A nesting ability of male siamese fighting fish *(Betta sple ndens)* under different level of turbidity derived from palm oil mill effluent

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Abstract. Contamination of Palm Oil Mill Effluent (POME) into water bodies potentially disrupting the visualization and affects behaviourial changes such nesting ability of male Siamese Fighting Fish (*Betta splendens*). The study examined the effects of turbidity due to POME contamination toward nest area as one of the indicators for the reproductive behavior of the *betta splendens.* The research used Completely randomized Design with different level of turbidity: <1 NTU (control), 20 NTU (treatmen A), 40 NTU (tretment B), 60 NTU (Treatment C). Preference tests were carried out in a 6-L aquarium (20x20x20 cm^3). Nest area was measured each hour for 8 hours. Nest area was captured using camera and measured with imageJ. Result showed there was no significant difference in nest area of *Betta splendens* in different level of turbidity. The range of nest area in sequence until the end of exposure were 0-0.8 cm^2, 0.7-2.5 cm^2, 4.6-6.7 cm^2, 4.5-7.2 cm^2, 7.6-8.7 cm^2, 9.4-11.11 cm^2, 9.7-12.5 cm^2, dan 11.6-14.14 cm^2. Turbidity due to POME collected from the last pond with low pollutant contamination has no impact on nesting ability of betta fish. Nest building behavior is often correlated with hormone concentrations due to pollutant contamination with a certain consentration of pollutant.

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1 Introduction

Indonesia is listed as the world's leader in palm oil production. Indonesia's palm oil plantation area reached 11 million hectares (Mha) and produced 31 million tons of palm oil in 2015 [1]. The palm oil waste produced is in the form of solid, liquid and gas waste which has the potential to reduce water quality and cause pollution [2]. POME causes damage to the reproduction of nile tilapia (*Oreochromis niloticus*) such as decreasing the performance of tilapia hormones and resulting in a decrease in gonadsomatic index and spermatocrit [3]. Exposure to POME reduces the concentration of estradiol, testosterone and progesterone hormones in tilapia *(Oreochromis niloticus)* [4]. Exposure to POME also caused histological alteration in the liver tissue of zebrafish (*Dario rerio*), including congestion, hemorrhage and hyperplasi [5]. Several related cases are thought to be the result of high levels of by-products from the palm oil processing process which contain various organic materials in the form of Biological Oxygen Demand (BOD, range: 25000–65714 mg/L), Chemical Oxygen Demand (COD, range: 44300–102696 mg/L), and Total Suspended Solid (TSS, range: 18000–46011 mg/L) as well as various heavy metals [6][7].

High concentrations of TSS in water bodies increase water turbidity. Previous study revealed that POME waste contamination in Bangka Regency has increased the turbidity of the Mabat river from 5.04 Nephelometric Turbidity Units (NTU) under normal conditions to 23.5-65.4 NTU [7]. This increase in turbidity has the potential to block the entry of sunlight which has a negative impact on photosynthetic activity, reduces the availability of dissolved oxygen and disrupts the visual performance of fish [8]. Increased turbidity affects interactions between male and female fish and reduces spawning effectiveness [26].

The Siamese fighting fish (Betta splendens) is widespread in Indonesia from rivers, wetlands and swamps [27][28]. The population of Siamese fighting fish has been decreasing due to various factors such as habitat loss and water pollution [29]. Additionally, the introduction of non-native species and competition for resources have also contributed to the decline in their population. In addition, high levels of turbidity indicate the presence of pollutants that accumulate in fish tissues and cause physiological and behavioral changes, which ultimately affect fish populations [25]. Therefore, although there is no direct relationship between turbidity and Betta fish populations, the impact of turbidity on their behavior and the presence of pollutants in turbid water may affect Betta fish populations indirectly. The decreasing population of Siamese fighting fish is a cause for concern, as these fish are not only popular in the aquarium trade but also play a crucial role in their natural ecosystems [30]. Increased turbidity can affect fish behavior due to visual limitations [5]

