. The COD value of Kuala Tambangan's estuary ranged from an average value of 19.5mg/ L in July 2022 to an average value of 60.65mg/ L in February 2023. Government Regulation No. 22 of 2021 states that COD is not among the standard measurements of seawater quality. This omission is due to the difficulty in accurately measuring seawater COD, primarily because of potential interference from high chloride (Cl) levels present in seawater during its analytical reaction. However, according to Decree No. 2 of the Minister of Environment of the Republic of Indonesia from 1988, the standard value for COD quality in seawater is 80mg/ L.

Assessment of water health quality based on examining physical and chemical parameters provides an overview of changes in water quality in general. However, it cannot directly provide information on the accumulating effects that influence biota. The physicochemical analysis ignores potentially hazardous substances and has detrimental ecological repercussions **(Kumar & Han, 2010)**. Therefore, it is vital to assess using histology characteristics, as fish spend their entire lives in the environment to produce a cumulative effect **(Widiyanto & Sulistayarsi, 2016)**. Analysis of water's physical and chemical test parameters supplemented with biological test parameters is more effective for determining a body of water's health quality **(Park *et al.*, 2023)**.

Using fish as a bio-indicator is beneficial because it can summarize the adverse effects of pollutants **(Keci *et al.*, 2013)**. After all, fish are susceptible to environmental changes **(Altshuler *et al.*, 2011)**. According to **Elrayess *et al.* (2022)**, using fish for measuring changes in water quality owing to pollution exposure by histopathological alterations is deemed effective. According to **Santoso *et al.* (2021)**, the mudskipper fish has bio-indicator potential since it can provide a picture of histopathological alterations as an early warning indication of disease and damage to cells, tissues, or organs. Fish histopathology is an appropriate and helpful bio-indicator for assessing the presence of heavy metals in the environment to estimate the environmental risk **(Mustafa, 2020)**.

The histological characteristics of *B. pectinirostris* healthy liver show lobules radial structures that are concentrated on the central vein and divided by hepatic sinusoids. The lobes are composed of parenchyma cells known as hepatocytes. Between sinusoids containing blood and bile channels, hepatocyte cells are found **(Safratilofa, 2017)**. The nuclei of liver cells are spherical and have a polyhedral form **(Siagian *et al*., 2016)**. *B. pectinirostris* kidneys contain glomerulus, Bowman's capsule, proximal convoluted tubule, distal tubule, collecting duct, and hematopoietic tissues **(Genten *et al.,* 2009)**.

Necrosis, fatty degeneration, and congestion were found in the livers of *B. pectinirostris* from the Kuala Tambangan estuary. Necrosis is a form of cell death characterized by a shrinking nucleus, cell lysis, nuclei protruding from the cell membrane, and the absence of cytoplasm. Necrosis of the liver is further distinguished by the diminution/shrinking of cell size **(Asniatih *et al.,* 2013)**. Necrosis is believed to result from the harmful effects of heavy metal pollution. This is consistent with the findings of **Mustafa *et al.* (2017)** who discovered that, *Luciobarbus xanthopterus* fish in the Tigris River, Baghdad, Iraq, that were exposed to the heavy metal Pb exhibited features of liver necrosis. **Reddy and Baghel (2012)** also detected necrosis in the liver parenchyma of *Mytus tengar* fish from the Hambal River in India exposed to metals from industrial waste. Fatty degeneration in the fish liver is a cellular response to harmful substances, leading to the accumulation of excess fat in the cytoplasm **(Triadayani *et al*., 2010)**. Fatty degeneration is defined by the appearance of empty, colorless circles caused by fat-filled liver tissue that cannot absorb dyes during the coloring process. It is believed that exposure to chemical pollutants in water contributes to fatty liver degeneration. According to a research by **Silviany (2004)**, exposure to Pb in the liver of *Cyprinus carpio L.* results in fatty degeneration.

Other histological alterations in the liver of *B. pectinirostris* include congestion, namely an increase in blood volume in blood vessels due to physical damage, and circulatory diseases caused by environmental toxins **(Hadi & Alwan, 2012)**. Congestion, characterized by blood occlusion, occurs due to circulatory abnormalities that impede the flow of oxygen and nutrients. In addition, congestion is defined by the dilatation of blood vessels full of blood, creating a blood-filled cell boundary. According to **Rosmaidar *et al.* (2017)**, congestion is an inflammatory response to heavy metal-induced changes in the biochemical structure of cells. According to **Zaghloul *et al.* (2020)**, heavy metal exposure causes histological alterations in vacuolar degeneration, red blood cell infiltration, congestion, degeneration, and necrosis in *Clarias gariepinus* fish in Fayoum Governorate, Egypt. Similar findings were reported by **Abou El-Gheit *et al*. (2012)**, who discovered necrosis, fatty degeneration, hemorrhage, and picnotics in the blooming phenomenon of Lake Qarun, Egypt, in the livers of *Solea vulgaris* and *Mugil* sp. This is further confirmed in the study of **Santos *et al.* (2022)** indicating that, exposure to Zn, Cu, and Fe causes histological abnormalities in the livers of *Luciobarbus bocagei* and

Pseudochondrostoma duriense in the Sabor River of Portugal. Histological alterations include hepatocyte vacuolization, endothelial rupture, necrosis, and fibrosis.

