



Differences in Soaking Time of Sea Urchin Gonad Extract (*Diadema setosum*) on Nile Tilapia (*Oreochromis niloticus*) Masculinization with a Dose of 4 mg/L

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Abstract

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Nile tilapia (*Oreochromis niloticus*) is a fish that is in demand because of its high economic value and thick meat. Tilapia is easy to breed, uncontrolled spawning can occur so that growth is inhibited. The growth of male tilapia is faster than females at the same age. To increase the growth rate, sex reversal is carried out towards males through immersion in steroid hormones. Bioactive substances contained in sea urchins are steroid compounds. The purpose of this study is to determine the effect of the length of immersion in sea urchin gonad extract (*Diadema setosum*) on the formation of male phenotypes of tilapia larvae and tilapia survival. The method used is a completely randomized design with 4 treatments and each treatment with 3 replicates at a dose of 4 mg/L with soaking times of 0, 12, 18, and 24 hours. Data are analyzed using SPSS 16 software with analysis of variance (One-way ANOVA). The results show that different lengths of immersion in sea urchin steroid extract at a dose of 4 mg/L affect the formation of male individuals. A soaking time of 18 hours is quite effective in directing the sex of fish to males by 66%. Immersion time also affects the survival of tilapia larvae.

Keywords: sea urchin gonads, Nile tilapia, sex reversal, steroids.

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INTRODUCTION

Tilapia (*Oreochromis niloticus*) is an economically important consumed fish species [1]. Tilapia has the advantages of being easy to breed, fast growth, tolerant of environmental conditions, thick meat, favored by the public, and easy to cultivate [2], [4]. This fish is easy to breed, uncontrolled spawning can occur, and

growth is inhibited [1], [4], [6].

The growth rate of male tilapia is faster than females [2], [4], [7]. Due to this phenomenon, the difference in fish biomass at harvest time can reach 30-50% [8]. In its development, mono-sex male tilapia cultivation is carried out. Monosex cultivation is done to obtain faster growth, control of wild spawning, and better performance [9].

One technique to obtain male mono-sex tilapia fry is through sex reversal techniques by administering male hormones (testosterone). The commonly used hormone is the synthetic hormone 17 α -methyltestosterone [4], [10]. However, its use has been banned in aquaculture because it is naturally challenging to degrade and has the potential to pollute the environment. The use of synthetic hormones is relatively expensive and harms the environment. According to Sukmara, et al [11], the administration of synthetic hormones in sex reversal can cause stress, resulting in low fish survival.

Some natural ingredients that can be used as alternatives to synthetic hormones include a purposing extract [12], bovine testis flour [13], sea cucumber offal extract [14], a combination of sea cucumber gonad extract and wild honey [15]. In this study, sea urchin extract was used as a safer alternative. Sea urchins contain 28 types of amino acids and fatty acids, vitamins A and B complex, and minerals [16]. In addition, sea urchins also contain naphthoquinone secondary metabolites that give anti-free radical effects [17]. According to Akaerina et al. [18] and Susanto et al. [19], extracts from the shell, spines, and gonads of sea urchins contain active compounds of the steroid, alkaloid, triterpenoid, flavonoid, and saponin groups. Administration of steroid hormones will change the sex of fish physiologically. Initially, this hormone will stimulate the reproductive process, starting from gonad differentiation, gametogenesis, ovulation, spermatogenesis, spawning, and mating behavior. Furthermore, these hormones will stimulate external sex characteristics, including changes in morphology and physiology during spawning and pheromone production [20].

According to [21], active steroid compounds contain the hormone testosterone and play a role in the formation of male genital organs, reproductive function, and sexual behavior. Therefore, this study aims to determine the effect of different immersion

lengths in sea urchin gonad extract (*Diadema setosum*) at a dose of 4 mg/L on the formation of male phenotypes of tilapia larvae.

METHODS

The research was conducted in October-December 2022 in the Aquatic Biology research laboratory of the Integrated MIPA building, Faculty of Mathematics and Natural Sciences, University of Lampung, while the preparation of sea urchin (*Diadema setosum*) steroid extracts was carried out at the Integrated Laboratory of the Center for Innovation and Technology, University of Lampung. The tools used in this study were an 8-liter fiber tub for acclimatization, a 5-liter glass aquarium for treatment, a 5-liter larval rearing tub, a blower, a 2-meter aeration hose, a 1-m sewage suction hose.

