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REPRODUCTIVE PERFORMANCE OF ZEBRAFISH (*Danio rerio*) AFTER EXPOSURE OF BENENG TARO LEAF EXTRACT

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ABSTRACT

Beneng taro leaves (*Xanthosoma undipes*) have potential benefits for fish, including reproductive performance, but their use has not been widely studied. Therefore, research on the effect of Beneng taro leaf extract on zebrafish (*Danio rerio*) reproduction is crucial as basis for use in aquaculture. This study aimed to analyze the reproductive performance of zebrafish after exposure to Beneng taro leaf extract solution. The reproductive performance parameters observed were egg number, egg morphometry, egg fertilization rate, and egg hatching rate. The research method used was an experiment with a Completely Randomized Design (CRD). Sublethal dose tests were conducted at concentrations of 250 - 450 ppm for 96 hours. Reproductive performance tests used a concentration of 350 ppm with exposure durations of 0, 24, 48, and 96 hours. The results showed that exposure to a sublethal concentration of 350 ppm did not affect zebrafish reproductive performance. Based on statistics, each treatment did not show significant differences. The average number of eggs in each treatment was not much different, namely 43 ± 8 - 46 ± 5, the fertilization rate was around 45 ± 2 – 50 ± 14%, and the hatching rate was around 44 ± 4 – 47 ± 8%. In egg morphometry, the egg diameter ranged from 1.15 ± 0.03 - 1.20 ± 0.02 mm and the yolk volume was 0.084 ± 0.003 - 0.184 ± 0.020 mm^3.

KEYWORDS: Egg morphometry, fertilization rate, hatching rate, number of eggs, spawning, toxicity.

ABSTRAK

Daun Talas Beneng (*Xanthosoma undipes*) memiliki kandungan yang berpotensi baik untuk ikan termasuk performa reproduksi, namun penggunaannya belum banyak dikaji. Oleh karena itu, penelitian tentang pengaruh ekstrak daun Talas Beneng terhadap reproduksi ikan zebra sangat penting sebagai dasar pemanfaatannya di dalam bidang akuakultur. Penelitian ini bertujuan untuk menganalisis performa reproduksi ikan zebra (*Danio rerio*) pascapemaparan larutan ekstrak daun Talas Beneng. Performa reproduksi yang diamati yaitu jumlah telur, morfometri telur, tingkat pembuahan telur, dan tingkat penetasan telur. Metode penelitian yang digunakan adalah eksperimen dengan Rancangan Acak Lengkap (RAL). Uji dosis subletal dilakukan pada konsentrasi 250 - 450 ppm selama 96 jam. Uji performa reproduksi menggunakan konsentrasi 350 ppm dengan lama pemaparan 0, 24, 48, dan 96 jam. Hasil penelitian menunjukkan pemaparan konsentrasi subletal 350 ppm tidak memberikan dampak negatif terhadap performa reproduksi ikan zebra. Berdasarkan statistik, setiap perlakuan tidak menunjukkan perbedaan nyata. Kisaran rata-rata jumlah telur setiap perlakuan tidak jauh berbeda yakni 43 ± 8 – 46 ± 5, tingkat pembuahan telur sekitar 45 ± 2 – 50 ± 14%, dan tingkat penetasan telur sekitar 44 ± 4 – 47 ± 8%. Pada morfometri telur, kisaran diameter telur sebesar 1,15 ± 0,03 - 1,20 ± 0,02 mm dan volume kuning telur sebesar 0,084 ± 0,003 - 0,184 ± 0,020 mm^3.

KATA KUNCI: Jumlah telur, morfometri telur, pemijahan, tingkat pembuahan, tingkat penetasan, toksisitas.

