

Biometric characteristics of the digestive system of *Tor tambroides* (Bleeker, 1854) exposed to palm oil mill effluent

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Abstract. One of the detrimental impacts of the palm oil processing company that might disrupt aquatic environments is liquid waste. The purpose of this study is to characterize the biometric circumstances of the digestive system of thai mahseer, *Tor tambroides* (Bleeker, 1854) contaminated by palm oil mill effluent (POME). The study was conducted from July to October 2023. The fish sample size ranged from 7 to 10 cm in length and a weight of 15 g. For 30 days, fish were contaminated by POME at concentrations of 0% (control), 10% LC₅₀ (A), 10% LC₅₀ (B), and 10% LC₅₀ (C). Analysis was carried out on the gastro-somatic index (GaSI), intestine somatic index (ISI), relative gut lengths (RGL), Relative Intestine Lengths (RIL), and hepato-somatic index (HSI). The research results show that POME can affect the biometrics of the digestive system of *T. tambroides*. Contamination to POME with a concentration of C (10% LC₅₀) affects GaSI of 0.46±0.02%, ISI of 1.96±0.05%, HSI of 0.79±0.03%, RGL of 44.82±4.08%, and RIL of 139.95±8.25%. The POME can disrupt the metabolism of the stomach, intestine, and liver cells of *T. tambroides*. High concentrations of POME can damage the lining of the stomach and intestines, resulting in a decrease in appetite.

Keywords: aquatic environments, intestine, gastro, gut.

Introduction. The Leuser Ecosystem Area (LEA) in Sumatra, designated as a strategic conservation zone via Presidential Decree No. 33 of 1998, represents a biodiversity hotspot of global significance (Djufri 2015). However, this ecosystem currently faces severe anthropogenic pressures, primarily driven by forest fragmentation, climate change, and extensive land conversion for agricultural expansion (Condro et al 2022). Data reveals that the LEA has been converted for various purposes, including infrastructure development and, most notably, oil palm plantations (Clement et al 2014; Sloan et al 2018).

While the palm oil industry is economically vital, it generates significant environmental externalities, specifically palm oil mill effluent (POME). POME is a high-strength wastewater characterized by high chemical and biological oxygen demand (COD and BOD), suspended solids, and residual oil. When discharged into waterways without adequate treatment, POME significantly degrades water quality, causing hypoxia and toxicity in aquatic habitats (Puasa et al 2021). This pollution poses a direct threat to ichthyofauna diversity (Muliari et al 2019). For instance, Defira & Muchlisin (2004) reported a decline in fish species richness in the Alas River (LEA), from 12 to 9 species over two decades, coinciding with increased agricultural activity.

Understanding the impact of pollutants requires more than biodiversity surveys; it necessitates an examination of physiological responses. Digestive and hepatic biometric parameters serve as reliable biomarkers for assessing the sublethal effects of xenobiotics on fish health. The liver, quantified via the hepato-somatic index (HSI), is the primary

organ for detoxification. Exposure to pollutants like POME often induces metabolic stress, leading to hepatomegaly (liver enlargement) or degeneration as the organ attempts to metabolize toxic compounds (Owolabi et al 2021).

Similarly, the gastrointestinal tract is the first line of defense against ingested pollutants. Parameters such as the gastro-somatic index (GaSI), intestine somatic index (ISI), and relative gut length (RGL) provide insights into the structural integrity and functional plasticity of the digestive system. Pollutants can disrupt mucosal linings, alter enzymatic activity, and force morphological changes in the gut, affecting the organism's nutrient absorption and overall energy budget (Kurbah & Bhuyan 2018). Despite their importance, the specific correlation between POME exposure and these digestive biometric alterations remains understudied.

Thai mahseer, *Tor tambroides* (Bleeker, 1854) is an indigenous freshwater species within the LEA waterways that is directly vulnerable to such environmental degradation (Akmal et al 2022; Muchlisin et al 2022). This species holds dual significance: it is a high-value commodity for consumption and ornamental purposes (Jaafar et al 2021) and is of conservation concern, currently listed as "Data Deficient" by the IUCN (Kottelat et al 2018). While previous studies on the genus *Tor* have extensively covered morphometrics (Akmal et al 2018), osteology (Akmal et al 2020), and diet composition (Muchlisin et al 2015), there is a critical gap in knowledge regarding its physiological resilience to industrial waste.

