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Turbidity effect derived from palm oil mill effluent altered predation period of siamese fighting fish (*Betta splendens*, Regan 1910)

Ilham Zulfahmi[1,*], Agung Setia Batubara[2], Adli Waliul Perdana[3], Zultira Harina Roza[4], Badratun Nafis[4], Furqan Maghfiriadi[5]

[1] *Department of Fisheries Resources Utilization, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia*
[2] *Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Kota Medan 2022, Sumatera Utara, Indonesia*
[3] *Department of Aquaculture, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.*
[4] *Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia*
[5] *Center of Aquatic Research and Conservation, Universitas Islam Negeri Ar-Raniry Banda Aceh 23111, Indonesia.*

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ABSTRACT

Contamination of palm oil mill effluent (POME) into water bodies potentially increases turbidity, resulting in disrupting aquatic organisms behaviour, including predation period. However, the effect of increased turbidity due to POME contamination toward the fish predation is still unexplored. Siamese fighting fish (*Betta splendens*) is one of the ideal test fish to investigate this effect. This study aims to analyze the effect of increased turbidity due to POME contamination toward the fish predation period of Siamese fighting fish. The research design was completely randomized with six treatments (0, 20, 40, 60, 80 and 100 NTU) followed by five repetitions for each treatment. The experimental data parameters include the predation rate of Siamese fighting fish during foraging activity, which was calculated from the percentage of the number of mosquito larvae consumed per 2 min during 15 min. The significance level of predation between treatments in each observation time period was analyzed using a one-way Analysis of Variance (ANOVA) with the confidence interval set at the 95% level. The results showed that at a turbidity level of 100 NTU, the predation period of Siamese fighting fish had been altered. In the period of 0-2 min, the predation rate decreased significantly. Meanwhile, the predation rate increased significantly during the period of 2-4 and 8-10 min. This finding should be considered to support POME remediation management, especially turbidity parameters.

Introduction

An inappropriate approach to managing palm oil mill effluent (POME) might threaten the aquatic ecosystem (Chan *et al.,* 2013; Zulfahmi *et al.,* 2021). POME contains organic matter in high concentrations such as Biological Oxygen Demand (BOD, range: 25000–65714 mg/L) and Chemical Oxygen Demand (COD, range: 44300–102696 mg/L), as well as various heavy metals (Wang *et al.*, 2015; Hashiguchi *et al.*, 2021). The contamination of POME has been reported to cause detrimental impacts on phytoplankton and fish (Muliari and Zulfahmi, 2016; Muliari *et al.,* 2018; Zulfahmi *et al.,* 2018; Muliari *et al.,* 2019; Muliari *et al.,* 2020). Exposure to POME decreased the gill operculum rate of Nile tilapia (*Oreochromis niloticus*) and altered liver tissue of zebrafish *(Dario rerio),* including congestion, hemorrhage, hyperplasia (Muliari *et al.,* 2018; Zulfahmi *et al.*, 2023). It also has caused a decrease in hatchability and increased larval deformities of Nile tilapia (Muliari *et al.,* 2020).

POME also contains a high concentration of Total Suspended Solid (TSS, range: 18000–46011 mg/L) which potentially increases water turbidity (Wang *et al.*, 2015). The turbid conditions might reduce the photosynthetic activity of algae and plankton in order to provide food and oxygen for other aquatic organisms (Muliari and Zulfahmi, 2016). In addition, suspended particles have impaired gill performance, resulting in decreasing growth rate of fish (Sutherland and Meyer, 2007)

Siamese fighting fish are one of the vulnerable organisms that suffer from the turbid condition generated by the contamination of POME. This might occur due to its wide distribution in Indonesian water, such as rivers, rice fields, swamps, and shallow fresh waters. Previous studies have revealed a decrease in the feeding ability of several

* Corresponding author.
Email address: ilham.zulfahmi@usk.ac.id

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types of fish due to turbid conditions (Sutherland *et al.,* 2002; Sweka and Hartman, 2003; Rowe *et al.,* 2003; Zulfahmi *et al.*, 2022). The predation rate of Arfak rainbowfish (*Melanotaenia arfakensis*) reared at higher turbidity levels (⩾ 100.12 NTU) was decreased to 23.2%-65.9% (Manangkalangi *et al.*, 2017). Pikeperch fish (*Sander lucioperca*) that inhabit turbid conditions tend to choose larger zooplankton (Zingel and Paaver, 2010). Turbidity also impacts the feeding ability of rosyside dace fish (*Clinostomus funduloides*), as indicated by the increase in the energy required for foraging activity (Zamor and Grossman, 2007). To date, the impact of turbidity on the feeding period of siamese fighting fish remains unknown. Thus, this study aims to examine the effect of turbidity due to contamination of POME on the feeding period of siamese fighting fish.