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INTRODUCTIONS

Beneng taro (*Xanthosoma undipes*) is a plant native to Pandeglang Regency, Banten. This plant is one of the typical agricultural commodities in the Pandeglang area (Susilawati *et al*., 2021). The main use of talas beneng is the tubers, while other parts such as leaves have not been optimally utilized. It has been reported that Taro Beneng leaves have a protein content of 0.30%, carbohydrates 7.16%, minerals, flavonoids, alkaloids, phenolics, saponins, and tannins (Hermita *et al.,* 2017; Hermita *et al.,* 2019). According to a study conducted by Adriana (2025), Taro Beneng leaves have phytochemical content, namely flavonoids of 19.23 mgGAE/g, alkaloids of 5.84 mgGAE/g, phenols of 50.66 mgGAE/g, tannins of 17.71 mgGAE/g, and saponins of 4.01 mgGAE/g.

The phytochemical content in Talas Beneng leaves has a significant biological role, especially as an antioxidant and bioactive agent that can affect the physiological functions of living things (Rahmawati *et al*., 2019; Sari *et al.,* 2020). Compounds such as flavonoids and phenolics function as antioxidants that are able to reduce oxidative stress, while also acting as phytoestrogens that affect hormonal balance (Chen *et al*., 2018). Saponins and tannins can also have negative effects to cell membrane permeability and potentially disrupt physiological balance, while tannins can bind to proteins and disrupt nutrient metabolism (Makkar *et al*., 2021). However, the effect of using talas beneng leaves for fish has not been widely studied.

Zebrafish (*Danio rerio*) are often used as test animal models for toxicity testing due to their sensitive hormonal regulatory system to foreign substances, is easy to maintain and spawn, transparent embryos, and affordable maintenance costs (Sudradjat, 2019; Khotimah & Ali, 2020). Toxicity test is a preliminary test to determine the toxic effects of a material (Tampungan, 2011) in which commonly *for the in vivo* toxicity test using zebrafish (Sudrajat, 2019). Research on the influence of Talas Beneng leaf extract on zebra fish reproduction is very important as a basis for the development of its use in the field of aquaculture.

Several toxicity studies on the reproductive performance of zebrafish have been conducted. Subchronic toxicity testing of liquid waste from oil palm at a concentration of 1 mL/L for 28 days had a significant impact on the Gonad Maturity Index (IKG) (Utami, 2023). Rumahorbo (2024) mentioned that exposure to Pb at a level of 0.2 mg/L for 96 hours resulted in damage to gonadal tissue that interfered with the oogenesis process and reduced gonadosomatic index (GSI) and ovulation. Based on this, it is necessary to conduct research that aims to analyze the reproductive performance of zebrafish (*Danio rerio*) after exposure to Talas Beneng leaf extract. The observed reproductive performance was the number of eggs, egg morphometry, egg fertilization rate, and egg hatching rate.

MATERIALS AND METHODS

The toxicity test research on the reproductive performance of zebrafish has first test to obtain sublethal doses of Beneng taro leaf extract followed by second on the reproductive performance of zebrafish after exposure using subletal doses. All zebra fish broodstock used were obtained from the Parung Ornamental Fish Market, Bogor, with a total length of 3-4 cm. Prior to the experimental work, 600 fish were first adapted for 7 days in a fiber tub measuring 100 x 65 x 40 cm^3 filled with 195 L water. The method used in this study is an experiment with a Complete Random Design (CRD). In the first stage, a test was carried out with five concentrations of talas beneng leaf extract, namely 250 ppm, 300 ppm, 350 ppm, 400 ppm, and 450 ppm which were repeated twice. In the second stage, the test was carried out with sublethal doses with four different exposure durations, namely 0 hours (as a control), 24 hours, 48 hours, and 96 hours repeated three times.