Therefore, this study aims to examine the effects of POME contamination on the digestive and hepatic biometrics of *T. tambroides* using quantitative indices and qualitative histological assessments. Providing a comprehensive understanding of how POME affects the vital organs of *T. tambroides* is crucial for developing evidence-based conservation strategies and preventing the local extinction of this valuable species due to aquatic pollution.

Material and Method

Sample collection. The study was conducted at the Hatchery and Cultivation Technology Laboratory, Aquaculture Study Program, Faculty of Agriculture, Malikussaleh University, Indonesia. The research period spanned from July to October 2023.

Research design. The study employed a completely randomized design (CRD) with four treatments, each replicated three times. The sub-chronic exposure concentrations were based on the LC50-96h value of POME for *Tor tambroides* (15.65 mg L⁻¹) as reported by Hayatun et al (2022). The exposure duration for the sub-chronic test was 96 hours. The experimental treatments were as follows: Control - 0% POME addition; Treatment A - 10% POME addition (1.565 mg L⁻¹); Treatment B - 15% POME addition (2.347 mg L⁻¹); Treatment C - 20% POME addition (3.130 mg L⁻¹).

Gastrointestinal biometrics. At the end of the exposure period, the fish were dissected to isolate the digestive organs. The liver and intestines were weighed using a digital balance and measured for length to determine the biometric parameters. GaSI and RGL were calculated following the formulas described by Tran et al (2021). ISI and relative intestine length (RIL) were determined according to Wagner et al (2009), while HSI was calculated following Okoboshi et al (2022).

$$\text{GaSI} = \frac{\text{weight of the fish's stomach (g)}}{\text{weight of the fish's body (g)}} \times 100$$

$$\text{ISI} = \frac{\text{weight of fish intestines (g)}}{\text{weight of the fish's body (g)}} \times 100$$

$$\text{HSI} = \frac{\text{weight of fish liver (g)}}{\text{weight of the fish's body (g)}} \times 100$$

$$\text{RGL} = \frac{\text{Length of fish gut (cm)}}{\text{Total length of fish (cm)}} \times 100$$

$$\text{RIL} = \frac{\text{Length of fish intestines (cm)}}{\text{Total length of fish (cm)}} \times 100$$

Statistical analysis. Biometric data were analyzed using One-Way Analysis of Variance (ANOVA). Differences were considered statistically significant at a 95% confidence level ($p < 0.05$). All statistical analyses were performed using SPSS software version 22.

Results. Exposure to POME induced significant non-linear biometric alterations in the digestive system of *T. tambroides*. At lower sublethal concentrations (10% and 15% LC_{50}), the fish exhibited a potential compensatory response characterized by significantly increased GaSI and RGL, alongside a marked reduction in the ISI. However, this physiological adaptation collapsed at the highest concentration (20% LC_{50}), where GaSI and RGL dropped significantly below control levels, contrasting with a sharp surge in ISI. Furthermore, metabolic stress appeared most pronounced at the intermediate concentration (15% LC_{50}), indicated by a peak in the HSI, suggesting that while lower doses may stimulate digestive morphometrics, higher concentrations disrupt homeostasis and induce organ toxicity.

Table 1
Biometric alterations in the digestive system of *Tor tambroides* exposed to POME

Parameters	Control	A (10% LC_{50})	B (15% LC_{50})	C (20% LC_{50})
Gastro-somatic index (GaSI)	0.87±0.04 ^b	1.00±0.03 ^c	1.11±0.02 ^d	0.46±0.02 ^a
Intestine somatic index (ISI)	1.53±0.07 ^b	0.83±0.03 ^a	0.81±0.02 ^a	1.96±0.05 ^c
Hepatosomatic index (HIS)	0.56±0.02 ^a	0.58±0.02 ^a	1.83±0.03 ^c	0.79±0.03 ^b
Relative gut length (RGL)	58.42±3.18 ^b	64.46±5.11 ^c	64.81±3.99 ^c	44.82±4.08 ^a
Relative Intestine length (RIL)	133.72±11.68 ^a	145.29±9.76 ^b	141.53±5.66 ^{ab}	139.95±8.25 ^{ab}

