INTRODUCTION

Indonesia has become the largest palm oil producer all over the world. Food and Agriculture Organization (FAO) noted that the total palm oil production of Indonesia in 2014 reached 29.27 million metric tons (FAO, 2016). Together with Malaysia, Indonesia was able to produce more than 85% of the world’s palm oil (Jayed et al., 2011; Fitzherbert et al., 2008). One of the negative impacts arising from the existence of palm oil mill industry is the increased pollution from Palm Oil Mill Effluent (POME), which can affect the aquatic environment (Alhaji et al., 2016). POME contains a high concentration of chemical oxygen demand (COD) ranging from 45,000–65,000 mg/L, biochemical oxygen demand (BOD) ranging from 21,500–28,5000 mg/L and high total suspended solids (TSS) ranging from 15,660–23,560 mg/L (Wong et al., 2009; Chan et al., 2013). Bello et al. (2013) reported that oil and grease content in POME being as high as 4,000 mg/L with a pH between 3.4 and 4.7.

High concentrations of COD and BOD harm aquatic organisms, for instance, decrease of oxygen consumption and cause of hypoxic condition (Maygaonkar et al., 2012; Verberk et al., 2016; Vithana et al., 2019). Oil and grease are considered as hazardous pollutants, particularly in the...
aquatic environments and can completely damage the ecology of the aquatic ecosystems (Bala et al., 2015). One previous study by Muliari and Zulfahmi (2016) revealed that contamination of POME had resulted in negative effects on the phytoplankton community. Furthermore, exposure to POME has led to a disruption of the fish liver and gill performances which can be signalled by histopathological changes in the tissue (Zulfahmi et al., 2017a; Muliari et al., 2018).

Nile Tilapia (Oreochromis niloticus) is a species of fish that is potentially impacted by POME. Contamination occurs due to several eco-biological characteristics such as their extensive distribution in aquatic environments and their availability at various stages throughout the season (Zulfahmi et al., 2014). Nile Tilapia has been used to assess the effects of several toxicants such as treated leachate (Gallão et al., 2019), copper oxide (Abdel-Khalek et al., 2015), and zinc oxide nanoparticles (Alkaladi et al., 2015).

Mekkawi and Hasan (2011) state that the disturbance of fish reproductive functions can potentially affect the dynamics of fish populations and has direct implications on sustainable fishery management. The presence of pollutants in a water body can result in a dangerously compromised fish reproduction system which potentially generates a scarcity of stock and the increased price of Nile tilapia. Al-Rawi (2015) proved that pollution can affects the physicochemical characteristics of the water, sediment and other biological components, so harms the quality and quantity of fish stocks.

Reproductive hormones play an important role in supporting the success of fish reproductive processes (Kime et al., 1996; Rurangwa et al., 1998). The disturbances of reproductive hormones affects the gonadosomatic index (GSI), fecundity, egg diameter, and vitellogenesis of fish (Hecker et al., 2002). Several studies have reported that pollutants have an impact on reducing the concentration of reproductive hormones. Ebrahim and Taherianfard (2011) reported a decrease in the concentration of estradiol, progesterone and testosterone hormones in Cyprinus carpio and Capoeta sp. due to heavy metal exposure. Luo et al. (2015) reported that cadmium exposure had caused a decrease in the concentration of estradiol in Nile Tilapia. To date, we lacked the information on the exposure of POME to the performance of fish reproductive hormones. Hence, the objectives of this study were to elucidate the toxic effects of POME on the performance of reproductive hormones (estradiol, progesterone, and testosterone) of female Nile Tilapia.

### MATERIALS AND METHODS

#### STUDY AREA, COLLECTION AND ACCLIMATIZATION OF FISH

Fish (N = 300; weight: 9.46 g ± 1.16; length: 7.75 cm ± 1.67) were obtained from the Department of Fish Hatchery Center (BBI) Batee Iliek, Bireuen Regency Aceh, Indonesia. All procedures for fish treatment were conducted according to the international standards of animal welfare (Canadian Council on Animal Care, 1993). Fish were acclimatized over a period of one week.