Water quality measurement tools include a pH meter, D.O. meter, thermometer, plastic bucket, scopnet, and lup. Some tools for making steroid extracts are a reflux instrument, rotary vacuum evaporator, centrifuge, 500 ml volumetric flask, 250 ml glass beaker, test tube, and drop pipette.

The materials used in this study were 240 14-day-old tilapia larvae (*Oreochromis niloticus*), steroid extract of sea urchin offal, tilapia feed (Paralytic Shellfish Poisoning/ PSP, Prima Feed 100/PF 100; Prima Feed 500/PF 500), maintenance media water, methanol and distilled water as solvents, and phenolphthalein/pp indicator for making sea urchin extract.

The study was conducted using the Completely Randomized Design method consisting of 4 treatments of immersion in sea urchin gonad extract at a concentration of 4 mg/L for 12, 18, and 24 hours, and control (without immersion) and each treatment with three replications. Each rearing tank contained 20 tilapia larvae at five fish/liter density.

The first stage is the preparation of the

rearing container and water filling. The container must first be cleaned using chlorine (NaOCL). The water used is well water that has been tanked for three days. The next stage is making sea urchin (*Diadema setosum*) steroid extract. Sea urchin gonad extract was obtained by maceration using methanol for 48 hours in a ratio of 1:3 (weight/volume), then shaken using a rotary vacuum evaporator at 37-400 C, and then the extract was stored at chilling temperature (0-4°C). The extract obtained as much as 4 mg was then dissolved in 1 liter of distilled water to obtain a solution with a concentration of 4 mg/L as a treatment material. The research implementation stage starts with preparing and acclimatizing test animals for three days. Then, selected by looking at the morphological characteristics of tilapia, such as body size less than 2 cm, completeness of limbs, and active body movements when swimming. Furthermore, the treatment was given with the immersion method. Each treatment tub was filled with 60 tilapia fish and soaked in sea urchin gonad extract at a concentration of 4 mg/L for 0, 12, 18, and 24 hours. After soaking in the treatment tanks, tilapia larvae were transferred to rearing tanks for 60 days. During rearing, fish were fed with PSP until 3 weeks old, PF 100 until 5 weeks old, and PF 500 until 8 weeks old every morning and evening. During the rearing process, water quality must always be maintained, therefore, water replacement and measurement of water quality, including water temperature, D.O., and pH were carried out every 7 days.

The parameters in this study are:

1. The percentage of males and females was calculated using the formula [22].

$$J (\%) = \frac{\text{the number of male fish}}{\text{number of fish sampled}} \times 100\%$$

$$B (\%) = \frac{\text{the number of female fish}}{\text{number of fish sampled}} \times 100\%$$

Description:

J = percentage of males (%)

B = percentage of females (%)

2. The survival rate was calculated using the formula [22].

$$SR = \frac{N_t}{N_o} \times 100\%$$

Description:

S.R. = fish survival rate (%)

Nt = number of fish at the end of the study

No = number of fish at the beginning of the study

3. Water quality during rearing
Water quality measurements include pH measured using a pH meter, water temperature with a thermometer, and dissolved oxygen (D.O.) measured with a D.O. meter. Data was analyzed with SPSS 16 software with analysis of variance (One Way ANOVA), and if the variance analysis data contained significant differences between treatments, it was continued with the LSD (least significant difference) test at the 5% level ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The study results of differences in the length of immersion in sea urchin gonad extract at a dose of 4 mg/L on sex formation and survival of tilapia during 60 days of maintenance in the aquarium can be seen in Table 1.