Sublethal concentration test

The test to determine the sublethal dose used 7 females per container. The test container used is in the form of a plastic measuring 19 x 13 x 9 cm^3 filled with 1 L of water. Dose dilution is carried out with the following formula (Miratsi *et al*. 2024):

$$C1 \times V1 = C2 \times V2$$

Description:
C1 = concentration of the parent solution (ppm)
V1 = volume of the parent solution taken (mL)
C2 = desired concentration of the rule (ppm)
V2 = volume of the desired solution (mL)

Zebrafish are put into each container containing a solution of talas beneng leaf extract according to the test concentration. Mortality observations were conducted at 24-hour, 48-hour, 72-hour, and 96-hour time intervals. The subletal dose used was a concentration of Talas Beneng leaf extract solution which was not enough to cause the death of all test animals.

Reproductive performance test

The reproductive performance test used the spawning ratio of 1 female: 1 male. The soaking and spawning container measures 19 x 13 x 9 cm^3 which is filled with 1 L of water and equipped with aeration.

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Before spawning, female fish are first soaked in a solution of Talas Beneng leaf extract according to the subletal dose, while male fish are not given soaking treatment. Female zebrafish were exposed to taro beneng leaf extract for 24 hours, 48 hours, and 96 hours. After that, the female fish is transferred to a spawning container that has been filled with male fish. The spawning process is carried out for 10 days. During spawning, zebrafish are fed dry artemia (pollardred brand) in the morning and evening at satiation. Fish egg harvesting is carried out in the morning between 08.00-09.00. The rest of the feed in the dissipon spawning container after egg harvest and water is refilled as much as was wasted at the time of spawning. The eggs were then counted and transferred into an incubated plastic container measuring 8 cm in diameter and 12 cm high filled with 200 mL of water. The incubation container does not use aeration. During spawning and egg incubation, physical-chemical measurements of the water, namely dissolved oxygen (DO), pH, and temperature were carried out at the beginning and end of the study.

Research parameters

The research parameter of the sublethal concentration test is female fish mortality. The parameters of the reproductive performance test research are the number of eggs, egg morphometry (diameter and yolk volume), fertilization rate, and hatching rate. Data on mortality, egg morphometry, and water physics-chemistry were analyzed descriptively using microsoft excel software. Data on number of eggs, fertilization rate, and hatching rate were analyzed using microsoft excel and SPSS software by applying the Kruskall-Wallis test. The data from the analysis is presented in the form of tables and figures. The equations used to analyze the test parameters are as follows:

The mortality formula can be calculated with the formula of Muchlisin *et al.* (2016):

$$\text{Mortalities}(\%) = \frac{\textit{initial number of fish - final number of fish}}{\textit{initial number of fish}} \times 100$$

The formula for the number of eggs can be calculated using the formula Herjayanto *et al.* (2016):

$$\text{Number of Eggs (eggs)} = \sum \text{Eggs released by the female}$$

The formula for egg fertilization rate (FR) can be calculated after 1 hour spawning using the formula of Larasati *et al.* (2017):

$$\text{Egg fertilization rate}\ (\%) = \frac{\textit{number of fertilized eggs}}{\textit{total number of eggs}} \times 100$$

The hatching rate (HR) formula can be calculated using the formula of Al Ishaqi and Sari (2019):

$$\text{Egg hatching rate}\ (\%) = \frac{\textit{number of hatched eggs}}{\textit{number of fertilized eggs}} \times 100$$

The observed egg morphometry is the diameter and volume of the yolk. The diameter of the egg is observed by placing the eggs in a petri dish and then placed under the lens of a microscope. Eggs were documented using the Las Ez application and then measurements were made using the ImageJ application. The volume of the yolk was measured using the same procedure as the observation of the egg diameter, but more specifically on the diameter of the elongated yolk and the shortened diameter of the yolk (Figure 1). The equations used to analyze the volume of egg yolks are as follows (Heming & Buddington, 1988):

$$\text{Volume of egg yolk}\ (\text{mm}^3) = \left(\frac{\pi}{6}\right) \text{LH}^2$$

Description:
L= elongated yolk diameter (mm)
H= shortened yolk diameter (mm)

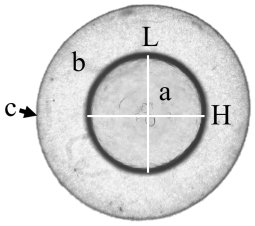


Figure 1. Zebrafish eggs. a) Yolk, b) Perivitelline space, c) Chorion.