Raw POME was collected from the Syaukat Sejahtera Palm Oil mill factory in a sterile container and brought back to the laboratory. Thirty liters (30 L) of stock solutions of POME (100 mg/L) was prepared and diluted to achieve the required concentration for the chronic test. Tanks (50 x 30 x 40 cm³) were used as fiberglass containing 25 L of water. Water was taken from an artesian well and aerated for 24 hours before being used. Fish rearing and chronic test were carried out at the Aquaculture Laboratory, Faculty of Agriculture, University of Almuslim (5.19’47.54” N, 96.78’72.44” S).

#### EXPERIMENTAL DESIGN

The chronic test was conducted by using completely randomized design. The concentration of POME in each treatment was selected based on the 96-h LC₅₀ of the POME on Nile Tilapia obtained from a previous study, this being 15.65 mg/L (Zulfahmi et al., 2017b). Treatments were designed as follows: control (0% palm oil mill effluent: 0 mg/L), treatment A (10% of the 96-h LC₅₀: 1.565 mg/L), treatment B (15% of the 96-h LC₅₀: 2.347 mg/L), and treatment C (20% of the 96-h LC₅₀: 3.130 mg/L). Ten fish were stocked into each of the 20 fiberglass tanks. Each of the control and treatment was maintained in five replicates. Fish were fed with commercial feed at 5% kg of body weight twice a day. Exposure period of POME lasted for 45 days. Fish faeces and excess food were stripped every day using the siphon method. Water was completely changed every 15 days, and new concentrations of POME were used.

#### MEASUREMENTS OF HORMONE, TOTAL LIPID CONTENT AND RELATIVE BILE VOLUME

At the end of the exposure period of 45 days, ten fish from each replicate was collected and sacrificed by using clove oil for hormone analysis. Hormone measurements were performed by ELISA method at Veterinary Clinic, Reproduction and Pathology of the Faculty of Veterinary Medicine, Bogor Agricultural University. One mL blood sample from each fish was taken by using syringe from the caudal vessels and then inserted into microtube to be centrifuged for 15 minutes at 4000 RPM. The serum and blood plasma...
were separated and stored at – 20°C until use. The DRG Instruments GmbH, Marburg, Germany (REF EIA-2693 for Estradiol, REF EIA-1561 for Progesteron, REF EIA-1559 for Testosterone) was used for the analysis. The intensity of Color assay solution, was scanned and analyzed by using ELISA reader (BIORAD xMark Microplate Spectrophotometer) at a wavelength of 450 nm. The concentrations of estradiol, testosterone, and progesterone hormones were calculated by using MPM 6 program. The relative bile volume was measured based on the equation described in Zulfahmi et al. (2014):

\[ \text{RBV} = \frac{\text{BV}}{\text{LV}} \times 100 \]

Where RBV: relative bile volume (%), BV: bile volume (mL), LV: liver and bile volume (mL). Measurements of total lipid content in gonads and liver were performed by the Soxhlet method (AOAC 2005).

**Statistical Analysis**

SPSS 22 (SPSS Inc., Chicago, IL) for Windows software was used for the statistical analysis of reproductive hormone concentration, relative bile volume and total lipid content in each treatment by using one way analysis of variance (ANOVA). Least significant difference (LSD) was performed to test differences value of the parameter between treatments. P values ≤ 0.05 were considered significant.

**RESULTS**

**Reproductive Hormone**

Results showed that exposure to POME caused a decrease in the concentration of estradiol and testosterone in female Nile Tilapia. The highest estradiol concentration observed in treatment B while the lowest observed in treatment C were 6.31 ± 1.65 ng/mL and 1.35 ± 0.03 ng/mL, respectively. There was no significant difference in the concentration of estradiol hormone in treatment A and treatment B. However, a significant decrease in the concentration of estradiol hormone was observed in treatment C (p < 0.05) (Figure 1). The testosterone concentration was highest in control and lowest in treatment C were 6.63 ± 2.62 ng/mL and 1.18 ± 0.11 ng/mL, respectively. Statistically, testosterone concentration decreased significantly in treatment C (p < 0.05) (Figure 2). Exposures to POME had no significant effect on the concentration of progesterone hormone (p > 0.05) (Figure 3).