Table 1. Sex formation and survival rate of tilapia at different immersion durations in 4 mg/L doses of sea urchin gonad extract for 60 days of rearing in the aquarium

Soaking Time	Gender		Survival Rate (%)
	Males (%)	Females (%)	
0 hour	51,16 ± 1,52a	48,83 ± 1,00	71,66 ± 2,08
12 hours	58,33 ± 1,52a	41,66 ± 1,52	80 ± 2,64

18 hours	66 ± 1,00b	34 ± 1,15	83,33 ± 1,52
24 hours	63,26 ± 1,52a	36,73 ± 1,73	81,66 ± 2,08

The results showed that the 18-hour immersion treatment significantly differed from the 0-hour immersion. The significant value of each treatment > 0.05. The highest percentage of males, 66%, was found in the 18-hour immersion, while the lowest was at 0 hours, 51.16%. The highest female phenotype was formed in 0 hours immersion at 48.83% and the lowest at 18 hours at 34%. ANOVA's results showed that 18 hours of immersion significantly affected the formation of males and their survival rate.

In this study, the measured water quality includes acidity (pH), temperature, and dissolved oxygen (D.O.) content measured every 10 days for 60 maintenance days. The results of water quality measurements can be seen in Table 2.

Table 2. Results of water quality measurements during 60 days of rearing

Parameter	Value Range	Tolerance Range
pH	6,7-6,9	6,8-7
Suhu (°C)	26-28	25-30
DO (mg/L)	3,6-3,9	3,6-4

The successful formation of the male phenotype of tilapia larvae is inseparable from the administration of sea urchin gonad extract. According to Aaerina et al. [18], sea urchin contains the highest steroid in the gonads at 7.10% and the lowest in the spines at 0.94%. The high content of sea urchin gonads is thought to be influenced by the many compounds that dissolve in methanol solvents. According to [21], active steroid compounds contain the hormone testosterone, which plays a role in the formation of male genital organs, reproductive function, and sexual behavior.

The sea urchin gonads contain 8 essential amino acids (lysine, methionine, phenylalanine, threonine, valine, arginine, tryptophan, and histidine) and nonessential amino acids such as aspartate, cysteine,

serine, glycine, and glutamate [23]. Zinc (Zn) and selenium (Sn) are minerals contained in sea urchin gonads and can affect testosterone levels [24].

According to Hamid and Toha [25] and Padang et al. [26], sea urchin gonads contain essential and nonessential amino acids. One of the crucial roles of amino acids in the formation of androgen hormones is testosterone, which increases libido and spermatozoa formation and enters the bloodstream as a regulator of secondary sexual characteristics. This statement is under the research of [27], that sea urchin gonads contain steroids, triterpenoids, and saponins, which are proven to be used in masculinization techniques. According to Susanto et al. [19] sea urchin gonad extract at a dose of 4 mg / L significantly affects the formation of male cupang fish (*Betta sp*) by 84.10%. Research by Lubis and Fitriani [28] using a dose of 4 mg/L honey on hickey fish obtained males of 77.33%. In addition, sea urchins contain a secondary metabolite, naphthoquinone, which has an anti-free radical effect [17]. According to Wang et al. [29], steroids are androgenic hormones that play a role in determining the expression of male phenotypes. Therefore, stimulation of these hormones can cause fish larvae to become masculine.

Steroid compounds are inherently bioactive substances thought to contain androgen hormones that play a role in sex reversal [30]. Administration of these steroid compounds appears to affect the gonads during male character formation and gonadal development stages. Testosterone is suspected to affect neurons through the hypothalamus, followed by synaptic secretion of gonadotropin-releasing factor [31]. This androgen hormone then enters the cytoplasm bound to specific receptors in the cytosol. These formed steroids then travel to the nucleus and are bound to acceptors in the genome [32].

The immersion method of hormones will enter the body by diffusion through the skin, gills, and digestive organs [33].

Steroids initiate changes in secondary sex characteristics behavior following the development and maturity of gametes [31]. Steroid formation in fish occurs in Leydig cells. Cholesterol is taken up and synthesized by Leydig cells. Cholesterol is converted to pregnenolone in mitochondrial organelles. Pregnenolone is converted to 17α -hydroxy-progesterone, the substrate for androgen synthesis. The main product of adult fish testes is 11-testosterone (11-KT), produced by testosterone (T) [34]. The final steroid plasma concentration depends on the rate of synthesis and the rate of deactivation by the liver [31]. According to [35], the absorption of dissolved components in water through the gills is quite large compared to the skin and digestive organs.