Gastro-Somatic Index (GaSI). The average GaSI value of *T. tambroides* in the control treatment (0% POME) was 0.87±0.04%. The average GaSI value tended to increase in treatments A (1.00±0.03%) and B (1.11±0.02%). Meanwhile, in treatment C, the average GaSI value decreased to 0.46±0.02% (Figure 1). The results of statistical analysis showed significant differences in GaSI values between the treatments ($p < 0.05$). The results of statistical analysis showed that the increase in the average GaSI value in the control treatment compared to treatment A, treatment B, and treatment C was due to contamination of POME, which provided a significant difference ($p < 0.05$). The same thing was also shown in treatment C, where the decrease in the average GaSI value that occurred gave a significant difference ($p < 0.05$) when compared with the average GaSI value in treatment B (2.347 mg L^{-1}).

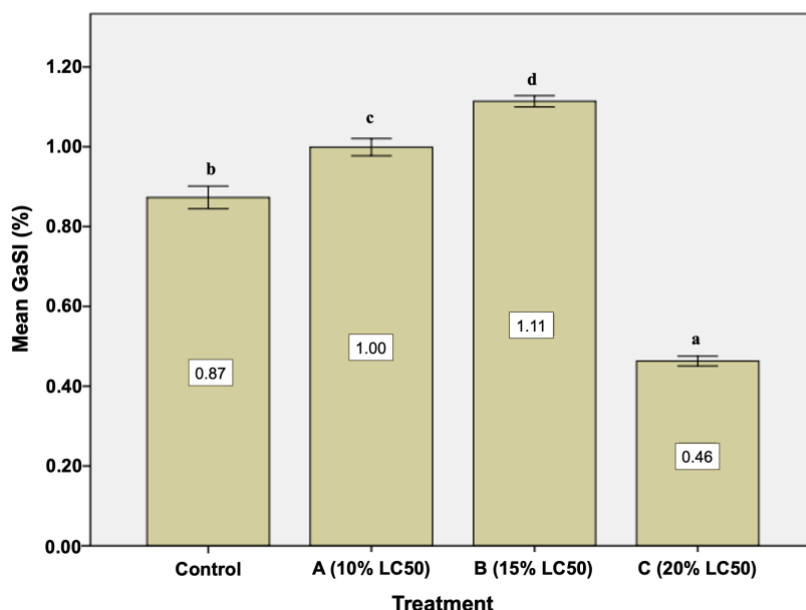


Figure 1. Average value of gastro somatic index of *T. tambroides* contaminated to POME at various concentrations at the end of the contamination period (30 days).

Intestine somatic index (ISI). The average ISI value of *T. tambroides* in the control treatment (0% POME) was $1.53 \pm 0.07\%$. The average ISI value tended to decrease in treatments A ($0.83 \pm 0.03\%$) and B ($0.81 \pm 0.02\%$). Meanwhile, in treatment C, the average ISI value increased to $1.96 \pm 0.05\%$ (Figure 2). The results of statistical analysis showed significant differences in ISI values between the treatments ($p < 0.05$). The results of statistical analysis showed that the increase in the average ISI value in the control treatment compared to treatment A, treatment B, and treatment C due to contamination of POME provided a significant difference ($p < 0.05$). A different thing was also shown in treatment C, where the increase in the average ISI value that occurred gave a significant difference ($p < 0.05$) when compared with the average GaSI value in treatment A and treatment B (2.347 mg L^{-1}).