Materials and Methods

Fish and POME Collection

A total of 40 male siamese fighting fish with a total length range of 3.5-4.5 cm and a total weight range of 1-2 g were purchased from local traders in Banda Aceh, Indonesia, and then acclimatized for one week in laboratory conditions. The acclimatization was done in transparent tanks with 500 ml of fresh water (1 NTU) (1 fish per tank). During acclimatization, fish were fed 5-6 mm mosquito larvae of the fourth instar to satiation level twice daily at 09.00 and 14.00 WIB (Western Indonesian Time). Fresh mosquito larvae (*Culex* sp.) were also purchased from a local market in Banda Aceh city of Aceh province. The fish were fasted for 24 hours before treatment. No aeration and heating systems were installed during the acclimatization and treatment test. The POME from the eighth settling pond was collected from the Palm Oil Processing Plant in Aceh Province. Furthermore, POME was transported to the laboratory using land transportation.

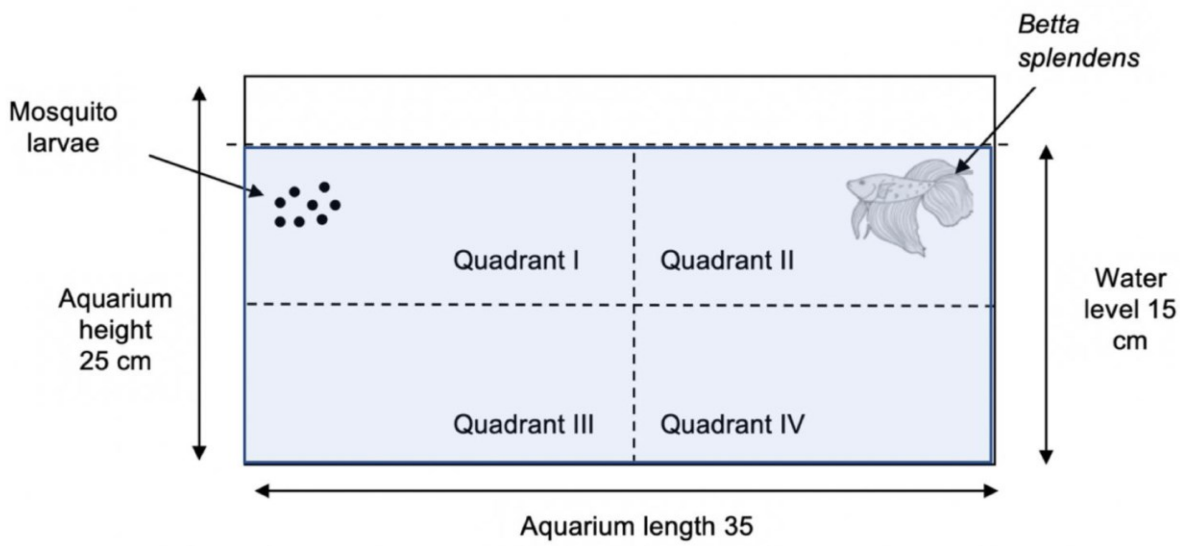

Figure 1. Ilustration of aquarium set-up (35 x 21 x 25 cm3, L x W x H)

Research Design

The research design was a Completely Randomized Design (CRD) with six treatments and five repetitions of each treatment. The range of turbidity in the treatment refers to the previous studies on POME contamination in water bodies (Zahara *et al.*, 2016). The details of each treatment were as follows: control treatment (turbidity level 0 NTU), treatment A (turbidity level 20 NTU), treatment B (turbidity level 40), treatment C (turbidity level 60 NTU), treatment D (turbidity level 80 NTU) and treatment E (turbidity level 100 NTU). The aquarium's turbidity value was validated using a turbidity meter (Lutron TU 2016). Preference tests were performed in a 10-L aquarium (35 ×21×25 cm3). Siamese fighting fish were placed on the right side of the treatment aquarium, and then 100 individual siamese fighting fish were added to the opposite side (Figure 1). The experimental data parameter includes the predation period of siamese fighting fish during foraging activity, which was calculated based on the proportion of mosquito larvae consumed every 2 min throughout a 15-minute timeframe.

