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Effects of Microplastic Exposure on Catfish(*Clarias gariepinus*) Feed to *Cyp19b* Gene

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Abstract

Microplastics that are eaten by fish will bioaccumulate and will settle in the fish's body, especially in the gills and stomach organs. Exposure to microplastics in farmed animals can cause damage to gonadal cells and increase the level of aromatase in fish body so that the synthesis of androgens to become estrogene is higher. *Cyp19b* is a gene responsible for the synthesis of the aromatase enzyme which is related to the androgen hormone. The purpose of this study was to analyse the effect of giving polystyrene to catfish (*C. gariepinus*) feed on changes in the *Cyp19b* gene sequence. This research was descriptive experimental research. Results and observation data were analysed using a comparative descriptive method. The study was conducted by examining the brain of catfish (*C. gariepinus*) to determine changes in the Cyp19b gene sequence. The first step was to extract DNA from the gonads and fish brains, then examine the Cyp19b gene sequence. The next step was constructed a phylogenetic tree to determine if there is a change in kinship in the *Cyp19b* gene sequence. The results showed a trend towards changes in the *Cyp19b* gene sequence which can be described in a phylogenetic tree.

Keywords: *Cyp19b; Polystyrene; Microplastics; C.gariepinus*

1. Introduction

Catfish (*Clarias gariepinus*) is one of freshwater species that has potential for aquaculture because of its delicious meat taste, affordable price, and easy to cultivate (Negara, et al., 2015). National catfish production from year to year continues to increase and continues to dominate freshwater aquaculture commodities from 2013-2018. In the last six years, there has been an average increase in catfish production of 11.77% per year (Hendrawan, et al., 2020). The production of catfish in Indonesia reached 347,511.48 tons in 2020, placing it in second place behind tilapia (KKP, 2020).

Microplastics in the waters have the potential to be consumed by aquatic organisms. The most common type of microplastics found in waters is polystyrene. Polystyrene is a plastic composed of several chemicals such as styrene, plasticizer, butyl hydroxy toluene (BHT), polystyrene and CFC (chlorofluorocarbon). The use of these additives can be dangerous if it occurs continuously and accumulates in the body which has effects such as carcinogens, reduces cell stability, disrupts the endocrine system or hormonal system and damages reproductive organs including increased aromatase encoded by the *Cyp19a* and *Cyp19b* genes (Al Mukminah, 2019; Yona, Di Prikah, & As'adi, 2020

When microplastics enter the body through the cell membrane, polystyrene will unite with the basic receptor protein and enter the cell nucleus (Sedayu, 2011). Polystyrene then will interact with DNA and attack the genes that control many biochemical reactions such as the synthesis and metabolism of hormones, enzymes, as well as growth and reproduction factors.

Microplastics that are consumed by fish will accumulate and remain in the fish's body, especially in the gills and stomach (Lu, et al., 2016). In addition, microplastics can also cause damage to the ovaries, brain, mouth area and even the liver (Wang, et al., 2019; Farrell & Kathryn, 2013). Ingested microplastics can cause fish to become intersexual or have erratic sex due to the presence of Endocrine-Disrupting Chemicals (EDC) which interfere with the hormone system, thus interfering with external fertilization of fish. Sex in fish is determined by more than one gene, such as the aromatase gene (cytochrome P450) *Cyp19a* which is expressed in the ovaries and *Cyp19b* which is expressed in the brain (Irmawati, et al., 2022).

Microplastics can cause stress, disrupted metabolism, damage to liver, kidney, gill, gonad tissue, reduce cell membrane stability, disrupt energy absorption by biota, inhibit growth and reproduction of aquatic organisms (Lusher et al., 2017; Su et al. , 2018).

The inhibition of the fish reproduction process can lead to reduced regeneration of a fish population in the ecosystem. Microplastics in waters cause reproductive disorders because microplastics can inhibit hormone responses. Exposure to microplastics can cause

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damage to gonadal cells and increase the level of aromatase in the fish's body so that the synthesis of androgens into estrogens is higher. Estrogen is a steroid hormone that plays a role in fish reproduction. Exposure to microplastics containing several chemicals such as styrene can cause the activation of estrogen cells which will capture estrogen around the brain so that estrogen increases. Aromatase is responsible for the aromatization of androgens to estrogens. There are two types of aromatase genes in fish bodies, namely type one *Cyp19a* (gonad type) and type two *Cyp19b* (brain type). Increased expression of the aromatase gene type one Cyp19a (gonadal type) can cause feminization or the formation of ovaries in fish, whereas decreased expression of the aromatase gene causes masculinization or the formation of testes. Cyp19b is a gene that is responsible for the synthesis of aromatase enzymes related to androgen hormones (Heriyati et al. 2015).

2. Material and Methods

2.1. Catfish (C. gariepinus)

Catfish (*C. gariepinus*) with average length of ±10-15 cm were obtained from People's Hatchery Unit (UPR) in Ngajum District, Malang and then adapted for 7 days in the laboratory. The fish were put into the maintenance aquarium according to the desired density, 1 fish per 2 liter of water (SNI, 2000). The stocking density of catfish was 19 fish in the aquarium. When in adaptation time, catfish were given commercial feed which was made in form of a wet paste. Feeding was carried out ad libitum as much as 3% of the biomass of the test animals (Rahma et al., 2015).

2.2. Manufacture of Microplastics

Styrofoam was the polystyrene source for this research, crushed using a wet blender. The process of destroying styrofoam was done 2-3 times, dried under the sun and covered using a plankton net to prevent the microplastics from flying into the air. After dried, the powder was then sifted using a 80 mesh flour sieve then stored in a plastic jar. In general, the size of microplastic particles that can be filtered by a flour sieve is at least > 177 µm. This is in accordance with the provisions of SNI (Marlisa et al., 2020).