In an effort to produce new individuals, male Betta fish will make bubbles and form a foam nest (bubblenest) on the surface of the water. Bubble nests play important rule in guarding eggs during spawning [10]. The results of research [5] reported that turbidity can influence the level of predation on emerald shiner fish (Notropis atherinoides). Fish reared at 40 NTU turbidity showed a reduction in predation due to reduced visual performance. Another study by [6] also reported that increased turbidity due to POME contamination of ~60 NTU had affected the eating behavior of Betta splendens fish as indicated by a reduction in the fish's home range and slow food detection efforts. The turbidity level of 8.7 NTU was caused by the addition of bentonite which caused a decrease in foraging behavior in E. fonticola [9]. The same case was also observed in different types of fish including *Oncorhynchus tshawytscha* and *Oncorhynchus mykiss,* that increasing turbidity caused a decrease in the number of fish that foraged and the amount of prey consumed [22][23][24]. Although no research has directly examined the impact of turbidity on the ability of fish to build nests, information from related studies can provide insight into how environmental conditions at different levels of turbidity can influence the overall behavior and abilities of fish. Therefore, this research aimed to investigated the impact of turbidity due to POME on the ability of Siamese Fighting Fish to build nests.

2 Material and Methods

2.1 POME and Fish Collection

A total of 20 L of POME from the eighth settling pond (last pond) (100 NTU) was collected from Palm oil Factory located in Aceh Province. Until the exposure period, POME was stored at a temperature <4ºC to avoid degradation. A total of 25 pairs of Siamese Fighting Fish with uniform colors (predominantly red) were collected from local traders located in Banda Aceh. The total length range of male and female Siamese Fighting Fish is 4-4.5 cm. The weight of male Siamese Fighting Fish ranges from 1.53-1.96 g, while the weight of the fish Female Siamese Fighting Fish range between 1.53-2.10 g. Male Siamese Fighting Fish are characterized by black spots on the dorsal fin area and red vertical lines on the gill covers. Meanwhile, female Siamese Fighting Fish are characterized by their bulging stomach shape accompanied by 2-3 gray vertical lines on the side of the body [11].

Fish acclimatization was carried out individually in transparent containers for one week (Photoperiod 12:12 hours light: dark; DO: 6.2 mg O^2/L; temperature: 25 C; PH: 7.5 and turbidity: <1 NTU). To avoid stressful conditions due to interactions between fish, each side of the acclimatization container was lined with opaque paper. Feed in the form of silk worms was given twice a day (09.00 and 16.00 WIB) ad libitum [6]

2.2 Experimental Design

The research design was a Completely Randomized Design (CRD) with 4 treatments accompanied by 3 repetitions for each treatment. The range of turbidity in the treatment refers to the results of previous research regarding pollution of palm oil liquid waste in Indonesian waters [7]. The details of each treatment are as follows: control (turbidity level <1 NTU), treatment A (turbidity level 20 NTU), treatment B (turbidity level 40 NTU), treatment C (turbidity level 60 NTU). The turbidity value for each treatment is obtained by mixing POME into the treatment container until it reaches the specified turbidity level. Validation of the media turbidity value was carried out using a turbidity meter (Lutron TU-2016, Taiwan).

The experimental container is an aquarium 20 x 20 x 20 cm^3 with a volume of 6 liters of water. Each aquarium is filled with one pair of Siamese Fighting Fish. Bubble attachment media in the form of clear plastic measuring 5x5 cm is placed on the water surface close to one end of the tank for the male to build a bubble nest. Nest area was documented every hour and measured using the ImageJ application version 1.5.3 (Figure 3). All maintenance and experimental stages were approved by the Animal Ethics Committee of Faculty of Science and Technology, Universitas Islam Negeri Ar-Raniry (Animal Ethics approval number: BIO/ET/2020/1970).