Low doses of hormones can cause less than optimal sexual drive, while high doses can cause sterility in fish [9]. To be able to distinguish the formation of male phenotypes of tilapia can be seen from physical characteristics such as tail, dorsal, and anal fins that are longer and wider. In female larvae, the three types of fins are narrower and shorter [36].

This study uses immersion so hormones more effectively enter the body through the circulation system and osmoregulation. Tilapia larvae used are <14 days old by considering the process of sexual differentiation so that the sex of the individual cannot be determined to be formed male or female. According to Hadayani et al. [37], several factors need to be considered in the process of sex direction, such as the continuous administration of hormones when the gonads have not yet formed and the use of the proper dose of hormones. In this study, immersion in sea urchin extract solution at a dose of 4 mg/L for 18 hours showed a percentage male formation of 66%. The longer the immersion time of the hormone used, the lower the male formation. Not all hormones may be well absorbed in the body. According to Zairin [38], the weakness of the immersion method is that not all absorbed hormones can reach the

target organ.

Immersion in sea urchin extract solution gives an optimal effect compared to the control (without immersion). The administration of hormones aims to disrupt the balance of hormones in the blood during sex differentiation [38]. In the treatment without immersion, female sex formation was higher at 48.83% (Table 1). Without hormonal stimulation in the process of gonad differentiation, sex formation occurs naturally. At the same time, immersion in sea urchin extract solution triggers hormonal stimulation that affects differentiation into males. Sea urchin gonad extract contains active compounds that can penetrate cell walls by inhibiting protein synthesis, thus causing changes in cell composition [39]. The naphthoquinone content in sea urchins also has potential as an antibacterial and anti-inflammatory, similar to aspirin [40]. Soaking time affects the survival of tilapia. The highest survival rate of tilapia larvae was obtained at an 18-hour soaking time of 83.33%. This is due to the age of fish larvae that are vulnerable to changes in environment and temperature. Factors that affect survival rates consist of biotic factors, including population density and age, the adaptability of organisms to the environment, and environmental abiotic factors [41].

Tilapia larval mortality occurred from the first day to the eighth day after treatment. Larval mortality is thought to be due to fish stress after transferring from treatment to rearing tanks.

The ideal pH for tilapia growth ranges from 6.8 to 7.0 [42]. pH in the maintenance media ranges from 6.7-6.9, still following what tilapia needs. The results of the respiration of organisms and waste of feed can cause the pH of the media to change. Low pH values can cause fish to be easily stressed and attacked by disease and reduce productivity and growth rates [43]. According to Effendi [22], temperature is an essential factor in the metabolic process of aquatic organisms. Temperature changes

can affect fish survival and death due to increased toxicity of dissolved contaminants and decreased dissolved oxygen.

Fish are classified as poikilothermal animals, where body temperature adjusts to the environment's temperature. All physiological processes of fish are strongly influenced by temperature. Low temperatures can increase the toxicity of dissolved contaminants and reduce dissolved oxygen levels, which can cause fish to be susceptible to mold and mortality [44]. [45] state that the ideal temperature range for tilapia spawning activities to produce eggs and larvae is 22-37°C. The maintenance temperature of tilapia in this study ranged from 26-28°C. Oxygen is needed as an energy source to oxidize food substances that enter the body [46]. Lack of dissolved oxygen levels will harm fish, such as stress and susceptibility to diseases that can cause fish death [47]. This study's dissolved oxygen (D.O.) content ranged from 3.6-3.9 mg/L. [48] state that the dissolved oxygen content in tilapia cultivation media is at least 4 mg/L, so D.O. in the study <4 mg/L is still considered optimal.

CONCLUSIONS

Differences in the duration of immersion in sea urchin extract at a dose of 4 mg/L affect the sex formation of tilapia larvae (*Oreochromis niloticus*). Immersion of 18 hours is a duration that is quite effective in forming males by 66%. Immersion duration also affects the survival of tilapia (*Oreochromis niloticus*).