RESULTS AND DISCUSSIONS

Sublethal dose test

Sublethal test results showed that all female zebrafish deaths occurred at concentrations of 450 ppm within 24 hours and 400 ppm within 48 hours. The 250 and 300 ppm treatments showed an identical mortality rate of 7% until the end of the 96-hour exposure period. At a concentration of 350 ppm, the percentage of mortality was 56% and rose to 71.5% which continued to be consistent until the end of the test (Figure 2).

Based on these results, the concentration and duration of exposure may affect the mortality rate in the test animals. Secondary metabolites of plants are usually toxic to aquatic organisms, and these levels of toxins increase with increasing doses of exposure (Indrayani *et al.,* 2020). According to Orozco *et al.* (2020), flavonoid compounds and saponins have the potential to produce reactive oxygen species (ROS) which can damage cell membranes, block the respiration process, and interfere with cellular metabolism in fish. This is what causes physiological

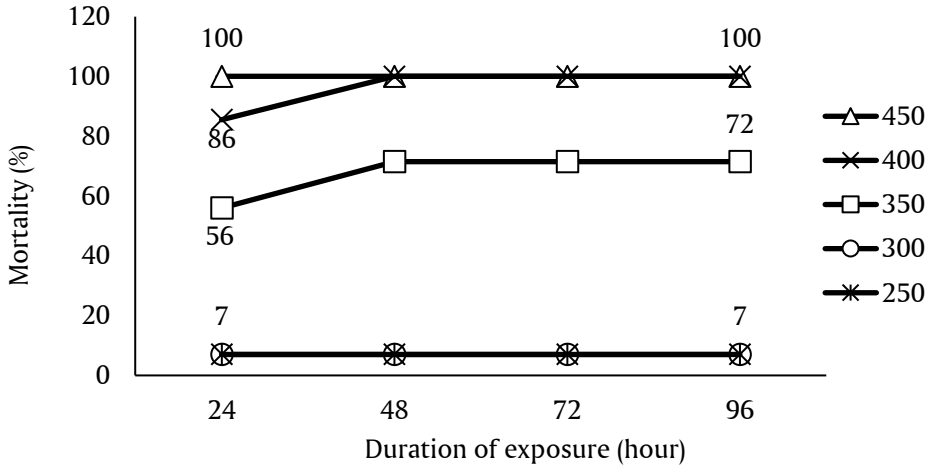


Figure 2. Zebrafish mortality after 96 hours of sublethal dose test exposure across five treatment concentrations (250, 300, 350, 400 and 450 ppm).
Notes: The symbols in the figure overlap due to identical values.

stress in fish to increase and leads to high mortality rates at certain extract concentrations.

A concentration of 350 ppm was selected as the sublethal dose used in the reproductive performance test. The selection of a concentration of 350 ppm is considered to provide a greater opportunity to assess the subletal impact on zebrafish reproductive performance without having to lose the population due to high mortality. The findings on papaya leaf extract have an LC_{50} in the range of 300-350 ppm which indicates a moderate level of toxicity (Ridho, 2021). In addition, the Organisation for Economic Co-operation and Development (OECD) 203 guidelines affirm that toxins that cause premature death within 24 hours fall under the category of acute toxicity.

Reproductive performance test

The reproductive performance after exposure showed no significant differences between the treatments given (Tabel 1).