Data Analysis

The data were presented in the form of the mean and standard deviation. The significance level of predation between treatments in each observation time period was analyzed using a one-way Analysis of Variance (ANOVA) with the confidence interval set at the 95% level. Statistical analysis was performed using SPSS 25 software.

Results

Predation Period

The period of highest predation in all treatments occurred between 0 and 4 minutes and tended to decrease after that. In the 0-2 minute range, the predation rate of betta fish in the control, treatment A (20 NTU), treatment B (40 NTU), treatment C (60 NTU), and treatment D (80 NTU) was not significantly different ($p > 0.05$), with the lowest predation rate of 31.39 ± 6.25% and the highest of 36.14 ± 6.95%. However, the predation rate decreased significantly in treatment E (100 NTU) with a value of 22.78 ± 4.57% ($p < 0.05$) (Figure 2). Moreover, in the 2-4 minute range, the predation rate of siamese fighting fish in the control, treatment A (20 NTU), treatment B (40 NTU), treatment C (60 NTU), and treatment D (80 NTU) was not significantly different ($p > 0.05$). The lowest predation rate was 22.05 ± 2.84%, and the highest was 34.51 ± 6.49%. Predation rate increased significantly in treatment E (100 NTU) with a value of 35.51 ± 3.49% ($p < 0.05$) (Figure 2). There was no significant difference in siamese fighting fish predation rate between treatments in the 4-6, 6-8, 10-12, 12-14, and 14-15 minutes ranges. The significance of the siamese fighting fish predation level was then observed in the 8-10 range. In this period, the predation rate of siamese fighting fish in the treatment E (100 NTU) increased significantly compared to the control with respective values of 12.67 ± 3.76% versus 6.84 ± 2.78% ($p < 0.05$) (Figure 2).

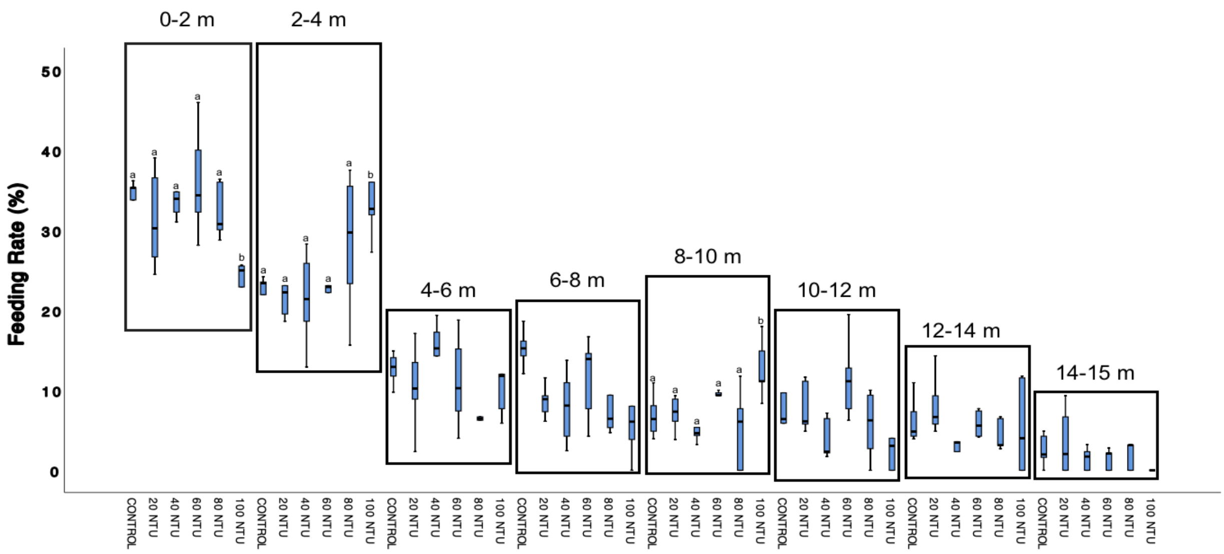

Figure 2. Predation period of siamese fighting fish between treatments during 15 min POME exposure.