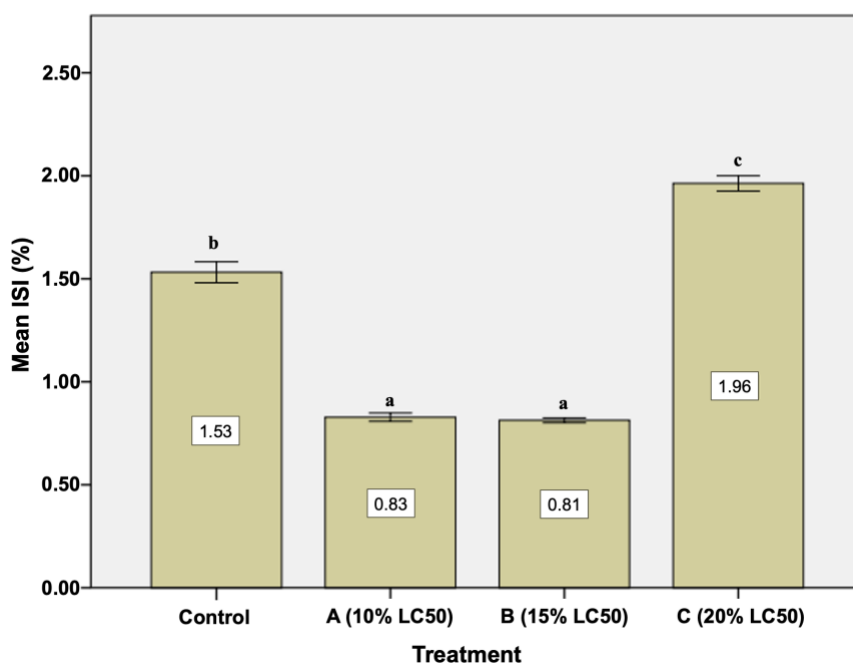


Figure 2. Average value of intestine somatic index (ISI) of *tambroides* contaminated to POME at various concentrations at the end of the contamination period (30 days).

Hepato-somatic index (HSI). The average HSI value of *T. tambroides* in the control treatment (0% POME) was $0.56 \pm 0.02\%$. The average HSI value tended to increase in treatments A ($0.58 \pm 0.02\%$) and B ($1.83 \pm 0.03\%$). Meanwhile, in treatment C, the average HSI value decreased to $0.79 \pm 0.03\%$ (Figure 3). The results of statistical analysis showed significant differences in HSI values between the treatments ($p < 0.05$). The results of statistical analysis showed that the increase in the average HSI value in the control treatment compared to treatment A, treatment B, and treatment C due to contamination to POME provided a significant difference ($p < 0.05$). The same thing was also shown in treatment C, where the decrease in the average HSI value that occurred gave a significant difference ($p < 0.05$) when compared with the average HSI value in treatment B (2.347 mg.L^{-1}).

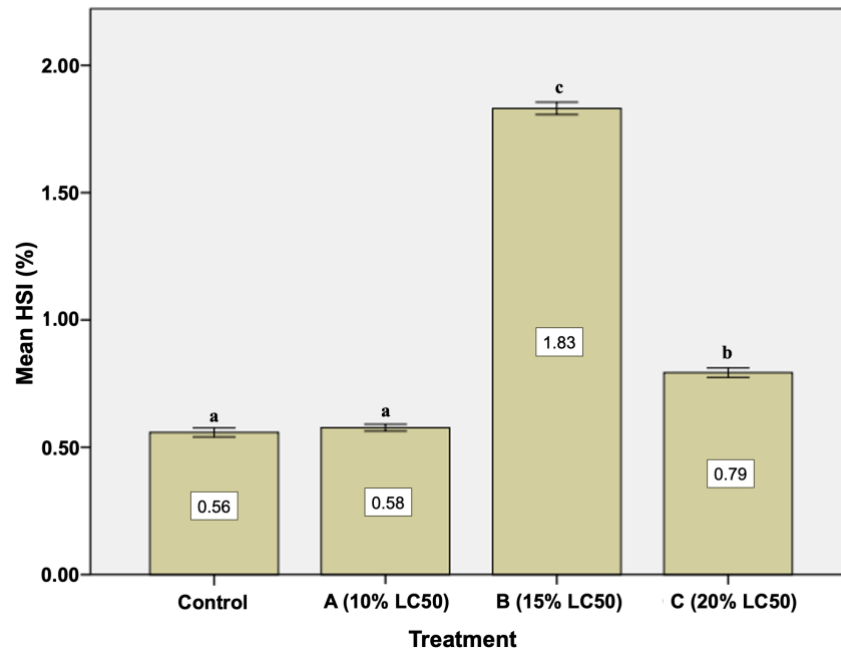


Figure 3. Average hepatosomatic index (HSI) value of *tambroides* contaminated to POME at various concentrations at the end of the contamination period (30 days).