2.3. DNA Extraction

Brain samples were taken and weighed as much as 50 mg (0.05 g), then a 1.5 ml tube was prepared and 100 µL of PBS (Phospate Buffer Saline) 1X pH 7.4 was added. Then the sample was put into the tube and cut into small pieces. small using sterile scissors. Added 200 µL T1 buffer then mixed using a vortex mixer for 10 seconds then added 25 µL pro K then vortexed for 20 seconds then incubated at 56°C in an 800 rpm shaker incubator for 1×24 hours. After incubation, the sample was centrifuged at 11000xg at 25°C for 25 minutes. The supernatant was transferred into a new tube and 200 µL of buffer B3 was added and vortexed for 20 seconds to homogenize. Then incubated again at 70°C for 60 minutes in a shaker incubator at 800 rpm. Then 210 µL absolute ethanol was added and vortexed for 10 seconds. The sample was transferred to the DNA column and centrifuged at 11000xg for 3 minutes at 25°C, then the DNA column was replaced and 500 µL BW buffer was added, then centrifuged at 11000xg for 2 minutes at 25°C. The DNA column was replaced and 600 µL of buffer B5 was added, then centrifuged at 11000xg for 3 minutes at 25°C, then transferred to a new tube and treated with dry silica membrane by centrifuging the sample at 11000xg for 3 minutes at 25°C. Then 25 µL of warm BE buffer (70°C) was added and incubated for 5 minutes at room temperature. Then the sample was centrifuged at 11000xg for 5 minutes at 25°C and a DNA sample was obtained. Then stored in a refrigerator at 40°C until DNA analysis was carried out. After obtaining the DNA, a quantitative test was carried out using a Nano Drop 2000 UV spectrophotometer. Calibration was carried out using an Elution Buffer as a DNA stock solvent. DNA is in a pure state when it has the A 260/280 range of 1.8-2.

2.4. DNA Amplification Using PCR (Polymerase Chain Reaction)

2 µl DNA extracted was then put into an tube (0.2 ml), and added with the master mix (Primer F (0.5 µl), Primer R (0.5 µl), ddH2O (2.75 µl), and 2× Go Taq Green PCR (0.25 µl)). Primer F and Primer R are presented in Table 5. The master mix was put in a 1.5 ml microtube, then homogenized. A 200 µl microtube was prepared and the master mix was put into the microtube with a predetermined concentration. 1 µl DNA template was added into the microtube, then put in the Thermalcycler which had been set for a certain time. Until the specified time, the microtubes were taken from the Thermalcycler and stored in the refrigerator for a long period of time.

Table 1. Designed specific primers and nucleotide sequences (Aggarwal, et al. 2014)

Gene	Primer	Primer Sequen
Brain Aromatase (CYP19b)	CYP19bF	5'TGGAGATGGTA ATCGCAGC3'
	CYP19bR	5'TTGGTTTGCCT GATGGTGC3'
β-actin	β ActF	5'ACAACGGATCC GGTATGTGC3'
	β ActR	5'TGGTGACAATA CCGTGCTCG3'

2.5. Electrophoresis

A 2% agarose gel was prepared, then poured into an electrophoresis tank that had been equipped with a comb (electrophoretic comb). After the agarose solution hardens (forms a gel), then the TBE buffer solution is poured on a flat electrophoresis apparatus. The agarose was placed on the electrophoresis apparatus, 1x TBE were added if the solution did not

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inundate the agarose as high as 2 mm. The solution to be tested was added with 2 µL of loading dye. This solution serves to amplify the DNA template and gives color. 10 µL of sample was taken and placed it in the agarose gel well. Placed using 2 hands to prevent tremors (shaking) in the hands~~,~~. After that the marker that has been used is placed at the end of the agarose as much as 1 µL. The process run to 60 minutes, finally Agarose was visualized using Gel Doc.

2.6. DNA Sequencing

The amplified PCR results were then sent to First Base Co (Malaysia) using Big Dye© terminator chemistry (Perkin Elmer), to obtain the base sequence that forms DNA or nucleotide sequences. The results of the DNA sequence were matched using the BLAST (Basic Local Alignment Search Tool) program, which is the NCBI program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) used to look for similarities in nucleotide or protein sequences with sequences database in GenBank.

2.7. PCR Data Analysis

The data from the sequencing results were analyzed and edited using BioEdit (Hall, 1999). Sequence data obtained is then matched with data available at GenBank NCBI online (requires stable internet speed) using the BLAST method. The results of the sequence analysis were performed using the MEGA ver. 7 and then aligned with data from GenBank.

3. Results and Discussion

3.1. Results

The results of the Fasta *Cyp19b* catfish can be seen in Table 2 below.