These results showed that the longest exposure to Talas Beneng leaf extract solution, 96 hours, not have a negative impact on the reproduction of female zebrafish. It is suspected that this is due to the content of bioactive compounds such as flavonoids, saponins, tannins, and alkaloids in the leaves of Talas Beneng (Hermita *et al.,* 2019). According to Zhang *et al.* (2022) flavonoids are known to have antioxidant abilities that can help protect reproductive cells from oxidational damage, thereby potentially supporting the oogenesis process and improving egg quality. Exposure for 96 hours is also thought to be too short to affect zebrafish fecundity because fecundity in fish is generally more influenced by long-term nutritional factors than short-term treatment (Engdaw & Geremew, 2024). Certain plant extracts only showed a significant decrease in the number of fish eggs after prolonged exposure to high concentrations (Siregar *et al.,* 2021). This suggests that duration of exposure and concentration are important factors in determining the biological effects of a compound on fish reproduction.

The rate of egg fertilization is a decisive initial step in the development of the fish embryo, since it is in this phase that the male and female gametes unite, followed by cell division as well as embryo

Table 1. Reproductive performance of zebrafish after exposure to 350 ppm Beneng taro leaf extract for 96 hours.

Treatment	Average		
	Number of Eggs	Egg Fertilization Rate (%)	Egg Hatching Rate (%)
A	46±5[a]	49±10[a]	47±8[a]
B	44±7[a]	47±5[a]	45±6[a]
C	43±8[a]	50±14[a]	47±13[a]
D	45±1[a]	45±2[a]	44±4[a]

Notes: Treatment refers to the duration of exposure of female zebrafish broodstock. A (control), B (24 hours), C (48 hours), D (96 hours). The same superscript letter indicate no significant differences (Asymp. Sig > 0.05) based on the result of the Kruskall-Wallis test.

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Table 2. Characteristics of zebrafish eggs after female broodstock exposure to 350 ppm Beneng taro leaf extract for 96 hours.

Treatment	Egg Diameter (mm)	Volume of Egg Yolk (mm^3)
A	1.15±0.03	0.084±0.003
B	1.17±0.04	0.086±0.015
C	1.13±0.06	0.090±0.040
D	1.20±0.02	0.184±0.020

Notes: Treatment refers to the duration of exposure of female zebrafish broodstock. A (control), B (24 hours), C (48 hours), D (96 hours

formation (Kimmel *et al*., 1995). The value of the egg fertilization rate in all treatments ranges from 45-50%. In zebrafish, the success of fertilization is highly dependent on the synchronization of male gamete maturity and the quality of eggs produced by females (Lawrence, 2021; Cabrita *et al.,* 2022). In addition, viability, motility, and gamete biochemical conditions are the main determinants of successful fertilization in fish (Cabrita *et al*., 2022). Therefore, a dose of 350 ppm is considered not to have a negative impact on egg quality.

The egg hatching rate is a parameter that is greatly influenced by the quality of the eggs, the success of previous fertilization, as well as the environmental conditions during incubation. In zebrafish, hatching success is highly dependent on embryonic integrity and physiological balance during early development (Lawrence, 2021). The egg hatching rate in all treatments ranged from 44-47%. The egg hatching rate showed the same pattern as the egg fertilization rate, where the dose of 350 ppm taro leaf extract did not have a significant effect. The content of compounds in Taro Beneng leaf extract such as flavonoids acts as antioxidants that are thought to protect embryos from oxidative stress during development, thus potentially increasing egg hatchability. Natural antioxidants can improve the success of embryonic development in fish by reducing cell damage due to free radicals (Zhang *et al*., 2022). The success of egg hatching in fish is more influenced by environmental factors than by the treatment of certain nutrients or phytochemical compounds administered or exposed to the female (Cabrita *et al*., 2022).

The morphometric character of zebrafish eggs obtained in this study is that it has a diameter of 1.13-1.20 mm and a yolk volume of 0.084 - 0.184 mm^3 (Table 2).

