DOI: http://dx.doi.org/10.15578/iaj.19.2.2024.189-200 *Indonesian Aquaculture Journal, 19 (2), 2024, 189-200*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

THE ADDITION OF COCONUT WATER THROUGH FEED EFFECTIVE ON MASCULINIZATION OF GUPPY FISH (*Poecilia reticulata*)

Sinar Pagi Sektiana[*)#], Muhammad Fiqi Zulendra[**)], Sinung Rahardjo[*)], and Rina[*)]

[*)]Jakarta Technical University of Fisheries

Pasar Minggu Street, Pasar Minggu, South Jakarta, DKI Jakarta, Indonesia

[**)] Fisheries Training and Counseling Center of Bitung

Tandurusa Street, Aertembaga, Bitung City, North Sulawesi, Indonesia

(Received: January 8, 2024; Final revision: October 22, 2024; Accepted: October 22, 2024)

ABSTRACT

The production of male guppies is more desirable due to it higher selling value than females. One way to produce male guppies is change it sex, namely masculinization which can be enhanced through the addition of coconut water, with high potassium level. Potassium can convert all cholesterol in the larvae into pregnenolone, which functions to convert estrogen into progesterone and produces testosterone. Hormones required in sex change can be supplemented by the oral method through feed. The research, which was conducted from November 2022 - March 2023, was divided into two stages, first is to determine the dosage of coconut water and the second one is to determine the appropriate coconut water sterilization method. Each stage used a completely randomized design with four treatments and four replicates. Doses of coconut water added to feed were 0.06 ml/g feed, 0.09 ml/g feed, 0.12 ml/g feed, and 0.15 ml/g feed. The sterilization methods conduct in this research are using UV light, autoclave, and ozone. Observation of male genitalia was done after 45 days by observing the urogenital organs and confirmed by hematoxylin-eosin histological staining after 52 and 59 days. The highest percentage of male guppies at 71.75% was produced by the dose of 0.15 ml/g feed, while sterilization treatment produces lower male percentage than control ($p<0.05$). No significant difference in fish survival was obtained. Fish survival ranges from 88% - 93%. Thus, the addition of 0.15 ml of coconut water to feed is the most effective treatment in producing male guppies.

KEYWORDS: *Coconut water; male guppies; masculinization, oral feed, sterilization*

INTRODUCTION

Masculinization has been identified as an appropriate method to induce male characteristics in fish (Dwinanti *et al*., 2018) with the aim of increasing the abundance of male fish (Malik *et al*., 2019). Male fish have been known to be more favorable than mixed-sex fish in a population (Chapman, 2000; Penman, 2004; Rakocy & McGinty, 1990; Tave, 1995) and increase favorable traits over female fish (Zairin, 2002). Rapid growth of male fish can be caused by various factors including increased body size, better physical appearance, and brighter colors, especially in ornamental fish species such as guppies and hickey fish (Himawan et al., 2018; Rachmawati *et al*., 2016; Rosmaidar, 2016).

Masculinization can be done by administering hormones, which is one of the easiest ways and can reduce mortality rates if the dose is appropriate. Hormone administration is not without drawbacks, for example, the success rate is not always optimal (Zairin, 2002). (Malik *et al*., 2019) in their study showed a mortality rate due to the inability to adapt to hormone-based therapy and the impact of lowering the pH level below the threshold. Male enhancement hormone therapy is usually administered orally with an immersion procedure based on effectiveness, efficiency, possibility of contamination and cost (Findayani & Dina, 2022).

The method of masculinization through oral feeding has several advantages, especially the ease of preparing feed containing hormones. This approach is considered efficient because it can reduce mortality rates, thus creating cost efficiency in the fish farming process through the utilization of very simple hormones (Zairin, 2002). Some previous similar stud-

[#] Correspondence: Jakarta Technical University of Fisheries
E-mail: sektiana@gmail.com

ies used materials such as 17α-methyltestosterone (Barades *et al*., 2020); bovine testes (Hutagalung, 2020); honey resin (Soelistyowati *et al*., 2010); and propolis (Tatalede *et al*., 2019) as alternative hormones. Currently, the soaking method using coconut water media is still the main approach in the masculinization process, although there are not many studies that discuss the use of coconut water added to feed as a medium for inducing masculinization in fish feed.