Table 2. FASTA of *Cyp19b*

1	A	>36339879 CTGAGATGGGGGCGAGTGGGTTGTGGACGGTGGG AAACCGAGCGCAGCGCCTGACACGCTTTCCATCAG CCTTTTCTTCATGCTGATGCTGCTCAAACAGAACCC AGATGTGGAGCTGAGGATAGTGGAGGAGATGAAC AGCGTGCTCAATGACAAAGATGCTGAAAACATTGA TTATCAGGTGTTGGAAGTGATGGAGAGTTTCATCA ATGAGTGTCTGAAGGTTTCATGGGGTGGTTGATTT CACAATGCGAAAAGCTGTCCAAGACCGACAAATC GAAGGCACCAGCATCCGAAAGGGAACCAACATGA TTCTTAACATTGGTCTCATGCACAAGACGGAGTTC TTCCCAAAAGCAAAAGCAAAGCAAAGAGTTCAGT CTGATGAACTTTGATGAAAACCGCCTATTGCGTAC ACCTCCTGCCCGGCGTCCCCCTTAAAA
2	B	>36340632 CATACCAAAAGGGGTGATATTTTGAAGGTTAGTCT GATGGCGCAGCGCCTGAGACACGCTTTCCATCAGC CTTTCTTCATGCTGATGCTGCTCAAACAGACCCAG ATGTGGAGCTGAGGATAGTGGAGGAGATGAACAG CGCTGCTCAATGAGGTTTGATCCCGTGGTTGATTTC ACAATGCGAAAAGCTGTGGAAGACGACAACATCA GAAGGCACCCAGCATCCGAAAGGGAACCAACATC ATTCTTAACATTGGTCTGATGCACACAAGACGGAG TTCTTCCCAAAAAGCACCAAGCATCCGAAAGGGAA CCAACATCATTCTTAACATTGGTCTCATGCACAAG ACGGAGTTCTTCCCCAAAAGCAAAAAGAGTTCAGT CTGATGAACTTTGATAAAACCGTGCCCAGTCGTTT CTTCCAGCCCTTTGGTAC
3	C	>36340365 GGGCTCCTTAATAGTTCGGGGATTAGATGCTGGAG ATTAGAGGAAGGAAGCGCAGCGCCTGACACGCTTT CCATCAGCCTATTCTTCATGCTGATGCTGCCAAAC AGAACCCAGATCTGGAGCTGAGGATAGTGGAGGA GATGAACAGCGTGCTCAATGACAAAGATGCTGAA AACATTGATTATCAGGTGTTGGAAGTGATGGAGAG TTTCATCAATGAGTGTCTGAGGTTCATGCCGTGTGT TGATTTCATTTCACAATGCGAAAGCTCTGGAAAGA CGACAACATCGAAGGCACCAGCATCCGAAAGGGA ACCAACATCATTCTTAACATTGGTCCATGCACAAG AACTTTGATAAAACCGACATAGCAGTGCTGATCCA GGTGTGCTGAATCTCTGTTTTCTGCTCTGCTTCCTT GTGGCTATGGTAGTATAAGAGCTAGTCCCTAATGT GTTCATCTCCTGCCCGGTGTCCCCCTTTAATACCCC TTTTAATAAG
4	D	>36340155 ACAAAAACATAGAGAATAGTGCGTCTTATACTACG ATAGTCCCAACGCACGCAGCGCCTGACACGCTTTC CATCAGCCTTTTCTTCATGCTGATGCTGCTCAACAG AACCCAGATGTGGAGCTGAGGATAGTGGAGGAGA TGAACAGCGTGCTCAATGACAAAGATGCTGAAAA CATTGATTATCAGGTGTTGGAAGTGATGGAGAGTT TCATCAATGAGTGTGTCTGAGGTTTCATCCCCGTGT GGTTGATTTCACAATGCGAAAAGCTCTGGAAGACG ACAACATCGAAGGCACCAGCATGCCTATATCTCCT CCTCCTCCTCCTCCTCCTCCACCACCAC
5	K	>K-CYP19B CTGGGGGTGGGGGGCGTGCTCGGTTATGCATGCTG CGGACGCAGCGCCTGACACGCTTTCCAATCAGCCT TTCTTCATGCTGATGCTCTCAAACAGAACCCAGAT GTGGAGCTGAGGATGAGTGGAGGAGATGATGAAC AGAGCGTGCTCAATGACAAAGATGCTGAAAACATT GATTATCAGGTGTTGGAAGTGATGGAGAGTTTCAT CGAAGGCACCAGCATCCGAAAGGGAACCAACATG ATTCTTAACATTGGTTTCGGAAGACGACAACATCG AAGGCACCAGCATCCGAAAGGGAACCAACATCAT TCTTAACATTGGTCTCATGCACAAGACGGAGTTCT TCCCAAAAGCAAAAAGAGTTCAGTCTGATGAACTT GATAAAACCGTGCCCAGTCGTTTCTTCCAGCGTCC CTAATGTGTACATCTCCTGCCCGGCGTCCCCCTTTA ATA

Note: A, B, C, D, K are treatments

3.2. Phylogenetics

The phylogenetic results of microplastic exposure to changes in *Cyp19b* gene in catfish (*C. gariepinus*) can be seen in Figure 1:

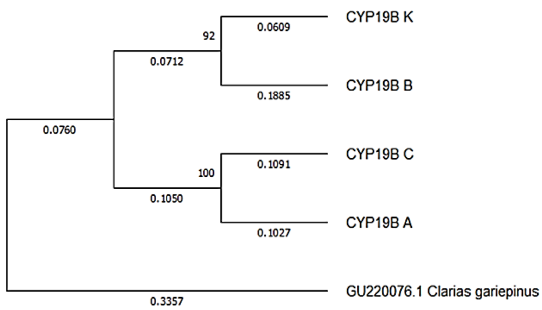

Fig 1. Phylogenetics of *Cyp19b*

3.3. Discussion

The phylogenetics of the *Cyp19b* in catfish exposed to microplastics resulted in 2 clades. A high level of kinship closeness of a population will have many similarities in morphology, genetics and is influenced by the environment (Mayr, 1970). Based on the 16S rRNA gene, the percentage of divergent sequences with a value of ±2-3% will change the status of the species, while a value of 11% in the percentage of divergent sequences is indicated as a

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new species (Kartavtsev and Lee, 2006). The closest kinship relationship was obtained from the control without microplastics in food at 6.09%. Meanwhile, from the feeding treatment with additional microplastics it was above 10%. So that the intake of microplastics in catfish feed that is cultured has a tendency to change the *Cyp19b* gene,

Styrofoam also contains a small amount of formaldehyde which has a long term effect and if it accumulates continuously it can damage tissues and irritate digestive organs. Styrofoam is made of styrene and benzene granules. These two active ingredients can cause various neurological diseases or nervous disorders, thyroid damage and are carcinogenic. Styrene itself is a substance that cannot be dissolved in the body and can not be excreted through the urine and over time will accumulate in the body. (Rahmayani, 2018). So microplastics carried by aquaculture water and eaten by fish will have an impact on the *Cyp19b* gene and are feared to have an impact on humans who consume them as well.