REFERENCES

- [1] A. K. Biswas, T. Morita, G. Yoshizaki, M. Maita, and T. Takeuchi, "Control of reproduction in Nile tilapia *Oreochromis niloticus* (L.) by photoperiod manipulation," *Aquaculture*, vol. 243, no. 1-4, pp. 229-239, Jan. 2005, doi: 10.1016/j.aquaculture.2004.10.008.
- [2] M. E. Shalaby, A. Ramadan, and A. E. Khattab, "Sex-Reversal of Nile Tilapia Fry Using Different Doses of 17 α -Methyl Testosterone at Different Dietary Protein Levels."
- [3] H. A. F. , Bombata and A. O. Somatun, "The Effect of Lyophilized Goat Tested Meal as First Feed on the Growth of 'Wesafu': An Ecotype Cichlid of Epe-Lagoon, in Lagos State, Nigeria," *Pakistan Journal of Nutrition* , vol. 7, no. 5, pp. 686-688, 2008.
- [4] R. Phelps and T. Popma, "Sex reversal of tilapia," in *Tilapia Aquaculture in the Americas*, vol. 2, B. Costa-Pierce and J. Rakocy, Eds., Baton Rouge, Louisiana: World Aquaculture Society, 2000, pp. 34-59.
- [5] Dunham RA, "Aquaculture and Fisheries Biotechnology Genetic Approaches," 2014.
- [6] K. Varadaraj and T. J. Pandian, "Production of All Female Sterile Triploid *Oreochromis mossambicus*," *Aquaculture*, 2020.
- [7] Popma T and Masser M, *Tilapia Life History and Biology*, 7th ed. SRAC Publication, 2019.
- [8] G. C. Mair, J. S. Abucay, J. A. Beardmore , and D. O. F. Skibinski, "Growth performance trials of genetically male tilapia (GMT) derived from YY-males in *Oreochromis niloticus* L.: On station comparisons with mixed sex and sex reversed male populations," 1995.
- [9] M. Zairin, "Endocrinology and its Role for the Future of Indonesian Fisheries. ," *Scientific Oration of Inauguration of Permanent Professor of Reproductive Physiology and Endocrinology of Aquatic Animals*. Bogor Agricultural University., Bogor, 2013.
- [10] Macintosh DJ and Litte DC, . "Nile Tilapia (*Oreochromis niloticus*) in Bromage N.R. and Ronald JB. Eds," in *Broodstock Management and Egg and Larval Quality*, USA: Blackwell Science, 2015, pp. 277-330.