17α-methyltestosterone is often used to induce masculinization as in the studies of (Ayuningtyas *et al*., 2015), (Takahashi, 1975) and (Zairin, 2002). While this method is highly effective, several limitations and risks need to be addressed. One of the primary risks associated with synthetic hormone use is the presence of hormone residues in fish and aquatic environments. These residues can contaminate surrounding ecosystems, affect non-target organisms, and potentially cause endocrine disruption in humans consuming. Additionally, hormone residues in aquaculture products can reduce consumer trust in food safety. In several countries, the use of synthetic hormones in fish farming is strictly regulated or prohibited for these reasons. In addition, the price is also relatively higher. Other limitations of hormonal masculinization methods include health risks to the fish, such as organ damage, hormonal imbalances, and reduced fertility in masculinized male fish. From an environmental perspective, excessive hormone usage can negatively impact microorganisms, wild organisms, and aquatic ecosystems. (KKP, 2014; Phelps & Popma, 2000; Sudrajat & Sarida, 2006 Sulystyaningsih *et al*., 2022). With the growing awareness of food safety and environmental sustainability, the development of environmentally friendly and consumer-safe masculinization methods has become an urgent need to support the sustainability of aquaculture.

In contrast to the use of these hormones, coconut water has been identified as a natural element with high potential to e masculinization (Angga *et al*., 2013; Arnu, 2020; Cahyani *et al*., 2021; Dwinanti *et al*., 2018; Malik *et al*., 2019; Masprawidinatra *et al*., 2015; Puspitha *et al*., 2023; Sulystyaningsih *et al*., 2022). The challenge in using coconut water as a masculinization medium is that its quality can decrease when mixed with air after the fruit opened, so it is necessary to pay attention to the right storage method. Sterilization method can extend the shelf life of coconut water, based on increasing the durability of food products by removing microbial contamination (Falguera *et al*., 2011; Hermana, 1991; Jaya, 2015; Kailaku *et al*., 2016; Nakano *et al*., 2011; Suharyono *et al*., 2009).

Currently, the soaking method using coconut water as a medium remains the primary approach in the masculinization process, compared to the feed-based method. However, it is expected that the feed-based method in this study can improve the percentage of males and achieve high survival rates in guppy fish. This study aims to determine the optimal dose of coconut water and identify the appropriate sterilization method.

MATERIALS AND METHODS

This research has two stages, the first research is to determine the dosage and the second research is to determine the optimal coconut water sterilization method in extending its shelf life. Both researches were conducted from November 2022 to March 2023 at the Hatchery Laboratory, Fisheries Business Experts Polytechnic of Jakarta, Indonesia. Materials used included: guppies (male and female), coconut water, dry feed (*Artemia nauplii*), and distilled water. This study used a completely randomized design with 4 treatments and 4 replicates for each treatment.

Fish Broodstock Provision

The guppy species used as broodstock in this research is *Poecilia reticulata*, old fashion mozaic roundtail strains (body length 4 cm, body weight 1 g) and obtained from an ornamental fish market in the Jakarta. The guppy gonad maturation process was carried out by putting the broodstock into the aquarium separately (male and female), then transferred into 16 aquariums measuring 40 x 30 x 15 cm (capacity of 18 liters / aquarium) containing 1 male and 2 females for further observation of the spawning process after gonad maturity.

Broodstock Spawning

The spawning process is characterized by males actively approaching females. Successful spawning is marked by the appearance of abdominal enlargement and black color on the urogenital part of the female. Based on previous research conducted, guppies mate within 4 days (Nurlina & Zulfikar, 2016) which is the basis for the mating period in this study. Next, the male is removed from the aquarium and put into a separate aquarium, while the female is observed, starting when the abdomen enlarges and the urogenital area is black.