**Total Lipid Content in Gonad and Liver**

The highest value of total lipid content in gonads observed in control while the lowest observed in treatment C were 6.50 ± 0.60% and 2.12 ± 0.24%, respectively. Statistically, there were no significant differences in the total lipid content in gonads in treatments A and B. Nevertheless, a significant decrease in total lipid content in gonad was observed at treatment C (p < 0.05) (Table 1). There were no significant differences observed in the total lipid content in liver between treatment (p > 0.05) (Table 1). Furthermore, results showed a positive correlation between the decrease of total lipid content ratio between gonad and liver. A significant difference in total lipid content ratio in control and treatment C was observed (p < 0.05). However, the lowest ratio (0.75) was detected in treatment C (Figure 4).
Figure 4: Total lipid content ratio between gonad and liver of female Nile Tilapia in each treatment. *The different letters indicate significant differences (p < 0.05).

Table 1: Total lipid content in gonad and liver of female Nile Tilapia in each treatment.

| Total Lipid Content | Treatments |
|---------------------|------------|
|                     | Control    | A         | B         | C         |
| Gonad (%)           | 6.50±0.60a | 4.13±0.86a| 4.47±1.31a| 2.12±0.24b|
| Liver (%)           | 4.84±1.39a | 4.00±1.54a| 3.77±1.68a| 2.27±0.33a|

*The different letters indicate significant differences (p < 0.05).

Figure 5: Relative bile volume of female Nile Tilapia in each treatment. *The different letters indicate significant differences (p < 0.05).

**DISCUSSION**

Fish reproductive system is potentially affected by environmental factors such as pollutants. Previous research have reported that exposure to POME cause the decrease in gonadosomatic index value, and shrinking oocyte diameter of female Nile Tilapia (Zulfahmi et al., 2018). The decrease in reproductive performance is strongly associated with the disturbances of reproductive hormones. Carboufan exposure reported a significant reduction of estradiol hormone and led to inhibition of the development of ovarian follicles in Clarias gariepinus and Heteropneustes fossilis (Ibrahim et al., 2014; Chatterjee et al., 2001). Ghazala et al. (2014) reported that the decreasing of hormones levels due to pollution helps to provide valuable biomarkers and useful tools in monitoring the impacts of stressors on fish. In our study, estradiol hormone concentrations of the female Nile Tilapia decreased significantly after exposure to POME. Sullivan and Yilmaz. (2018) stated that estradiol hormone plays an important role in inducing calcitonin, which affects vitellogenin secretion and development of oocytes. The decrease of estradiol concentration in different fish has also been reported when they were exposed to heavy metals (Ph, Hg, Cd & As) (Ebrahimi and Taherianfar, 2011), dioxin (Heiden et al., 2008), and polycyclic aromatic hydrocarbons (Thomas et al., 1995). The disruption of reproductive hormone production is caused by disruption of the hypothalamic-pituitary system of fish. Kime (1995) stated that pollutants could negatively regulate estrogen signalling by inducing oxidative metabolism of estrogens, suppressing and efficacy of the estrogen receptors, and inhibiting estradiol-regulated gene expression.

Testosterone hormone plays an active role in the maturation stage of the gonads at the end of the reproductive cycle and acts as an intermediate product in synthesizing estradiol (Barannikova et al., 2002). In the present study, testosterone hormone in Nile Tilapia decreased significantly after exposure to POME at 3.130 mg/L. A similar finding was also observed in different fish species when they were exposed to bisphenol S (Ji et al., 2013) and cadmium (Gárriz et al., 2019). The reduced testosterone hormone concentration is due to the disruption of enzymatic reactions that synthesize the hormone. Zhang et al. (2014) reported that two specific aromatase enzymes (CYP19a and CYP19b) that are responsible for catalyzing testosterone hormone production. CYP19a is mainly expressed in the gonads and CYP19b is mostly found in the SEP brain. Carnevali et al. (2018) reported that the concentration of estradiol hormone in fish is the result of synthetization of testosterone hormone; thus the decreased testosterone level can cause the decreased level of estradiol (Carnevali et al., 2018). Testosterone is converted into estradiol within granulosa cells via aromatization, where, aromatase is the enzyme that converts testosterone into estradiol (Huffman et al., 2013).