The sublethal effect often indirectly causes large changes in egg size and yolk volume. Generally, zebrafish produce eggs that are transparent and have an egg diameter of 0.7-1.27 mm (Spench *et al.,* 2008; Utomo, 2009) and egg yolk volume 0.085-0.094 mm^3 (Utomo, 2009). Based on this, the egg diameter and volume of zebra egg clusters in this study are still in the normal range. Therefore, exposure to Taro Beneng leaf extract in this study did not have a negative effect on the physiological condition of female zebrafish (Parichy, 2015; Lawrence, 2016) so that it does not have an impact on changes in the size of the egg diameter and the volume of the yolk. Generally, the diameter of the egg and the volume of the yolk are influenced by the feed nutrients consumed by the mother (Utomo, 2009; Nurfiana, 2020).

The physico-chemical parameters of the water during the study were still within the tolerance range to support spawning and incubation of zebrafish eggs (Table 3).

Table 3. Water quality parameters during the experimental period.

Medium	Treatment	pH	Temperature (^oC)	DO (mg/L)
Spawning	A	7.86 – 8.37	29.0	6.3 – 6.7
	B	7.78 – 8.27	29.0 – 30.0	6.2 – 6.4
	C	7.86 - 8,.29	29.0	6.3 – 6.5
	D	7.79 – 8.2	28.0	6.1 – 6.6
Incubation	A	7.98 – 8.15	28.0 – 29.0	4.8 – 5.6
	B	7.97 – 8.17	28.0 – 29.0	5.1 – 5.7
	C	8.00 – 8.18	28.0 – 29.0	5.2 – 5.6
	D	7.97 – 8.09	28.0 – 29.0	5.1 – 5.2

Notes: Treatment refers to the duration of exposure of female zebrafish broodstock. A (control), B (24 hours), C (48 hours), D (96 hours).

CONCLUSIONS

Exposure to a sublethal dose of 350 ppm Taro Beneng leaf extract for 96 hours was not effective in showing significant differences in the reproductive performance of zebrafish in terms of egg number, egg morphometry, egg fertilization rate, and egg hatching rate.

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LIST OF REFERENCES

Adriana, M. D. (2025). Analysis of the chemical profile and functional properties of taro leaves (*Xanthosoma undipes* K. Koch) with different extraction methods. Thesis. Source: Faculty of Agriculture. Sultan Ageng Tirtayasa University. 101 pp.

Al Ishaqi, A. M., & Sari, P. D. W. (2019). The *spawning of koi* (Cyprinus carpio) *using semi-artificial method: the observation of fecundity, fertilization rate and hatching rate. Journal of Fisheries and Marine* p–ISSN, 2089, 3469.

Alafia, A. A., Lai, K. S., Syahida, A., Mahmood, M., & Shaharuddin, N. A. (2019). Phytochemical evaluation, embryotoxicity, and teratogenic effects of *Curcuma longa* extract on zebrafish (*Danio rerio*) embryos. *Evidence-Based Complementary and Alternative Medicine*, 1–11. https://doi.org/10.1155/2019/3807207.

Cabrita, E., Sarasquete, C., & Martínez-Páramo, S. (2022). Factors influencing fish egg and larval quality. *Aquaculture, 548*, 737634.

https://doi.org/10.1016/j.aquaculture.2021.737634

Engdaw, F., & Geremew, A. (2024). Broodstock nutrition and reproductive performance in fish. *Frontiers in Aquaculture, 3*, 1281640.

https://doi.org/10.3389/faquc.2024.1281640

Hasibuan, D., Komariyah, S., Putriningtias, A., & Batubara, P. A. P. (2023). The use of fermented taro leaf flour in gourami fish seed feed. *Indonesian Aquaculture Journal*.

Heming, T. A., & Buddington, R. K. (1988). Yolk absorption in embryonic and larval fishes, p: 407-446. In W.S. Hoar and D.J. Randall (Eds). Fish physiology. Volume XI., Part A. The hysiology of developing fish, eggs and larvae. Academic Press. New York.