Coconut Water

Coconut water used came from *Cocos rubescens* which was then added to the dry *Artemia nauplii* as feed by spraying it according to the specified treatment dose. The feed containing coconut water was

given for the first time to the female guppy when the female's abdomen looked large and the urogenital area was black for 14 days using the adlibitum method. The sterilization process of coconut water was carried out after the best dose was obtained (stage-II of the research).

Masculinization Method

First Research: Masculinization using unsterilized coconut water

In this first stage, the objective to be achieved is to determine the optimal dose of coconut water in feed, before sterilization to extend the shelf life. The dosage used refers to the research conducted by (Sektiana *et al*., 2023). The first phase of the research was conducted from November 2022 to January 2023 at the Hatchery Laboratory, Fisheries Business Experts Polytechnic of Jakarta, Indonesia. A completely randomized design with 4 treatments and 4 replications for each treatment was applied (Table 1).

Second Research: Masculinization using sterilized coconut water

The second research after finding the optimal coconut water dosage by feeding is to determine the appropriate sterilization method to extend the shelf life of coconut water. The perishable characteristics of coconut water after opening from the fruit are caused by the oxidation process and microbial activity, therefore it is necessary to sterilize it so that microbial contamination cannot damage the coconut water used. Sterilization of coconut water for use in fish masculinization has not been previously conducted. This research was conducted from January to March 2023 at Fisheries Business Experts Polytechnic of Jakarta, using completely randomized design with 4 treatments and 4 replications (Table 2).

Measurement of Potassium Content in Coconut Water

Fresh coconut water (without sterilization) and coconut water with three sterilization procedures (using UV light, Autoclave, and Ozone) were measured for potassium content at the Spice and Medicinal Plant Research Institute (Balittro) in Bogor, West Java. Potassium plays a role in sex hormone changes in guppies. Coconuts used as a source of coconut water in this research were obtained from Serang - Banten. Coconut water samples, both sterilized and unsterilized, were put into 250 ml bottles and then tested for potassium levels.

Table 1. Research design of dosage of coconut water addition to dry feed

Repeat Count	Dose of Coconut Water			
	D1 (0.06 ml/g feed)	D2 (0.09 ml/g feed)	D3 (0.12 ml/g feed)	D4 (0.15 ml/g feed)
1	$D1_1$	$D2_1$	$D3_1$	$D4_1$
2	$D1_2$	$D2_2$	$D3_2$	$D4_2$
3	$D1_3$	$D2_3$	$D3_3$	$D4_3$
4	$D1_4$	$D2_4$	$D3_4$	$D4_4$

Table 2. Research design of coconut water sterilization method

Repeat Count	Coconut Water (D)	Sterilization Methods			
		S1 (No Sterilization)	S2 (UV Light)	S3 (Autoclave)	S4 (Ozone)
1	*Optimal Dosage*	$DS1_1$	$DS2_1$	$DS3_1$	$DS4_1$
2		$DS1_2$	$DS2_2$	$DS3_2$	$DS4_2$
3		$DS1_3$	$DS2_3$	$DS3_3$	$DS4_3$
4		$DS1_4$	$DS2_4$	$DS3_4$	$DS4_4$

Observation of male guppies

Sex observations were made morphologically and histologically with hematoxylin-eosin staining. Observations of male guppies were carried out morphologically by looking at the presence of gonopodium in the urogenital region (Sarida *et al*., 2011) and confirmed by histological procedures using hematoxylin-eosin staining through a microscope (Sary & Zainuddin, 2017). Histological procedures begin with tissue fixation, selection, washing, block making, slicing, and staining (Astuti *et al*., 2019; Sulistyorinie *et al*., 2020). Because hematoxylin is alkaline and attached to the male gonads, it can be distinguished when making histological preparations based on the purplish-blue color. Meanwhile, eosin turns pink because it is connected to the female gonads and is acidic (Dikna, 2022). Male percentage was calculated using

the formula (Zairin, 2002) as follows:

$$\text{Male Percentage (\%)} = \frac{\text{Number of Male Fish}}{\text{Total Fish Stocking}} \times 100$$