To date, information related the effect of pollutants to hormone progesterone level in fish is still not widely studied. This study indicates that exposures to POME have no sig-
significant effect on the concentration of progesterone hormone of Nile tilapia. According to Kime (1995), unlike estradiol and testosterone, the induction of progesterone hormone tends to take place during the oocyte final maturation process. In this study, we assume that until the end of the exposure period, the test fish did not yet enter the oocyte final maturation process. Thus, the effect of POME on the progesterone hormone tends to not detected. Besides that, Barannikova et al. (2002) also stated that the hormone progesterone tends to perform in gametogenesis than oogenesis development. Amutha & Subramanian (2013) reported that there was no significant effect on the concentration of progesterone hormone in female tilapia exposed to cadmium. Otherwise, the decreased concentration of the progesterone hormone in male Nile tilapia was detected.

The presence of pollutant exposure results in reproductive hormone dysfunction, which impacts the disruption of vitellogenin receptors in oocytes (Kime, 1999). Disruption of vitellogenesis is often associated with damage to liver tissue (Zulfahmi et al., 2015; Paraso et al., 2017). It is known that one of the products of the vitellogenesis is the availability of an adequate amount of egg yolk as a reserve food for the embryo. The fish liver is responsible for synthesizing lipid into egg yolks through the vitellogenesis. In the present study, total lipid content in the gonad of Nile Tilapia decreased significantly after exposure to POME at 3.130 mg/L. A similar result was reported in Danio rerio when exposed to di-isononyl phthalate (Carnevali et al., 2018). Zulfahmi et al. (2017a) revealed that POME exposure could cause hemorrhage, congestion, inflammatory cell infiltration, hydrophilic degeneration, and necrosis in the liver tissue of Nile Tilapia, so that the liver performance is decreased. The decrease in liver performance is thought to be the cause of the decrease in the ratio of lipid content in the gonad.

The results of the present study revealed that exposure of POME at 2.347 mg/L increased relative bile volume of Nile Tilapia; however, in higher concentrations of POME the relative bile volume value decreased significantly. Zulfahmi et al. (2014) also revealed that there was an increase in the value of relative bile volume of Nile Tilapia when exposed to mercury. Several studies have shown that many pollutants are subsequently excreted through the bile as conjugated metabolites (Neves et al., 2012; Blanco et al., 2019). In the present study, this may reflect the increase of relative bile volume in treatment B (2.347 mg/L). However, in higher concentration or more extended exposure period of pollutants, the alteration of liver cells is increased; thus the synthesis of pollutants in bile becomes disrupted and reduced the relative bile volume.

CONCLUSION

The present study revealed that exposure to POME had significant impacts on estradiol and testosterone hormone of female Nile Tilapia. The occurrence of decreased hormones resulted in a decrease in total lipid content in fish gonad. The disturbance to lipid synthesis can also be indicated from the elevation values of relative bile volume in fish exposed to POME. Further research is needed to analyze the effect of POME on vitellogenesis and the performance of the hypothalamic-pituitary-gonadal system of Nile Tilapia.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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REFERENCES

• Abdel-Khalek AA, MA Kadry, SR Badran, MAS Marie (2015). “Comparative toxicity of copper oxide bulk and nano particles in Nile tilapia; Oreochromis niloticus: biochemical and oxidative stress.” J. Basic Appl. Zool. 72: 43–57. http://dx.doi.org/10.1016/j.jobaz.2015.04.001.

• Amutha C, P Subramanian (2013).” Cadmium alters the reproductive endocrine disruption and enhancement of growth in the early and adult stages of Oreochromis mossambicus.” Fish Physiol. Biochem. 39(2): 351-361. https://doi.org/10.1007/s10695-012-9704-3

• Alhaji MH, K Sanaullah, SF Lim, A Khan, CN Hipolito, MO Abdullah, SA Bhawani, T Jamil (2016). “Photocatalytic treatment technology for palm oil mill effluent (POME) – a review.” Process Safety Environ. Protect. 102: 672–686.