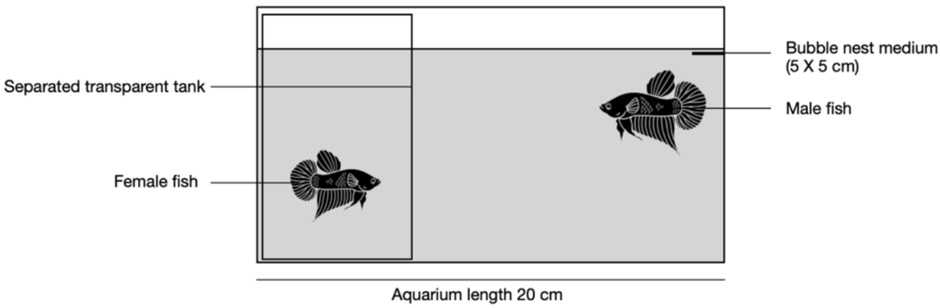

Fig. 1. Schematic diagram of aquarium setup during introduction period (20 x 20 x 20 cm^3). The aquarium is given a partition with a size of 8x8 with a volume of 1L of water. The bubble attachment medium is transparent plastic (5x5 cm^2).

3 Result and Discussion

Nest size in each treatment increased with increasing duration. The results of statistical analysis showed that there were no significant differences between treatments from the first hour to the last hour during the exposure period ($p>0.05$). The range of nest sizes built by Siamese Fighting Fish between treatments from the first hour to the eighth hour respectively are 0-0.8 cm^2, 0.7-2.5 cm^2, 4.6-6.7 cm^2, 4.5-7.2 cm^2, 7.6-8.7 cm^2, 9.4-11.11 cm^2, 9.7-12.5 cm^2, and 11.6-14.14 cm^2

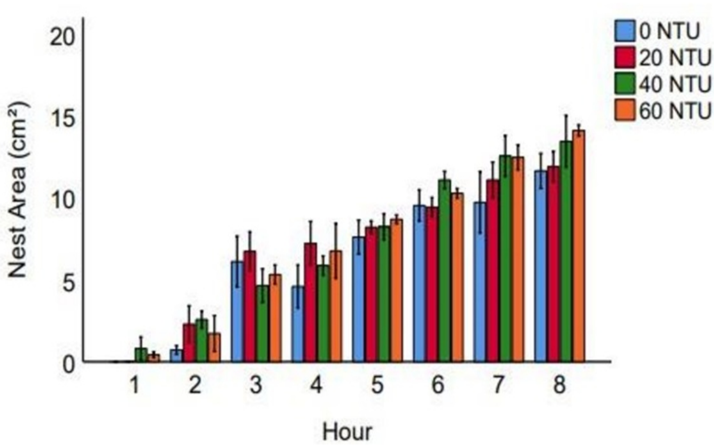

Fig. 2. Nest area of Siamese Fighting Fish in each treatment

A number of studies have reported the impact of turbidity on aquatic organisms [12][13]. Turbidity is closely related to visual function which directly impacts changes in behavior [14]. In natural environments, turbidity has been recognized as a major threat to the diversity of aquatic systems [15]. Male Siamese Fighting Fish have a vital role in reproductive success through nest formation, egg placement and parental care [16]. The behavior of male Betta fish before spawning is usually to release bubbles as a nest for their eggs later. According to [31] Betta fish are bubble nesters, that is, they make foam nests before spawning and put the eggs in them. The bubble substrate is useful so that the eggs do not sink to the bottom of the water, usually these bubbles are placed on aquatic plants [32]. The nest built by the male

Betta fish acts as a container for the eggs and provides a safe environment for the initial growth and development of the offspring [33]. The function of the bubble nest is to protect the embryo and provide more oxygen or nutrients to the embryo. The size of the bubble nest built by male fish can influence the reproductive choices of female fish and the survival rate of fish larvae [17].