Survival Rate

Guppy larvae were reared for 45 days after hatching (Dwinanti *et al*., 2018; Priyono, 2013). Feeding of guppy larvae was done 3 times a day using the adlibitum method. During rearing, 20% of the total water volume was replaced every 2-3 days, while water quality (pH, temperature, and dissolved oxygen) was measured every morning and evening. The percentage of survival rate was calculated using the formula from (Malik *et al*., 2019) as follows:

$$\text{Survival rate (\%)} = \frac{\text{Number of fish at the end of rearing}}{\text{Number of fish at the beginning of rearing}} \times 100$$

Total Plate Count of Bacterial

Total plate count testing was conducted to determine the number of bacterial colonies in coconut water before and after UV sterilization, autoclave sterilization, and ozone sterilization. The total plate count testing was conducted at the Department of Food Science and Technology Laboratory, Bogor Agricultural University - West Java

Statistical Analysis

Data on coconut water potassium value, sex percentage and survival rate were analyzed using ANOVA to determine significant differences ($P<0.05$) between treatments, followed by Tukey's multiple comparison test to determine differences between treatments. Maximum dose prediction was calculated using polynomial regression in Microsoft Excel.

RESULTS AND DISCUSSION

First Research: Masculinization using unsterilized coconut water

Potassium value of coconut water

The results of this research showed the potassium content of coconut water ranged from 1,300 mg/l to 2,300 mg/l with an average of 1,833 mg/l (Table 3). The potassium content of young coconut water obtained in this study is consistent with the findings of Muchlisin (2016), Ibrahim (2020) and Kailaku *et al*. (2015), which range from 772.40 to 1,700 mg/L. The use of young coconut water refers to the fact that young coconut water contains a higher concentration of potassium compared to other inorganic ions. Older coconuts, on the other hand, have a lower potassium content (Bhagya *et al*., 2012; Dwinanti *et al*., 2018; Hikmawandari *et al*., 2019; Kamala Devi & Velayutham, 1978; Yong *et al*., 2009). Potassium is the most abundant mineral in coconut water, depending and age and type (Rana *et al*., 2018; Rethinam, 2006).

Table 3. Potassium of *Cocos rubescens*

No	Type of Coconut Water	Repeat Count	Potassium (mg/L)
1	*Cocos rubescens*	1	1,300
2		2	2,300
3		3	1,900

Coconut water, through its potassium content, influences the masculinization of guppy fish. Potassium in coconut water converts cholesterol in all larval tissues into pregnenolone. Pregnenolone then transforms estrogen into progesterone, playing a role in the formation of testosterone, leading to the development of male fish (Ganong, 1983; Islama *et al*., 2017; Martin, 1979; Yamazaki, 1983). Behind all the advantages and benefits of coconut water, there is a crucial drawback, namely the short shelf life of coconut water after the coconut is opened (Az-zahra *et al*., 2019). Sterilization of coconut water and proper storage (Sari & Hadiyanto, 2013) are among several solutions that have the potential to extend the shelf life of coconut water. Phase 2 of this research provides an explanation of the different methods used to sterilize coconut water.

Sex observation of guppies

Morphologically, male guppies have more varied colors than females (Figure 1a-b). The difference between male and female fish can be seen from the presence of gonopodium in the urogenital of male fish which is not found in females (Sarida *et al*., 2011; Sulistyo *et al*., 2021). Figures 1 c and show histological differences using hematoxylin-eosin staining which shows male gonads with a purplish blue color, while female gonads are pink. Another difference is that the gonads in male guppies produce sperm, while females form eggs (Zairin, 2002). Sex identification through morphological observations in this research gave results that were in accordance with the results of hemactosylin-eosin histology staining.