Cytochrome P450 aromatase (Cyp19) is an enzyme involved in the conversion of androgenic steroids (testosterone) to estrogens (estradiol). According to Chiang, et al. (2001), the *Cyp19b* gene is located in the link group of chromosome 25. The regulation of this gene determines the ratio of androgen (CY19) to estrogen (CY18). Therefore, the expression of this enzyme is essential for reproduction as well as sex differentiation. Fish have two Cyp19 genes (*Cyp19a* and *Cyp19b*) each of which encodes a different protein and has its own regulatory mechanism. In general, the *Cyp19* gene differs significantly in its expression ontogeny. *Cyp19a* has more gonad shape than Cyp19b brain shape. However unlike *Cyp19a*, the expression patterns of *Cyp19b* can be clearly separated into the two populations, suggesting an association with sex differentiation (Aggarwal, et al., 2014).

Expression of *Cyp19b* in the hypothalamus and limbic areas of the brain influences behavior and the direction of sex change. Estrogen produced in the brain can be further metabolized into catechol components which can also bind to estrogen receptors around the brain (Tong & Bon-chu, 2003). Aromatase enzyme activity correlates with estradiol targets and functions to regulate sex, reproduction, and behavior. The activity of the aromatase enzyme is limited to areas with a gonadal structure, i.e. larvae with low aromatase activity will lead to the formation of testes, while high aromatase activity will lead to the formation of ovaries interpretation of the results of the analysis related to the problem and objectives. In the discussion, the novelty aspect of the research results is written, which is compared with the results of previous research or literature, which is marked by the inclusion of citations.

4. Conclusion

This study suggested that the exposure to microplastics on the feed for catfish (*C. gariepinus*) aquaculture affects the genetic distance of the catfish *Cyp19b* gene and changes in its genes

References

Abdel-Kader, H., H., & Mourad, M. H. (2019). Bioaccumulation of heavy metals and physiological/histological changes in gonads of catfish (*Clarias gariepinus*) inhabiting lake maryout, alexandria, egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, ***23*** (2), 363–377.

Adrial, A., Nur, I., & Yusnaini. (2018). Potensi Tepung Kulit Buah Manggis (*Garcinia Mangostana*) yang Ditambahkan dalam Pakan terhadap Profil Darah Ikan Komet (*Carrasius Auratus*). *Media Akuatika*, ***3*** (3), 689–701.

Aggarwal, N., Goswami, S. V., Khandelwal, P., & Sehgal, N. (2014). Aromatase activity in brain and ovary: Seasonal variations correlated with circannual gonadal cycle in the catfish, Heteropneustes fossilis. *Indian Journal of Experimental Biology*, ***52*** (5), 527–537.

Al Mukminah, I. (2019). Bahaya Wadah Styrofoam dan Alternatif Penggantinya. *Farmasetika*, ***4*** (2), 32–34.

Amalo, F. A. (2017). Identifikasi Daging Ayam Broiler Dengan Pengamatan Struktur Histologis. *Jurnal Kajian Veteriner*, ***5*** (1), 11–20.

Amenyogbe, E., Chen, G., Wang, Z., Lu, X., Lin, M., & Lin, A. Y. (2020). A Review on Sex Steroid Hormone Estrogen Receptors in Mammals and Fish. *International Journal of Endocrinology*, ***2020***.

Andriayani, U., & Syahbanu, I. (2018). Pembuatan Polyblend dari Limbah Styrofoam dan A - Selulosa. *Jurnal Kimia Khatulistiwa*, ***7*** (3), 40–46.

Arief, M., Fitriani, N., & Subekti, S. (2014). Pengaruh Pemberian Probiotik Berbeda pada Pakan Komersial terhadap Pertumbuhan dan Efisiensi Pakan Ikan Lele Sangkuriang (*Clarias* sp.). *Jurnal Ilmiah Perikanan Dan Kelautan*, ***6*** (1), 49–53.

Arukwe, A., Ibor, O. R., & Adeogun, A. O. (2017). Biphasic modulation of neuro- and interrenal steroidogenesis in juvenile African sharptooth catfish (*Clarias gariepinus*) exposed to waterborne di-(2-ethylhexyl) phthalate. *General and Comparative Endocrinology*, ***254***, 22–37.

Asniatih, M. I., & Sabilu, K. (2013). Studi histopatologi pada ikan lele dumbo (*Clarias gariepinus*) yang terinfeksi bakteri Aeromonas hydrophila. *Jurnal Mina Laut Indonesia*, ***3*** (12), 13–21.

Ath-thar, M. H. F., Ambarwati, A., Soelistyowati, D. T., & Kristanto, A. H. (2018). Keragaan genotipe dan fenotipe ikan uceng *Nemacheilus fasciatus*

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(Valenciennes, 1846) asal Bogor, Temanggung, dan Blitar. *Jurnal Riset Akuakultur*, ***13*** (1), 1–10.

Ayuningtyas, W. C., Yona, D., Julinda S., S. H., & & Iranawati, F. (2019). Kelimpahan Mikroplastik Pada Perairan Di Banyuurip, Gresik, Jawa Timur. *JFMR-Journal of Fisheries and Marine Research*, ***3*** (1), 41–45. https://doi.org/10.21776/ub.jfmr.2019.003

Banaee, M., Soltanian, S., Sured Gholamhosseini, A., Haghi, B. N., Akhl & Derikvandy, A. (2019). Evaluation of single and combined effects of cadmium and micro-plastic particles on biochemical and immunological parameters of common carp (*Cyprinus carpio*). *Chemosphere*, ***236***, 124335.