- [11] Sukmara, "Sex Reversal in Gapi Fish (*Poecilia reticulata* Peters) by Immersion of Larvae in 5 ml/L Honey Solution.," Bogor Agricultural University, Bogor, 2017.
- [12] H. Arfah, D. T. Soelistyowati, and A. Bulkini, "Masculinization of betta fish *Betta splendens* by embryo immersion in extract of purwoceng *Pimpinella alpina*," *Jurnal Akuakultur Indonesia*, vol. 12, no. 2, pp. 144–149, 2013.
- [13] D. Meyer, Mraco G, W. Chan, and C. Castillo, "Use of Fresh Bull and Hog Testis in Sex Reversal of Nile Tilapia Fry," Honduras, 2008.
- [14] G. N. Susanto, Supono, and F. D. Ikrom, "Sex reversal of juvenile freshwater crayfish (*Cherax quadricarinatus*) influenced by steroid extract of sea cucumber and 17 α -methyltestosterone hormone at different temperatures," in *AIP Conference Proceedings*, American Institute of Physics Inc., Aug. 2018. doi: 10.1063/1.5050123.
- [15] G. N. Susanto, Sutyarso, and W. Widiyanto, "Monosex male formation of juvenile redclaw crayfish using natural steroid hormone from gamma sea cucumber and different doses of honey bee," in *Journal of Physics: Conference Series*, IOP Publishing Ltd, Jan. 2021. doi: 10.1088/1742-6596/1751/1/012050.
- [16] Aziz, "Some Notes on the Sea urchin Fishery," *Journal of Oseana*, 2015.
- [17] A. N. Shikov, O. N. Pozharitskaya, A. S. Krishtopina, and V. G. Makarov, "Naphthoquinone pigments from sea urchins: chemistry and pharmacology," *Phytochemistry Reviews*, vol. 17, no. 3. Springer Netherlands, pp. 509–534, Jun. 01, 2018. doi: 10.1007/s11101-018-9547-3.
- [18] F. Olivia Akaerina, T. Nurhayati, and R. Suwandi, "Isolasi dan Karakterisasi Senyawa Antibakteri dari Bulu Babi," *J Pengolah Has Perikan Indones*, vol. 18, no. 1, pp. 61–73, Apr. 2015, doi: 10.17844/jphpi.2015.18.1.61.
- [19] G. N. Susanto, Sutyarso, H. Busman, N. R. Kurniawan, and S. M. Hasanah, "Genital reversal of betta fish by immersion using steroid extract of sea urchins," in *IOP Conference Series: Earth and Environmental Science*, IOP Publishing Ltd, Mar. 2021. doi: 10.1088/1755-1315/674/1/012057.
- [20] F. Yamazaki, "Sex control and manipulation in fish," 1983.
- [21] Sarida, "Effectiveness of Steroid Extract of Sand Sea Cucumber (*Holothuria scabra*) in the Production of Male Galah Shrimp (*Macrobrachium rosenbergii* de Man).," in *Proceedings of the National Seminar of Science and Technology II*, University of Lampung, Nov. 2018, pp. 197–208.
- [22] Effendi M.I, *Fisheries Biology*. Yogyakarta: Nusantara Library Foundation, 2004.
- [23] National Oceanology Institute, *Foodstuffs from the Sea*. Jakarta: National Institute of Oceanology-LIPI, 2003.
- [24] D. , Prianggines, Y. , Winanto, and R. Ali, "Perilaku Seksual dan Kadar testoteron Darah Tikus Putih (*Rattus norvegicus*) Strain Wistar Akibat Pemberian Pakan Gonad bulu Babi (*Diadema setosum*)," in *Proceedings of the 1st Annual National Seminar on Marine and Fisheries Biotechnology*, Semarang, 2012, pp. 81–90. [Online]. Available: <https://www.researchgate.net/publication/348295053>
- [25] A. Hamid and A. Toha, "Ulasan Ilmiah: Manfaat bulu babi (echinoidea), dari sumber pangan sampai organisme hias (Function of Sea Urchin (Echinoidea), from Food to Decoration Animal)."
- [26] A. Padang, T. Tuasikal, dan Rochman Subiyanto, "Kandungan Gizi Bulu Babi (Echinoidea) (Nutrient Contains in Sea Urchin (Echinoidea))," vol. 12, no. 2, pp. 220–227, 2019, doi: 10.29239/j.agrikan.12.2.
- [27] J. Tupan, B. Br Silaban, J. Teknologi,