• Alkaladi A, NAN El-Deen, M Afifi, OAA Zinadah (2015). “Hematological and biochemical investigations on the effect of vitamin E and C on Oreochromis niloticus exposed to zinc oxide nanoparticles.” Saudi J. Biolog. Sci. 22 (5): 556-563. http://dx.doi.org/10.1016/j.sjbs.2015.02.012

• Al-Rawi SM (2005). Contribution of manmade activities to the pollution of the tigris within mosul area/iraq.” Int. J. Environ. Res. Publ. Health. 2 (2): 245– 250.

• AOAO (2005). Official Methods of Analysis of the Association of Official Analytical International. In W. Horwitz (Ed.), Method 989.05 (18th ed. Arlington, USA: AOAC International.

• Bala JD, J Lalung, N Ismail (2015). “Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains.” International Journal of Recyel. Organic Waste Agric. 4 (1): 1-10. https://doi.org/10.1007/s40093-014-0079-6
Barannikova IA, VP Dyubin, LV Bayunova, TB Semenkova (2002). "Steroids in the control of reproductive function in fish." Neurosci. Behav. Physiol. 32 (2): 141-148. https://doi.org/10.1023/A:1013923308125

Bello MM, MM Nourouzi, LC Abdullah, TSY Choong, YS Koay, S Keshani (2013). "POME is treated for removal of color from biologically treated POME in fixed bed column: applying wavelet neural network (WNN)." J. Hazard. Mater. 262: 106–113. https://doi.org/10.1016/j.jhazmat.2013.06.053

Blanco M, J Rizzi, D Fernandes, N Colin, A Maceda-veiga, C Porte (2019). "Assessing the impact of waste water effluents on native fish species from a semi-arid region, NE Spain." Sci. Total Environ. 654: 218-225. https://doi.org/10.1016/j.scitotenv.2018.11.115

Canadian Council on Animal Care (CCAC) (1993). Guide to the Care and Use of Experimental Animals, 2: 30–45. CCAC, Ottawa, ON.

Carnevali O, S Santangeli, I Forner-Piquer, D Basili, F Chan YJ, C. Mei-Fong, L Chung-Lim (2013). "Optimization of palm oil mill effluent treatment in an integrated anaerobic-aerobic bioreactor." Sustain. Environ. Res. 23 (3): 153-170.

Chatterjee S, AK Dasmahapatra, R Ghosh (2001). "Disruption of the pituitary-ovarian axis by carbofuran in catfish, Heteropneustes fossilis (Bloch)," Comp. Biochem. Physiol. Part C. 129: 265–273. https://doi.org/10.1016/S1532-0456(01)90203-0

Ebrahimi M, M Taherianfard (2011). "The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River." Iranian J. Fisher. Sci. 10 (1): 13–24.

Food and Agricultural Organization [FAO]. 2016. Indonesian and Malaysia oil palm yield 2010-2014. www.fao.org/faostat/en/

Fitzherbert EB, MJ Struebig, A Morel, Danielsen, CA Bruhl, Gárriz Á, S Pamela, P Carriquiriborde, LA Miranda (2019). "Effects of heavy metals on the quality of fish; application to effects of heavy metals." Aquat. Toxicol. 36 (1): 223-237. https://doi.org/10.1016/S0166-445X(96)00806-5

Ebrahimi M, M Taherianfard (2011). "The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River." Iranian J. Fisher. Sci. 10 (1): 13–24.