Relative gut lengths (RGL). The average RGL value of *T. tambroides* in the control treatment (0% POME) was $58.42 \pm 3.18\%$. The average RGL value tended to increase in treatments A ($64.46 \pm 5.11a\%$) and B ($64.81 \pm 3.99\%$). Meanwhile, in treatment C, the average RGL value decreased to $44.82 \pm 4.08\%$ (Figure 4). The results of statistical analysis showed significant differences in RGL values between the treatments ($p < 0.05$). The results of statistical analysis showed that the increase in the average RGL value in the control treatment compared to treatment A, treatment B, and treatment C due to contamination of POME provided a significant difference ($p < 0.05$). The same thing was also shown in treatment C, where the decrease in the average RGL value that occurred gave a significant difference ($p < 0.05$) when compared with the average RGL value in treatment B (2.347 mg L^{-1}).

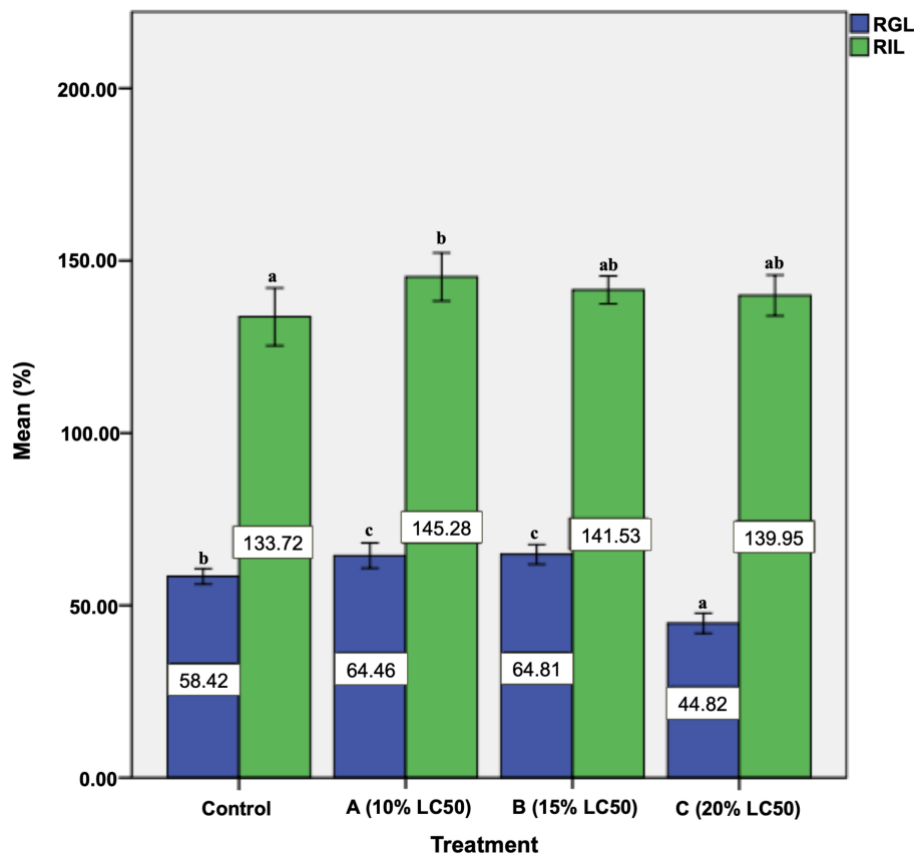


Figure 4. Average values of relative gut length (RGL) and relative intestine lengths (RIL) of *tambroides* contaminated to POME at various concentrations at the end of the contamination period (30 days).