The kidneys are organs that assist the body in eliminating metabolic waste products (**Nallakrishna *et al.*, 2015**). Alteration to the proximal tubular cell indicates kidney damage since this cell is the most susceptible to damage (**Suhita *et al.*, 2013**). In the Kuala Tambangan estuary, *B. pectinirostris* kidneys undergo dilatation of the lumen of the tubules, inflammation, and necrosis. The dilation of tubular structure is the dilatation of the tubular lumen. The enlargement of the lumen with normal tubular epithelium characterizes dilation. **Palipoch *et al.* (2011)** reported similar results, namely the presence of Pb-induced dilation in the kidneys of *Oreochromis niloticus* from Chacheongsao, Thailand. Additionally, the kidney of *B. pectinirostris* exhibits inflammation in the distinctive company of a Melanomacrophage Center (MMC). MMC is a solid, spherical cell containing several melanin pigments. MMC is prevalent in healthy fish, but the number increases during stress. **Abdel-Moneim *et al.* (2012)** stated that the occurrence of MMC in *Oreochromis niloticus* in Al-Hassa irrigation, Saudi Arabia, is due to a chemical exposure. In addition, the kidneys of *B. pectinirostris* contained necrosis, characterized by visible cell borders and indistinct or absent cell nuclei. Kidney necrosis is believed to be caused by hazardous chemical substances. This finding is consistent with **Hadi and Alwan (2012)** research on *Tilapia zilliiwhich* fish in Lake Umhfein, Libya, which found that aluminum pollution in water causes histological alterations in the liver and kidneys such as necrosis. Due to heavy metal exposure, **Zaghloul *et al*. (2020)** reported histological abnormalities in glomerular shrinkage, tubular degeneration, and necrosis in the kidney of *Clarias gariepinus* in Fayoum Governorate, Egypt. Biomonitoring of Pb and Cd in the Iraqi Tigris River employing *Luciobarbus xanthopterus* as a bio-indicator is characterized by heavy metal bioaccumulation and histopathology in the liver, kidneys, and gills (**Mustafa, 2020**). Vacuolization, degeneration, and infiltration of mononuclear cells occur in the liver. The kidneys exhibit hydropic degeneration and epithelial cells detached from the basal lamina, whereas the gill epithelial cells exhibit hyperplasia, detachment, and necrosis of secondary lamellae. **Hussain (2021)** reported that the histological structure of the liver and kidneys of *Cirrhinus mrigala* fish from the Chenab River in Pakistan were damaged due to exposure to Fe, Pb, and Cu from household waste and transportation operations.

Histopathology provides a rapid method to detect the effect of irritants in various organs. The liver is the primary organ for metabolism, detoxification of xenobiotics and excretion of harmful substances. The liver has the ability to degrade toxic compounds but its regulating mechanism can be overwhelmed by elevated concentrations of these compounds, which could subsequently result in structural damage. Two of the main alterations observed in both fish species were vacuolar degeneration changes and areas of necrosis. These alterations have been described in other species of fish living in contaminated environments, suggesting that these alterations might be related to the exposure to environmental chemicals present (**Javed *et al*., 2016**). In Teleostei fish, the kidney is another organ that is negatively impacted by contaminants (**Thophon *et al*., 2013**). Xenobiotics that come to the kidneys through the blood circulation damage the renal corpuscle and the tubules. The effects of toxic substances at the molecular and cellular levels appear in the form of degeneration and neoplastic damage in the kidneys (**Abbaszadeh & Şişman, 2020**).

Boleophthalmus pectinirostris in the Kuala Tambangan estuary may have developed liver and kidney histopathology due to the exposure to chemical pollutants, specifically heavy metals. The histopathology that occurs is thought to be caused by continuous long-term exposure to toxic chemicals or heavy metals, which negatively affects the physiological function of fish. If this issue is not resolved, the estuary water's quality will gradually deteriorate. As a result, it is essential to identify polluting sources after monitoring, specifically by examining and testing heavy metals in fish organs, soil, and water.

CONCLUSION

Based on the pollution index in July 2022 and February 2023, the Kuala Tambangan estuary waters are classified as slightly polluted. In the liver of *B. pectinirostris*, there were slight alterations in necrosis, fatty degeneration, and congestion. In the kidney, tubular lumen dilatation, inflammation, and necrosis occur. The *B. pectinirostris* can potentially be a bio-indicator for the standard of aquatic health. To keep estuary waters in good health, routine monitoring efforts are required.

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Histopathological Assessment in Liver and Kidney of Boleophthalmus pectinirostris Collected from The Kuala Tambangan Estuaryof South Kalimantan, Indonesia

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