Gallão M, KG Gnocchi, LR Carvalho, BF Silva, AN Barbosa, AR Chippari-Gomes (2019). "The Impact of Sublethal Concentrations of Treated Leachate on Nile tilapia (Oreochromis niloticus)." Bullet. Environ. Contam. Toxicol. 98 (5): 638-642. https://doi.org/10.1007/s00239-018-01018-6

Heiden TCK, CA Struble, ML Rise, MJ Hessner, RJ Hutz, MJ Carvan (2008). "Molecular targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) within the zebrafish ovary: Insights into TCDD-induced endocrine disruption and reproductive toxicity." Reprod. Toxicol. 25 (1): 47-57. https://doi.org/10.1016/j.reprotox.2007.07.013

Huffman LS, LA O’Connell, HA Hofmann (2013). "Aromatase regulates aggression in the African cichlid fish Astatotilapia burtoni." Physiol. Behav. 112: 77-83. https://doi.org/10.1016/j.physbeh.2013.02.004

Ibrahim ATA, AS Harabawy (2014). "Sublethal toxicity of carbofuran on the African catfish Clarias gariepinus: Hormonal, enzymatic and antioxidant responses." Ecotoxicol. Environ. Safety. 106: 33-39. http://dx.doi.org/10.1016/j.ecoenv.2014.04.032

Jayed MH, HH Masjuki, MM Kalam, TMI Mahlia, M Husnawan, AM Laiquat (2011). "Prospects of dedicated biodiesel engine vehicles in Malaysia and Indonesia." Renew. Sustain. Energy Rev. 15: 220–235. https://doi.org/10.1016/j.rser.2010.09.002

Ji K, S Hong, Y Kho, K Choi (2013). "Effects of bisphenol S exposure on endocrine functions and reproduction of zebrafish." Environ. Sci. Technol. 47 (15): 8793-8800. doi:10.1021/es40329a

Kime DE (1995). "The effects of pollution on reproduction in fish." Rev. Fish Biol. Fisheries. 5 (1): 52-95. https://doi.org/10.1007/BF01103366

Kime DE, M Ebrahimii, K Nysten, I Roelants, HDM Moore, F Ollevier (1996). "Use of computer assisted sperm analysis (ca) for monitoring the effects of pollution on sperm quality of fish; application to effects of heavy metals." Aquat. Toxicol. 36 (1): 223-237. https://doi.org/10.1016/S0166-445X(96)00806-5

Kime DE (1999). "A strategy for assessing the effects of xenobiotics on fish reproduction." Sci. Total Environ. 225: 3-11. https://doi.org/10.1016/S0048-9697(98)00328-3

Luo Y, D Shan, H Zhong, Y Zhou, W Chen, J Cao, Z Guo, J Xiao, F He, Y Huang, J Li, H Huang, P Xu (2015). "Subchronic effects of cadmium on the gonads, expressions of steroid hormones and sex-related genes in Tilapia (Oreochromis niloticus)," Ecotoxicology. 24 (10): 2213-2223. https://doi.org/10.1007/s10646-015-1542-5

Maygaonkar PA, PM Wagh, U Permeswaran (2012). "Biodegradation of distillery effluent by fungi." Bioso. Discov. 3 (2): 251–259.

Mekkawy IA, AA Hassan (2011). "Some reproductive parameters of Synodontis Schall (Bloch and Schneider, 1801) from the river Nile, Egypt." J. of Fisheries Aquat. Sci. 6 (4): 456–471.

Mulliari, I Zulfahmi (2016). "Impact of palm oil mill effluent towards phytoplankton community in Krueng Mane River, North Aceh." J. Perikanan dan Kelautan. 6 (2): 137-146. http://dx.doi.org/10.33512/jpk.v6i2.1107

Mulliari, I Akma, Zulfahmi, J Rizki, J NWK, Karja, C Nisa (2018). "Histopathological changes in gill of Nile Tilapia (Oreochromis niloticus) after palm oil mill effluent exposure." IOP Conference Series: Earth Environ. Sci. 216: 1 – 5.

Neves RLS, TF Oliviera, RL Zioli (2012). "Polyyclic aromatic hydrocarbons (PAHs) in fish bile Mugil liza as biomarkers for environmental monitoring in oil contaminated areas." Marine Pollut. Bullet. 54 (1): 1818–1824. https://doi.org/10.1016/j.marpolbul.2007.08.013