Herjayanto, M., Carman, O., & Soelistyowati, D. T., (2016). Spawning Behavior, Reproductive Potential of Female Fish, and Optimization of Rainbow Fish Spawning Techniques *Iriatherina werneri Meinken*, 1974. Indonesian Journal of Ichthyology. 16(2): 171–183.

Hermita, N., Fatmawaty, A. A., & Hastuti, D. (2019). Phytochemical content test of wild taro leaves (*Xanthosoma Undipes* K. Koch) around the coral mountain area, Banten. *Journal of Agros Agriculture*, 231-235.

Hermita, N., Ningsih, E. P., & Fatmawaty, A. A. (2017). Analysis of proximate and oxalic acid on wild taro leaf fronds. *Journal of Agroscience and Technology*, 2(2).

Indrayani, N., Yuliana, R., & Setyawan, D. (2020). Acute toxicity assessment of plant extracts on freshwater fish. *International Journal of Fisheries Science*, 5(1), 12–20. https://doi.org/10.1111/ijfs.2020.50112.

Khotimah, H., & Ali, M. M. (2020). Zebrafish (*Danio Rerio*) as a model animal in biomedical research and how to maintain it. *Sanus Med J.* 1(1):1-10.

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203(3), 253–310. https://doi.org/10.1002/aja.1002030302.

Larasati, S., F. Basuki, & T. Yuniarti. (2017). Effect of Pineapple Juice with Different Concentrations on the Degree of Fertilization and Hatching of Catfish Eggs *(Pangasius pangasius).* Journal of Aquaculture Management and Technology. 6 (4): 218-225.

Lawrence, (2021). The husbandry of zebrafish (*Danio rerio*): A Review. *Aquaculture, 269,* 1-20. https://doi.org/10.1016/j.aquaculture.2007.04.077

Lawrence, C. (2007). The husbandry of zebrafish (*Danio rerio*): A review. *Aquaculture, 269* (1–4), 1–20. https://doi.org/10.1016/j.aquaculture.2007.04.077 EPA HERO+1.

Lawrence, C. (2016). Advances in zebrafish husbandry and management. *ILAR Journal*, 57(2), 1–13.

Lei, P., Zhang, W., Ma, J., Xia, Y., Yu, H., Du, J., Fang, Y., Wang, L., Zhang, K., Jin, L., Sun, D., & Zhong, J. (2023). Advances in the utilization of zebrafish for assessing and understanding the mechanisms of nano-/microparticles toxicity in water. *Toxics*, *11*(4), 380. https://doi.org/10.3390/toxics11040380.

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Makkar, H. P. S., Francis, G., & Becker, K. (2021). Bioactivity of phytochemicals in aquaculture. *Aquaculture Research, 52*(4), 1503–1515. https://doi.org/10.1111/are.15021.

Miratsi, L., Kurniawan, W. B., & Indriawati, A. (2024). Application of electrocoagulation method in improving the quality of FeCl3 solution. 6H2O. Indonesian Journal of Physics Research, 5(1), 19-28.

Misaghi, A., Shaluei, F., Mohammadi, Y., & Mirrasooli, E. (2020). Influence of water temperature on embryonic development of zebrafish (*Danio rerio*). *Journal of Applied Aquaculture, 32*(3), 265–276.

Muchlisin, Z, A., Arisa, A, A., Muhammadar, A, A., Fadli, N., Arisa, I, I., & Siti Azizah, M, N. (2016). Growth performance and feed utilization of keureling (T*or tambra*) fingerlings fed a formulated diet with different doses of vitamin E (alpha- tocopherol). *Archives of Polish Fisheries* 23: 47-52.

Nguyen, T. H., Nguyen, P. D., Quetin-Leclercq, J., Muller, M., Huong, D. T. L., Pham, H. T., & Kestemont, P. (2021). Developmental toxicity of plant extracts on zebrafish embryos. *Journal of Ethnopharmacology*, 267, 113538.