- and H. Perikanan, "Karakteristik fisik-kimia bulu babi *diadema setosum* dari beberapa perairan pulau ambon (The Physical-Chemical Characteristics of Diadema Setosum From Some Waters at Ambon Island)," 2017.
- [28] M. Arrasyidin Lubis and M. Fitriani, "Maskulinisasi ikan cupang (*Betta* sp.) menggunakan madu alami melalui metode perendaman dengan konsentrasi berbeda Masculinization Betta Fish (*Betta* Sp.) Use Natural Honey Through Immersion Method with Different Concentration."
- [29] R. S. Wang, S. Yeh, C. R. Tzeng, and C. Chang, "Androgen receptor roles in spermatogenesis and fertility: Lessons from testicular cell-specific androgen receptor knockout mice," *Endocrine Reviews*, vol. 30, no. 2. pp. 119–132, Apr. 2009. doi: 10.1210/er.2008-0025.
- [30] E. Riani, K. Syamsu, E. Kaseno, and H. Triaji, "Pemanfaatan steroid teripang sebagai aprodisiaka alami dan untuk pengembangan budidaya perikanan (udang galah dan ikan hias)," Bogor, 2006.
- [31] L. Hachfi, S. Couvray, I. Océanographique, and P. Ricard, "Impact of Endocrine Disrupting Chemicals [EDCs] on Hypothalamic-Pituitary-Gonad-Liver [HPGL] Axis in Fish," 2012. [Online]. Available: <https://www.researchgate.net/publication/285468828>
- [32] C. Rougeot, B. Jacobs, P. Kestemont, and C. Melard, "Sex control and sex determinism study in Eurasian perch, *Perca fluviatilis*, by use of hormonally sex-reversed male breeders," *Aquaculture*, no. 211, pp. 81–89, 2002, [Online]. Available: www.elsevier.com/locate/aqua-online
- [33] M. Jr. Zairin, *Sex Reversal Producing Male or Female Fish Seeds*. Jakarta: Penebar Swadaya, 2002.
- [34] Schultz RW and Nóbrega RH, "Regulation of spermatogenesis," *Encyclopedia of fish Physiology: from genome to environment*. Academic Press, San Diego, 2011.
- [35] Connell and Miller, *Chemistry and Ethotoxicology of Pollution*. Jakarta: UI Press, 2006.
- [36] H. Saanin, *Taxonomy and Key to Fish Identification*. Jakarta: Bina Cipta, 2014.
- [37] N. Handayani, G. N. Susanto, and D. S. Murwani, "Pengaruh ekstrak steroid teripang (*Holothuria scabra* jaeger) dengan lama perendaman yang berbeda terhadap maskulinisasi juvenil lobster air tawar (*Cherax quadricarinatus*)," in *Prosiding SNSMAIP III-*, 2012, pp. 215–219.
- [38] Zairin, *Sex Reversal Producing Male or Female Fish Seeds*. Bogor: Penebar Swadaya, 2004.
- [39] R. Affandi and U. M. Tang, *Physiology of Aquatic Animals*. Riau: Riau: UNRI Press, 2012.
- [40] S. Soleimani, M. Yousefzadi, S. moein, H. Rezadoost, and N. A. Bioki, "Identification and antioxidant of polyhydroxylated naphthoquinone pigments from sea urchin pigments of *Echinometra mathaei*," *Medicinal Chemistry Research*, vol. 25, no. 7, pp. 1476–1483, Jul. 2016, doi: 10.1007/s00044-016-1586-y.
- [41] Ferdous Z, Masum MA, and Ali MM, "Effect of stocking density on growth performance and survival of monosex tilapia *Oreochromis niloticus* fry," *Intl. J. Res. Fish. Aquac*, no. 4, pp. 99–103, 2011.
- [42] Harrysu, *Tilapia Fish Farming*. Yogyakarta: Kanisius, 2012.
- [43] S. Suyanto, *Nila*. Jakarta: Swadaya, 2014.
- [44] A. T. Law, Y. H. Wong, and A. Bolong Abol-Munafi, "Effect of hydrogen ion on *Macrobrachium rosenbergii* (de Man) egg hatchability in brackish water," *Aquaculture*, no. 214, pp. 247–251, 2002, [Online]. Available: www.elsevier.com/locate/aqua-online
- [45] A. F. Andria and S. Rahmaningsih, "Kajian Teknis Faktor Abiotik pada Embung Bekas Galian Tanah Liat PT. Semen Indonesia Tbk. untuk Pemanfaatan Budidaya Ikan dengan

- Teknologi KJA [Technical Study of Abiotic Factors in Clay Embankment Used at PT. Semen Indonesia Tbk for Utilization of Fish 2, pp. 95–105, Nov. 2018, doi: 10.20473/jipk.v10i2.9825.
- [46] D. Setyohadi, G. D. R. Wiadnya, and Soemarno, "Effect of Aeration and Recirculation of Bio-Filter on Growth and Production of Galah Shrimp, *Macrobrachium rosenbergii* (de Man).," *Bioscience*, vol. 1, no. 1, pp. 39–46, 2011.
- [47] Salmin, "Dissolved Oxygen (D.O.) and Biological Oxygen Demand Cultivation with KJA Technology]" *Jurnal Ilmiah Perikanan dan Kelautan*, vol. 10, no. (B.O.D.) as an Indicator to Determine Water Quality" *Oceanography Research Center LIPI*, Jakarta, 2013.
- [48] M. Pramleonita, N. Yuliani, R. Arizal, and S. E. Wardoyo, "Parameter fisika dan kimia air kolam ikan nila hitam (*Oreochromis niloticus*)," *Jurnal Sains Natural*, vol. 8, no. 1, p. 24, Jan. 2018, doi: 10.31938/jsn.v8i1.107.