Para so MGV, JK Morales, AA Clavecillas, MS Lola (2017). "Estrogenic effects in feral male common carp (Cyprinus carpio) from Laguna de Bay, Philippines." Bullet. Environ. Contamin. Toxicol.98 (5): 638–642. https://doi.org/10.1007/...
• Rurangwa E, I Roelants, G Huyskens, M Ebrahimi, DE Kime, F Ollevier (1998). “The minimum acceptable spermatozoa to egg ratio for artificial insemination and the effects of heavy metal pollutants on sperm motility and fertilization ability. In the african catfish (Clarias gariepinus, burchell 1822)” J. Fish Biol. 53 (2): 402-413. https://doi.org/10.1111/j.1095-8649.1998.tb00989.x

• Thomas P, L Budiantara, L Forlin, T Andersson (1995). “Reproductive life history stages sensitive to oil and naphthalene in Atlantic croaker.” Marine Environ. Res. 39:147–150. https://doi.org/10.1016/0141-1136(94)00072-W

• Verberk WC, I Durance, IP Vaughan, SJ Ormerod (2016). “Field and laboratory studies reveal interacting effects of stream oxygenation and warming on aquatic ectotherms.” Global Change Biol. 22 (5): 1769-1778.

• Vithana CL, LA Sullivan, T Shepherd (2009). “Optimised treatment of palm oil mill effluent.” Int. J. Environ. Waste Manag. 3 (3/4): 265-277. https://doi.org/10.1504/IJEWM.2009.026343

• Woodman AG (1941). “Extraction Methods, Soxhlet Fat Extraction Food Analysis, Typical Methods and Interpretation of Results.” McGraw- Hill, New York, 607 pp.

• Sullivan CV, O Yilmaz (2018). “Vitellogenesis and yolk proteins, fish”. Encycl. Reprod. 2nd edn. Elsevier.

• Zhang Y, S Zhang, H Lu, L Zhang, W Zhang (2014). “Genes encoding aromatases in teleosts: evolution and expression regulation.” Gen. Comparat. Endocrinol. 205 (1): 151–158. https://doi.org/10.1016/j.ygeno.2014.05.008

• Zulfahmi I, A Ridwan, TFL Djamar (2014). “Kondisi biometrik ikan nila, Oreochromis niloticus (Linnaeus 1758) yang terpapar merkuri [Biometric condition of nile Tilapia, Oreochromis niloticus (Linnaeus 1758) after mercury exposure].” J. Iktiologi Indonesia. 14 (1): 37-48. [in Indonesian]. https://doi.org/10.32491/jii.v14i1.94

• Zulfahmi I, A Ridwan, TFL Djamar (2015). “Perubahan struktur histologis insang dan hati ikan nila (Oreochromis niloticus Linnaeus 1758) yang terpapar merkuri [Histological changes of liver and Gill of Nile Tilapia (Oreochromis niloticus Linnaeus 1758) after mercury exposure].” J. Edukasi dan Sains Biol. 4 (1): 35-31. [in Indonesian].

• Zulfahmi I, Muliai, Y Akmal (2017a). “Indeks hepatosomatik dan histopatologi hati ikan nila (Oreochromis niloticus Linnaeus 1758) yang dipapar limbah cair kelapa sawit [Hepatosomatic Index and Liver Histopathology of Nile Tilapia (Oreochromis niloticus Linnaeus 1758) Exposed to Palm Oil Mill Effluent].” Prosiding SEMDI-UNAYA (Seminar Nasional Multi Disiplin Ilmu UNAYA) 1: 301-314. [in Indonesian].

• Zulfahmi I, Muliai, I Mawaddah (2017b). “Toksisitas limbah cair kelapa sawit terhadap ikan nila (Oreochromis niloticus Linnaeus 1758) dan ikan bandeng (Chanos chanos Froskall 1755) [Toxicity of Palm Oil Mill Effluent on Nile Tilapia (Oreochromis niloticus Linnaeus 1758) and Milk Fish (Chanos chanos Froskall 1755)].” Agricola. 7: 44-55. [in Indonesian].

• Zulfahmi I, Muliai Y, Akmal, AS Batubara (2018). “Reproductive performance and gonad histopathology of female Nile Tilapia (Oreochromis niloticus Linnaeus 1758) exposed to palm oil mill effluent.” Egyptian J. Aquat. Res. 44: 327 – 332. https://doi.org/10.1016/j.ejar.2018.09.003