Discussion

The increased turbidity of water has been reported to affect the fish's appetite. (Chapman *et al.,* 2010; Leahy *et al.,* 2011). Fish will increase their non-visual sensory capabilities for detecting both food and the threat of predators under turbid conditions (Nieman and Gray, 2019; Johansen and Jones, 2013). The time it takes for Betta fish *(Betta splendens)* to consume mosquito larvae can vary based on different factors such as water temperature and the size of the larvae (Tyagnes-Hanindia *et al.,* 2023). In forraging efforts, smaller fish may take longer to consume mosquito larvae due to their smaller size and reduced predation efficiency. A study conducted in Gwalior, India, toward three well-known species including *Betta splendens* found that small-sized female fish species had a greater predation efficacy on mosquito larvae (Tyagnes-Hanindia *et al.*, 2023). A study observed that *Betta splendens* can consume *Aedes aegypti* mosquito larvae within an average of 12.03 minutes for males and 11.70 minutes for females (Thongprajukaew *et al.*, 2019). However, we suspect that the time it takes for Betta fish to consume mosquito larvae can be relatively quick, with variations based on environmental conditions such as turbidity and the specific characteristics of the fish and larvae. The result of this study show that in early predation period (0-2 minutes), the predation rate of siamese fighting fish at the highest turbidity (100 NTU) decreased significantly compared to other treatments. Decreased predation rates due to increased turbidity (caused by sedimentation and algal blooms) have also been reported in several fish, for instance *Notropic atherinoides*, *Neopomacentrus azysron*, *Gasterosteus aculeatus*, and *Dascyllus aruanus* (Helenius *et al.,* 2013; Sweka and Hartman, 2001; Ardjosoediru and Ramnarine, 2002).

A significant increase in predation rates was observed in siamese fighting fish reared at a turbidity level of 100 NTU during period of 2-4 minutes. Siamese fighting fish were able to adapt by enhancing non-visual alternative sensors, allowing them to detect prey more effectively. The existence of behavioral adaptations allows fish to forage effectively when experiencing visual disturbances (Figueiredo *et al.,* 2016). In certain cases, the presence of turbidity can actually increase the level of predation. Meanwhile, some species of fish were better able to avoid predators at high turbidity levels (Wojtas *et al.,* 2015).

During the observation period of 4-8 minutes, there was no significant change in the predation period between treatments. During this period, fish was thought to reduce movement to maximize digestive activity. However, siamese fighting fish reared in low turbidity conditions were able to use space more actively than siamese fighting fish at high turbidity. Increased turbidity had boosted the energy allocation of siamese fighting fish in foraging activity, resulting in decreased movement. Similar results were also reported in zebrafish (*Danio rerio*) reared in turbid conditions (>300 NTU), showing decreased aggression and activity with control (<5 NTU). Normal fish tended to be more active than fish in turbid condition. A decrease in fish movement rates was also reported in *Acanthochrom polyacanthus* that inhabit high turbidity condition due to sedimentation (Helenius *et al.,* 2013). The significance of the predation rate was back to observed in siamese fighting fish reared at a turbidity level of 100 NTU during period of 8-10 min. This may indicate a substantial amount of energy in predation activity dictated by turbid conditions. As how as a study on planktivorous damselfishes *(Pomacentridae)* found that attack success was reduced by up to 56% at turbidity levels as low as 4 NTU cause by sedimentation (Johansen and Jones, 2013). This case indicates that turbid conditions create a more difficult situation for them to catch prey. Reduced attack success has a major impact on energy acquisition and is potentially detrimental to ecological performance many species (Wolanski *et al.,* 2009; Johansen and Jones, 2013). In addition, at this period, the energy required for digestive processes was relatively low, therefore fish tended to restart their predation activity to meet the energy requirements. At period of 10-12 min, 12-14 min, and 14-15 min siamese fighting fish was thought as a period of fullness followed by a decrease in appetite.

Conclusion

Increased turbidity due to POME at a turbidity level of 20-80 NTU during the observation period did not show any significant differences. Meanwhile contamination up to 100 NTU has affected the predation period of siamese fighting fish. A significant level of predation was recorded in the period 0-2, 2-4, and 8-10 min. In the period of 0-2 min, the predation rate decreased significantly. Meanwhile, in the period of 2-4 and 8-10 min, the predation rate increased significantly. The altered predation periods suggest that increased turbidity affects the feeding rate of Siamese fighting fish, potentially leading to lower predation levels with longer exposure to higher turbidity. This may indicate a substantial amount of energy in predation activity dictated by turbid conditions. Further research related to the correlation effect between other environmental factors, such as temperature and the turbidity level toward the feeding behaviour of Siamese fighting fish, is necessary.

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