Bhaisare, L. Y., Zade, S. B., Nagwanshi, A. M., Netam, A. K., & Chaudhary, D. D. (2022). Impacts of Benzyl Butyl Phthalate on Histo-Architecture of Gonads of African Catfish *Clarias gariepinus* (Burchell, 1822). *Polish Journal of Environmental Studies*, ***31*** (2), 1049–1059.

Carnevali, O., Santangeli, S., Forner-Piquer, I., Basili, D., & Maradonna, F. (2018). Endocrine-disrupting chemicals in aquatic environment: what are the risks for fish gametes? *Fish Physiology and Biochemistry*, ***44*** (6), 1561–1576. https://doi.org/10.1007/s10695-018-0507-z.

Cera, A., Cesarini, G., & Scalici, M. (2020). Microplastics in freshwater: What is the news from the world? *Diversity*, ***12*** (7).

Chen, C. Y., Lu, T. H., Yang, Y. F., & Liao, C. M. (2020). Toxicokinetic/toxicodynamic-based risk assessment of freshwater fish health posed by microplastics at environmentally relevant concentrations. *Science of the Total Environment*, ***2020***, 144013.

Cordova, M. R., & Wahyudi, A. J. (2016). Microplastic in the Deep-Sea Sediment of Southwestern Sumatran Waters. *Marine Research in Indonesia*, ***41*** (1), 27–35. https://doi.org/10.14203/mri.v41i1.99

Darseno, S. P. (2010). *Buku Pintar Budi Daya dan Bisnis Lele.* AgroMedia. Jakarta. https://books.google.co.id/books.

Darus, N., Tamimi, M., Tirawaty, S., Muchtazar, M., Trisyanti, D., Akib, R., & & Ranggi, K. (2020). An Overview Of Plastic Waste Recycling In The Urban Areas Of Java Island In Indonesia. *Journal of Environmental Science and Sustainable Development*, ***3*** (2), 1–10.

Deng, S.-P., Liu, Z.-H., & Wang, D.-S. (2018). Mechanisms of Feminization and Sex Differentiation in Southern Catfish. *Sex Control in Aquaculture*, ***2***, 509–524. https://doi.org/10.1002/9781119127291.ch25

Dewi, R. R. S. P. S., & Tahapari, E. (2017). Performa Ikan Lele Afrika (*Clarias gariepinus*) Hasil Seleksi Terhadap Pertumbuhan, Sintasan, Koversi Pakan, Rasio RNA/DNA, dan Nilai Bioekonomi. *Jurnal Media Akuakultur*, ***12*** (1), 11–17.

Ding, J., Zhang, S., Razanajatovo, R. M., Zou, H., & Zhu, W. (2018). Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (*Oreochromis niloticus*). *Environmental Pollution*, ***238***, 1–9.

Driscoll, R. M. H., Faber-Hammond, J. J., O'Rourke, C. F., Hurd, P. L., & Renn, S. C. P. (2020). Epigenetic regulation of gonadal and brain aromatase expression in a cichlid fish with environmental sex determination. *General and Comparative Endocrinology*, ***296*** (3), 113538.

Ekvall, M. T., Lundqvist, M., Kelpsiene, E., Šileikis, E., Gunnarsson, S. B., & Cedervall, T. (2019). Nanoplastics formed during the mechanical breakdown of daily-use polystyrene products. *Nanoscale Advances*, ***1*** (3), 1055–1061.

Ermawati, R., Jati, B. N., Rumondang, I., Oktarina, E., & Naimah, S. (2016). Pengaruh Residue Catalytic Cracking (RCC) dan Zeolit terhadap Kualitas Crude Oil Hasil Pirolisis Limbah Plastik Polietilena. *Jurnal Kimia Dan Kemasan*, ***38*** (1), 47. https://doi.org/10.24817/jkk.v38i1.1978

FAO. (2018). *The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals*.

Fuentes, N., & Silveyra, P. (2019). Estrogen receptor signaling mechanisms. In *Advances in Protein Chemistry and Structural Biology*, **116**. Elsevier Inc. https://doi.org/10.1016/bs.apcsb.2019.01.001

Ghufran, M., Kordi, H. K., & Andi, T. (2011). *Pembenihan Ikan Laut Ekonomis Secara Buatan*. Lily Publisher.

Golpour, A., Broquard, C., Milla, S., Dadras, H., Baloch, A. R., Saito, T., & Pšenička, M. (2020). Determination of annual reproductive cycle in male sterlet, Acipenser ruthenus using histology and ultrasound imaging. *Fish Physiology and Biochemistry*, ***47*** (3), 703–711.

Hanif, K. H., Suprijanto, J., & Pratikto, I. (2021). Identifikasi Mikroplastik di Muara Sungai Kendal, Kabupaten Kendal. *Journal of Marine Research*, ***10*** (1), 1–6. https://doi.org/10.14710/jmr.v9i2.26832

Hastuti, S., & Subandiyono. (2015). Kondisi Kesehatan Ikan Lele Dumbo (*Clarias gariepinus*, Burch) yang Dipelihara dengan Teknologi Biofloc. *Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology*, ***10*** (2), 74–79. https://doi.org/10.14710/ijfst.10.2.74-79

Hayati, A. (2019). *Biologi Reproduksi Ikan*. Airlangga University Press.

Heriyati, E., Alimuddin, Arfah, H., & Sudrajat, A. O. (2015). Ekspresi gen aromatase pada pengarahan diferensiasi kelamin ikan nila (*Oreochromis niloticus* Linnaeus 1758) menggunakan madu. *Jurnal Iktiologi Indonesia*, ***15*** (1), 39–50.