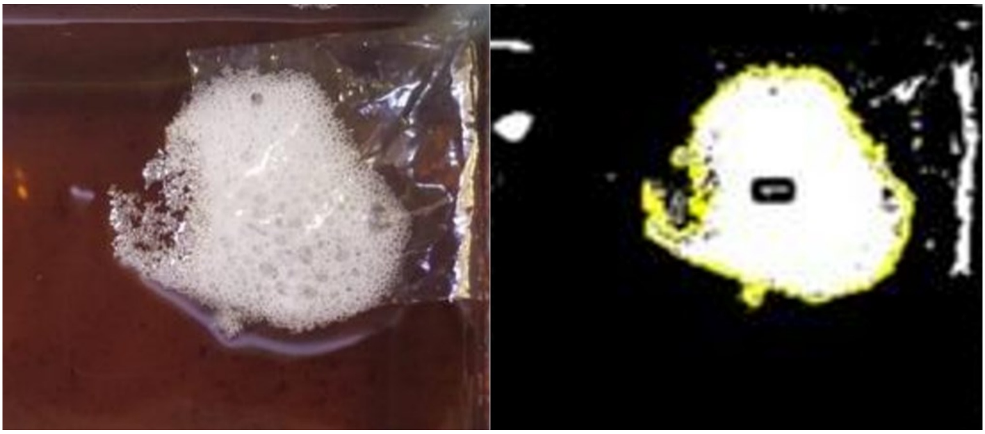

Fig. 3. The description of bubble nest and the measurements methode using ImageJ.

Nest building by male Betta fish involves several stages. These stages include preparing raw materials, making nests, and monitoring. The purpose of making betta fish nests is to maintain the health of the fish, maintain the survival of the fish, and help the development of the fish. The time needed to make a betta fish nest is very short, provided that a nest made in 1 hour can keep fish for 2-3 months. Betta fish nests can be beard-shaped, small, or large, depending on existing needs and guarding conditions [34]. Mucus-coated nest bubbles are formed from air and mucus in the buccal cavity of Siamese fighting fish. This mucus is very thick, it can allow the bubbles to survive longer in the water. Male fish can collect these bubbles to make nests. Betta fish are all unique, so the frequency of making nests is also unique. Some bettas may develop them regularly, while others may develop them less frequently or not at all. Daily, weekly, or monthly are the common time periods for male Betta fish to build a nest. Some nests are large, some are small, and their thickness can also vary. Regular bubble nest building can be a sign that Betta fish are happy and healthy, and suitable for mating [35]

The results of this study indicate that increasing turbidity does not affect the fish's ability to build nests. We suspect that the motivation to build a nest is influenced by internal factors, while turbidity is an external factor that only plays a small role during the nest building period. According to [17] larger male fish build larger bubble nests. This suggests that large nests will be able to accommodate more fish eggs and larvae which can only be built by larger males. However, the number of eggs per clutch did not correlate with male body size or nest bubble area. Habitat arrangement provides better results in building bubble nests in aquariums [18]. [10] reported that Siamese Fighting Fish pairs exposed to fluoxetine did not have bubble nests of consistent and optimal size. Exposure to fluoxetine causes a decrease in locomotion [19], a decrease in androgen hormones and sexual motivation in male fish [20]. As a result, during the spawning period some of the eggs fall to the bottom of the container and take the male's time to collect the eggs and return them to the bubble nest [10].

Fish have varied nest building strategies in turbid conditions through special sensory adaptations. Sensory adaptation is an alternative for fish to navigate and build nests in murky water. One of the sensory adaptations is the electrical signals utilized by fish for passive and active electrolocation and communication in poor visual conditions. Electrical signals have evolved in fish that live in turbid water and tend to be active at night [36]. Active electrolocation involves the production of electrical signals, the discharge of electrical organs by specially modified cells (electrocytes). Electrocytes are formed from muscle fibers or from nerve endings in apteronotids [37][38]. The electric field around the fish is detected by electroreceptors in the body and especially in the head [39][40]. Electroreceptors can detect differences between living and inanimate objects as well as differences in size, shape, and distance by monitoring changes in signal amplitude, resistance, and capacitance [41]. In the context of nest building, fish have special sensory adaptations to navigate and build nests in turbid water by utilizing the lateral line system. This system allows fish to detect changes in water pressure, helping to find suitable nesting sites even in low visibility conditions. All fish have a special mechanical sensor (mechanosensory) lateral line system to detect disturbances in the water. Due to the viscosity and density of water, disturbances created by the presence of potential prey and other moving objects are detected via mechanoreception [21].

4 Conclusion

No significant differences were observed of nest area of Siamese Fighting Fish between treatments from the first hour to the last hour of exposure period ($p>0.05$). The results of this study indicate that increasing turbidity does not affect the fish's ability to build nests. This suggests that large nests will be able to accommodate more fish eggs and larvae that only larger males can build.

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