Nurfiana, I. N. (2020). Effect of egg stimulant on feed on gonad maturity level and zebra egg diameter (*Danio rerio*) (Thesis). Brawijaya University.

Orozco, A. I., Vargas, M., & Serrano, L. (2020). Oxidative stress induced by plant secondary metabolites in aquatic organisms. *Ecotoxicology*, 29, 1200–1212. https://doi.org/10.1007/s10646-020-02228-1.

Parichy, D. M. (2015). Advancing biology through zebrafish research. *Developmental Biology*, 2(3), 1–10.

Pratiwi, R. A. (2021). The effect of water quality variations on the spawning success of zebrafish *(Danio rerio).* Thesis; University of Lampung.

Ridho, R. (2021). *Analysis of the toxicity of papaya leaf extract to zebrafish. Indonesian Journal of Aquaculture*, 10(2), 88–96. https://doi.org/10.1234/jaki.v10i2.88.

Rudiyanti, S., & Ekasari, D. (2009). Growth and survival rate of carp (*Cyprinus carpio* Linn) at various concentrations of pesticide regent 0.3 G. *Journal of Fisheries Science.* 5(1):39-47.

Rumahorbo, P. L. (2024). Toxicity test for histological analysis of the gonads of betok fish (*Anabas testudineus*) exposed to lead heavy metals (Pb). Thesis. Title: Aquatic Ecology Program. Department of Fisheries. Faculty of Agriculture. Sriwijaya University. 26 pp.

Siregar, R. A., Widodo, M. S., & Pratiwi, D. Y. (2021). Effect of plant extract exposure on reproductive performance of zebrafish (*Danio rerio*). *Indonesian Journal of Aquatic Biology*, 6(1), 12–18.

Spence, R., Gerlach, G., Lawrence, C., & Smith, C. (2008) The behaviour and ecology of the zebrafish, *Danio rerio*. *Biological Reviews*, vol. 83, no. 1, pp. 13–34.

Sudradjat, S. (2019). Test the toxicity of the drug with zebrafish larvae. *J.K. Medicine* 25(2):88-3.

Susilawati, P. N., Yursak, Z., Kurniawati, S., & Saryoko, A. (2021). Technical instructions for cultivation and processing of taro varieties of benek. Banten Agricultural Technology Assessment Center (BPTP). Attack.

Tampungan, W. A. (2011). Toxicity test of areca nut stem *extract (Areca vestiaria)* on Artemia salina Leach. *Journal of Bios Logos*, 1(1).

U.S. Environmental Protection Agency. (2012). *Ecological effects test guidelines, OCSPP 850.1075: Acute Toxicity Test for Freshwater Fish*. https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances.

Utami, C. S. (2023). Effect of Growth and Reproductive Performance of Female Zebrafish (*Danio rerio*) exposed to oil palm liquid waste at subchronic concentrations. Doctoral dissertation; Ar-Raniry State Islamic University.

Utomo, N. B. P. (2009). Improving the reproductive quality of ornamental fish through the provision of a combination of essential fatty acids and vitamin E in feed for zebra test fish (*Danio rerio*). Dissertation. Graduate School, Bogor Agricultural University. Bogor.

Westerfield, M. (2000). The Zebrafish Book: A guide for the laboratory use of zebrafish *(Danio rerio)*. University of Oregon Press.

Zainol Abidin, I. Z. (2020). The effects of piper sarmentosum aqueous extracts on zebrafish embryo development. *Scientific Reports*, 10, 14238. https://doi.org/10.1038/s41598-020-70962-7.

Zhang, Y., Liu, X., & Wang, L. (2022). Role of antioxidants in fish reproduction and embryo development. *Aquaculture Reports, 23*, 101034. https://doi.org/10.1016/j.aqrep.2022.101034