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Herjayanto, M., Carman, O., & Soelistyowati, D. T. (2017). Embriogenesis, Perkembangan Larva dan Viabilitas Reproduksi Ikan Pelangi Iriatherina Werneri Meinken, 1974 pada Kondisi Laboratorium. *Akuatika Indonesia*, ***2*** (1), 1. https://doi.org/10.24198/jaki.v2i1.23389

Hernowo, A. P., & Suyanto, S. R. (2010). *Pembenihan dan Pembesaran Lele*. Penebar Swadaya.

Ho, B. T., Roberts, T. K., & Lucas, S. (2018). An overview on biodegradation of polystyrene and modified polystyrene: the microbial approach. *Critical Reviews in Biotechnology*, ***38*** (2), 308–320.

Hussain, Z., & Ali, S. (2019). *Comparative Study on Breaking Strength of Burnt Clay Bricks Using Novel Based Completely Randomized Design (CRD) Civil Engineering Journal Comparative Study on Breaking Strength of Burnt Clay Bricks Using Novel Based Completely Randomized Design (CRD)*. ***5*** (5). https://doi.org/10.28991/cej-2019-03091320

Iswanto, B., Suprapto, R., Marnis, H., & Imron, I. (2017). Morphological Characteristics of a Red Strain of The Egyptian African Catfish (*Clarias gariepinus* BURCHELL 1822). *Indonesian Aquaculture Journal*, ***11*** (2), 49–59.

Itnawita, H., T. A, A., Khoirul, & Eriesa, &. (2014). Analisis Kandungan Formaldehid Dalam Minuman Dengan Kemasan Plastik Polyethylen Tereftalat (PET) Yang Beredar Di Kodya Pekanbaru. *Photon: Jurnal Sain Dan Kesehatan*, ***4*** (2), 39–42.

Jusmaldi, J., Solihin, D. D., Affandi, R., Rahardjo, M. F., & Gustiano, R. (2018). Kematangan gonad dan tipe pemijahan ikan lais, *Ompok miostoma* (Vaillant, 1902) di Daerah Aliran Sungai Mahakam Kalimantan Timur. *Jurnal Iktiologi Indonesia*, ***17*** (2), 201. https://doi.org/10.32491/jii.v17i2.359

Kaleli, A., Kulikovskiy, M. S., & Solak, C. N. (2017). Some New Records for Marine Diatom Flora of Turkey from Akliman, Sinop (Black Sea). *Turkish Journal of Fisheries and Aquatic Sciences*, ***17***, 1387–1395. https://doi.org/10.4194/1303-2712-v17

KKP. (2020). *Total Produksi Perikanan Budiddaya*. Statistik-KKP. https://statistik.kkp.go.id/home.php?m=total&i=2#panel-footer

Kruger, T., Barnhoorn, I., Jansen van Vuren, J., & Bornman, R. (2013). The use of the urogenital papillae of male feral African sharptooth catfish (*Clarias gariepinus*) as indicator of exposure to estrogenic chemicals in two polluted dams in an urban nature reserve, Gauteng, South Africa. *Ecotoxicology and Environmental Safety*, ***87***, 98–107.

Larsen, N. E., Venkadasalam, V. P., & Ganea, P. A. (2020). Prompting Children's Belief Revision About Balance Through Primary and Secondary Sources of Evidence. *Frontiers in Psychology*, ***11*** (7), 1–12.

Lestari, D. F., & Syukriah. (2020). Manajemen Stres Pada Ikan Untuk Akuakultur Berkelanjutan. *JURNAL AHLI MUDA INDONESIA*, ***1*** (1), 51–105.

Li, M., Sun, L., & Wang, D. (2018). Roles of estrogens in fish sexual plasticity and sex differentiation. *General and Comparative Endocrinology*, ***277*** (11), 9–16. https://doi.org/10.1016/j.ygcen.2018.11.015

Lusher, A., Hollman, P., & Mandoza-Hill, J. (2017). Microplastics in fisheries and aquaculture. In *FAO Fisheries and Aquaculture Technical Paper*, ***615*** (7). http://www.fao.org/3/a-i7677e.pdf

Mahyudin, K. (2013). *Panduan lengkap agribisnis Lele*. Niaga Swadaya.

Manan, A., & Pratiwi, H. C. (2015). Teknik Dasar Histologi pada Ikan Gurami (*Osphronemus gouramy*). *Jurnal Ilmiah Perikanan Dan Kelautan*, ***7*** (2), 153–158.

Marlina, N., Murniati, K., & Kasymir, E. (2021). Analisis Risiko Usaha Budidaya Ikan Lele Dumbo di Kecamatan Kota Gajah Kabupaten Lampung Tengah. *JIIA*, ***9*** (1), 48–53.

Marlisa, Diana, H., Yerizam, M., Junaidi4, R., & Fadarina. (2020). Performance Test of Disc Mill and Vibrating Screen (Discreen) in Making Mocaf Flour (Modified Cassava Flour). ***1*** (1), 87–91.

Mayr, E. 1970. Population Spesies and Evolution. Harvard University Press. England

Mukherjee, S. (2016). *The Gene: An Intimate History*. Scribner.

Najiyati, S. (1997). *Memelihara lele dumbo di kolam taman.* Niaga Swadaya. https://books.google.co.id/books?hl=id&lr=&id=vz-LEFRT8E0C&oi=fnd&pg=PA1&dq=Najiyati,+S.+(1997).+Memelihara+Lele+Dumbo+di+Kolam+Taman.+Jakarta:+Niaga+Swadaya.+49+hlm.+&ots=72GtF3FSr2&sig=K3Qy_SHCSfC0zStwP8_p1jLyiCQ&redir_esc=y#v=onepage&q&f=false

Nasichah, Z., Widjanarko, P., Kurniawan, A., & Arfiati, D. (2016). Analisis kadar glukosa darah Ikan Tawes (*Barbonymus gonionotus*) dari Bendung Rolak Songo Hilir Sungai Brantas. *Prosiding Seminar Nasional Kelautan*, ***2006***, 328–333.