Relative intestine lengths (RIL). The average RIL value of *T. tambroides* in the control treatment (0% POME) was $133.72 \pm 11.68\%$. The average RIL value tended to increase in treatment A ($145.29 \pm 9.76\%$), while in treatments B ($141.53 \pm 5.66\%$) and C ($139.95 \pm 8.25\%$), the average RIL value decreased (Figure 4). The results of statistical analysis showed significant differences in RIL values between the treatments ($p < 0.05$). The results of statistical analysis showed that the increase in the average RIL value in the control treatment compared to treatment A, treatment B, and treatment C due to contamination of POME provided a significant difference ($p < 0.05$). The same thing was also shown by treatment C, where the decrease in the average value of RIL that occurred gave a significant difference ($p < 0.05$) when compared with the average value of RIL in treatment B (2.347 mg L^{-1}).

Discussion. Biometric parameters serve as critical indicators of fish health, reflecting the physiological response of aquatic organisms to environmental pollutants. In this study, the exposure of *T. tambroides* to POME resulted in significant alterations in digestive and hepatic biometrics, indicating metabolic disruption and adaptive physiological mechanisms.

The HSI is a primary indicator of the metabolic status and energy reserves in fish (Pyle et al 2005). Our results demonstrated an increase in HSI values in Treatments B and C compared to the control. This enlargement of the liver (hepatomegaly) is likely a compensatory physiological response to the toxic stress imposed by POME. The liver is the principal organ for detoxification; thus, exposure to xenobiotics often induces the proliferation of smooth endoplasmic reticulum and enzyme production to metabolize pollutants, leading to cellular hypertrophy (Topić-Popović et al 2023). This finding aligns with Elhetawy et al (2023), who observed increased HSI values due to pollutant-induced cellular adaptation. However, at the highest concentrations, a decline in HSI was observed, which may indicate the exhaustion of hepatic compensatory mechanisms or the onset of

liver tissue necrosis, disrupting vital physiological processes such as vitellogenesis (Dane & Şişman 2020).

The digestive tract exhibited significant stress responses characterized by increased GaSI and ISI values. The elevation in GaSI is attributed to the inflammatory response of the stomach lining to the high chemical oxygen demand (COD) and suspended solids found in POME. As noted by Ezraneti & Windarti (2015), irritation of the gastric mucosa stimulates the excessive secretion of mucus as a protective barrier, which, combined with tissue swelling (edema), increases the organ's mass. Although some studies suggest that environmental stress can lead to reduced GaSI due to low feeding intensity (Kurbah & Bhuyan 2018), the increase observed in this study suggests that the pathological swelling outweighed the effects of empty stomach weight.

Similarly, the increase in ISI values correlates with intestinal damage. Visual observations revealed pale intestinal tissues and accumulation of waste, indicative of impaired peristalsis. Physiologically, this can be linked to oxidative stress. Contaminants in POME likely triggered the production of reactive oxygen species (ROS), overwhelming the antioxidant defenses and causing oxidative damage to the intestinal epithelium (Surai & Fisinin 2015). This oxidative stress can inhibit intestinal wall movement and reduce capillary permeability (Suwandi et al 2012), leading to the retention of fluids and waste, which artificially inflates the somatic index of the intestine.

The RGL and RIL are traditionally used to categorize feeding habits, forming a continuum from carnivory to herbivory (Kurbah & Bhuyan 2018; Isibor et al 2023). In this study, *T. tambroides* maintained RGL values typical of omnivorous species (2–3 times body length). However, POME exposure appeared to disrupt the structural integrity of the gut. The observed high RIL values in treated fish are likely a consequence of the loss of structural tone due to necrosis of the columnar epithelium and submucosal cells, as described in pesticide-exposed fish (Ghanbahadur et al 2015). The disruption of the mucosal layer impairs nutrient absorption (Pyle et al 2005), potentially forcing the fish to physiologically maximize intestinal surface area (distension) in a failed attempt to compensate for reduced nutrient uptake efficiency.

Conclusions. The deviations in biometric indices (HSI, GaSI, ISI, RGL) in *T. tambroides* are not merely morphological changes but are indicative of underlying physiological stress responses, specifically hepatic detoxification overload, gastrointestinal inflammation, and oxidative stress induced by the toxicity of POME.

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Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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