Ndubuisi, U. C., Chimezie, A. J., Chinedu, U. C., Chikwem, I. C., & Alexander, U. (2015). Effect of pH on the growth performance and survival rate of *Clarias gariepinus* fry. *International Journal of Research in Biosciences*, ***4*** (3), 14–20.

© Copyright by Indonesian Aquaculture Society 2023

Nugroho, E. (2011). *Kiat agribisnis lele*. Niaga Swadaya.

Nurhakim, Y. I. (2018). *Panen Melimpah Budidaya Lele Di Kolam Terpal: Solusi Beternak Di Lahan Sempit*. Yusnu Publisher.

Pascottini, O. B., Hostens, M., Dini, P., Vandepitte, J., Ducatelle, R., & Opsomer, G. (2016). Comparison between cytology and histopathology to evaluate subclinical endometritis in dairy cows. *Theriogenology*, ***86*** (6), 1550–1556.

Phillips, M., Henriksson, P. J. G., Tran, N., Chan, C. Y., Mohan, C. V, Rodriguez, U. P., Suri, S., Hall, S., & Koeshendrajana, S. (2016). Menjelajahi masa depan perikanan budidaya Indonesia (Exploring Indonesian aquaculture futures). *WorldFish*.

Qian, C., Wang, R., Wu, C., Wang, L., Ye, Z., Wu, J., & Ji, F. (2018). A fast and visual method for duplex shrimp pathogens detection with high specificity using rapid PCR and molecular beacon. *Analytica Chimica Acta*, ***1040***, 105–111.

Qiang, L., & Cheng, J. (2021). Exposure to polystyrene microplastics impairs gonads of zebrafish (*Danio rerio*). *Chemosphere*, ***263***, 128161. https://doi.org/10.1016/j.chemosphere.2020.128161

Rahardjo, M. F., & Gumelar, A. D. (2019). *Ekologi Reproduksi dan Pertumbuhan Ikan*. PT Penerbit IPB Press.

Rahma, F. W., Mahasri, G. dan, & Sumartiwi, L. (2015). Pengaruh Pemberian Ekstrak *Sargassum* sp. dengan Pelarut Metanol pada Pakan terhadap Jumlah Eritrosit dan Differensial Leukosit Ikan Lele Dumbo (*Clarias gariepinus*). *Jurnal Ilmiah Perikanan Dan Kelautan*, ***7*** (2), 213–218.

Rahmayani, S. (2018). *Perlindungan Konsumen Terhadap Penggunaan Produk Pendahuluan Undang-Undang Nomor 8 Tahun 1999 tentang Perlindungan Konsumen (yang selanjutnya disebut UUPK Tahun 1999) merupakan landasan hukum utama yang mengatur tentang perlindungan konsumen di Indonesi*. ***2*** (1), 165–177.

Retnowati, D., Anshori, M., Fudhla, A. F., Ardiasyah, G., Ardhyani, I. W., & & Puspita, A. D. (2020). Pelatihan budidaya pembesaran ikan lele di desa tanjungan driyorejo gresik. *Jurnal Pengabdian Masyarakat*, ***2*** *(2)*.

Rimalia, A. (2020). Variasi Lama Kejutan Panas pada Suhu yang Sama terhadap Tingkat Penetasan Telur (HR) Ikan Lele Dumbo (*Clarias gariepinus*). *Al-Hayat: Journal of Biology and Applied Biology*, ***3*** (1), 37–39.

Sabia, H., & Chaube, R. (2017). Selection of Appropriate Inhibitor for Ovarian Type Selection of Appropriate Inhibitor for Ovarian Type Cytochrome P450 Aromatase Gene of Heteropneustesfossilis with the. *International Journal on Recent and Innovation Trends in Computing and Communication*, ***5*** (6), 578–598.

Samsundari, S., & Wirawan, G. A. (2015). Analisis penerapan biofilter dalam sistem resirkulasi terhadap mutu kualitas air budidaya ikan sidat (A*nguilla bicolor*). *Jurnal Gamma*, ***8*** (2).

Satriyatama, A., Amaldi, H., & Ibrahim, M. M. (2019). Komposit Grafit-Polistirena Diperkuat Poliuretan sebagai Penyerap Gelombang Akustik. *Jurnal Metalurgi Dan Material Indonesia (JMMI) Grafit*, ***2*** *(3): 14-2*.

Schirinzi, G. F., Llorca, M., Seró, R., Moyano, E., Barceló, D., Abad, E., & Farré, M. (2019). Trace analysis of polystyrene microplastics in natural waters. *Chemosphere*, ***236***. https://doi.org/10.1016/j.chemosphere.2019.07.052

Schreck, C. B., & Tort, L. (2016). The concept of stress in fish. In *Fish physiology* ***35*** (1–34). Elsevier.

Setiasih, N. L. E., Putu, S., Luh, G. S. S. H., & Ni, N. W. S. (2019). Struktur Histologi Uterus Anjing Kintamani pada Periode Dewasa Kelamin. *Buletin Veteriner Udayana*, ***11*** (1), 39–43.

Shanthanagouda, A. H., & Khairnar, S. O. (2018). Breeding and spawning of fishes: Role of endocrine gland. *International Journal of Fisheries and Aquatic Studies*, ***6*** (4), 472–478.

Sinjal, H., Ibo, F., & Pangkey, H. (2014). Evaluasi Kombinasi Pakan dan Estradiol_17β Terhadap Pematangan Gonad dan Kualitas Telur Ikan Lele Dumbo (*Clarias gariepinus*). *Jurnal LPPM Bidang Sains Dan Teknologi*, ***1*** (1), 97–112.

Su, L., Deng, H., Li, B., Chen, Q., Pettigrove, V., Wu, C., & Shi, H. (2018). The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. *Journal of Hazardous Materials*, ***365***, 716–724. https://doi.org/10.1016/j.jhazmat.2018.11.024

Sulistyani, M., & Huda, N. (2018). Perbandingan Metode Transmisi dan Reflektansi Pada Pengukuran Polistirena Menggunakan Instrumentasi Spektroskopi Fourier Transform Infra Red. *Indonesian Journal of Chemical Science*, ***7*** (2), 195–198.

Sulistyo, E. N., Rahmawati, S., Amalia Putri, R., Arya, N., & Amertha Eryan, Y. (2020). Identification of the Existence and Type of Microplastic in Code River Fish, Special Region of Yogyakarta. *EKSAKTA: Jurnal Ilmu-Ilmu MIPA*, ***20*** (1), 85–91. https://doi.org/10.20885/eksakta.vol1.iss1.art13

Sumantri, A., Mulyana, & Mumpuni, F. S. (2017). Pengaruh Perbedaan Suhu Pemeliharaan terhadap Histopatologi Insang dan Kulit Ikan Komet (*Carassius auratus*). *Jurnal Mina Sains*, ***3*** (4), 1–7.

Suyanto, N. S. R. (2004). *Budidaya Ikan Lele (ed. Revisis)*. Niaga Swadaya.

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Thakur, S., Verma, A., Sharma, B., Chaudhary, J., Tamulevicius, S., & Thakur, V. K. (2018). Recent developments in recycling of polystyrene-based plastics. *Current Opinion in Green and Sustainable Chemistry*, ***13***, 32–38.

Trianto, H. F., Pratiwi, S. E., & Suprianto, A. (2015). Perbandingan kualitas pewarnaan histologis jaringan testis dan hepar menggunakan fiksasi formalin metode intravital dan konvensional. *Jurnal Kesehatan Khatulistiwa*, ***1*** (1), 2–15.

Triyanto, Affandi, R., Kamal, M. M., & Haryani, G. S. (2020). No: 64/ Tahun XXXII Juni 2020 ISSN 0251-5168 PUSAT PENELITIAN LIMNOLOGI-LIPI. *WARTA LIMNOLOGI PUSAT PENELITIAN LIMNOLOGI-LIPIJ*, ***1*** (64), 24–30.

Urbinati, E. C., Zanuzzo, F. S., & Biller, J. D. (2019). Stress and immune system in fish. *Biology and Physiology of Freshwater Neotropical Fish*, ***5***, 93–114. https://doi.org/10.1016/B978-0-12-815872-2.00005-1

Utami, N. P., Suherman, Y. M., & Haetami, K. (2012). Pertumbuhan *Chlorella* sp. yang dikultur pada perioditas cahaya yang berbeda. *Jurnal Perikanan Kelautan*, ***3*** (3).

Victoria, A. V. (2017). Kontaminasi Mikroplastik di Perairan Tawar. *Jurnal Teknik ITB*, ***1***, 1–10.

Wagiman, W., Yusfiati, Y., & Elvyra, R. (2014). Struktur Ginjal Ikan Selais (*Ompok hypopthalmus* bleeker, 1846) Di Perairan Sungai Siak Kota Pekanbaru. *JOM FMIPA*, ***1*** (2), 1–9.

Wibowo, T. W., Boesono, H., & Setiyanto, I. (2014). Uji performansi alat pemisah limbah cair berminyak (Oily Water Separator) untuk kapal perikanan dalam skala laboratorium. *Journal of Fisheries Resources Utilization Management and Technology*, ***3*** (4), 1–9.\

Winarno, H., & Pujantara, R. (2015). Pengaruh Komposisi Bahan Pengisi Styrofoam Pada Pembuatan Batako Mortar Semen Ditinjau Dari Karakteristik Dan Kuat Tekan. *Jurnal SCIENTIFIC PINISI*, ***1*** (1), 1–12. https://ojs.unm.ac.id/pinisi/article/view/2114

Wootton, R. J., & Smith, C. (2014). *Reproductive Biology of Teleost Fishes*. Wiley. https://books.google.co.id/books?id=_YnjBAAAQBAJ

Yona, D., Di Prikah, F. A., & As'adi, M. A. (2020). Identifikasi dan Perbandingan Kelimpahan Sampah Plastik Berdasarkan Ukuran pada Sedimen di Beberapa Pantai Kabupaten Pasuruan, Jawa Timur. *Jurnal Ilmu Lingkungan*, ***18*** (2), 375–383. https://doi.org/10.14710/jil.18.2.375-383

Yulianto, T., Putra, W. K. A., Hardiyanti, S. M. T., Fitriana, S., & Yulianto., F. (2019). Tingkat kematangan gonad ikan sembilang dengan induksi hormon hCG berbeda. *Jurnal Perikanan Dan Kelautan*, ***9*** (1), 95–109.

Yuniar, I. (2017). *Biologi Reproduksi Ikan*. Hang Tuah University Press.

Yunus, R. (2006). *Teori Darwin dalam pandangan sains dan Islam*. Gema Insani.

Zhao, F., Wei, P., Wang, J., Yu, M., Zhang, X., Tian, H., Wang, W., & Ru, S. (2017). Estrogenic effects associated with bisphenol a exposure in male zebrafish (*Danio rerio*) is associated with changes of endogenous 17β-estradiol and gene specific DNA methylation levels. *General and Comparative Endocrinology*, ***252***, 27–35.

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