Percentage of Male Guppies

The results of statistical analysis showed that there was a significant difference in the percentage of male guppies between treatment 4, which was the addition of 0.15 ml coconut water to the feed with treatment 2 (0.09 ml/g feed) and treatment 1 (0.06 ml/g feed). While there was no significant difference in

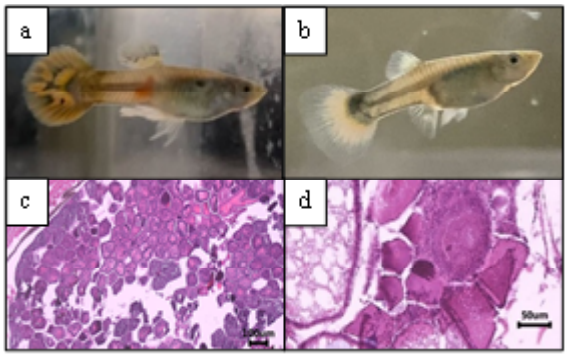

Figure 1. Sex differences based on visual morphology in guppies (a & b) at 45 days and by hematoxylin-eosin histological staining (c & d) at 52 days.

Notes: [a] Male [b] Female [c] Male [d] Female

treatment 3 (0.12 ml/g feed) to other treatments (Table 4). Based on the analysis, it can be said that different doses of coconut water have a significant effect on the percentage of male guppies, according to the results by (Malik *et al*., 2019) which showed a higher concentration of coconut water can increase the percentage of male guppies. Masculinization influenced by several factors including sex differentiation, fish species, hormone type and dosage, treatment duration, starting time, and water temperature. The establishment of sex differentiation that has not yet occurred is strongly influence the succes of masculinization. In guppies, sex differentiation occurs at eight days before birth during embryonic development and 12 days after birth at the larval stage (Hunter & Donaldson, 1983; Piferrer, 2001; Priyono, 2013; Strüssmann *et a*l., 2005; Yamamoto, 1969).

Table 4. Percentage of Male Guppies with different doses of coconut water

No	Dose of Coconut Water (ml/g feed)	Percentage of Male Guppies (%)
1	0.06	65.75 ± 2.75[a]
2	0.09	66.50 ± 2.38[a]
3	0.12	70.25 ± 2.06[ab]
4	0.15	71.75 ± 1.70[b]

Furthermore, a polynomial regression equation was created to ascertain the optimal dose of coconut water addition to the feed in the masculinization.The regression analysis shows that the average dose of coconut water affects 94% of male guppies, while 6% of male guppies can be influenced by variables not studied in this research. Based on the third-order polynomial curve, the optimal dose of coconut water added to fish feed for broodstock was 0.15 ml/g feed, which resulted in a male sex ratio of 71.75%, 6% higher than the control treatment which only yielded 65.75%. This first stage research found a higher proportion of male guppies than previous studies, which used a control treatment without coconut water (Dwinanti *et al*., 2018; Malik *et al*., 2019; Perdana *et al*., 2022; Sektiana *et al*., 2023; Sulistyo *et al*., 2021).

Survival Rate

The statistical analysis based on Table 5 below shows that there is no significant difference in the percentage survival of guppies among the different treatments. This indicate that masculinization through addition of coconut water on dry feed at different doses does not pose a risk to guppies. In some previous studies, the masculinization process in organisms through immersion can cause high mortality rates (Findayani & Dina, 2022; Malik *et al*., 2019; Novitasari *et al*., 2022; Priyono, 2013). This may be due to improper dosing in addition to other factors.

Table 5. Percentage of Guppies Survival with different doses of coconut water

No	Dose of Coconut Water (ml/g feed)	Percentage of Guppies Survival (%)
1	0.06	89.25 ± 5.31[a]
2	0.09	90.00 ± 2.16[a]
3	0.12	88.25 ± 2.06[a]
4	0.15	89.25 ± 3.86[a]

Water Quality

The results of research on water quality (Table 6) show that the values obtained are still within the tolerance limits to support the survival of guppies. Water quality needs to be maintained so that it is in accordance with the needs of fish which is useful for promoting growth during maintenance. Temperature plays an important role in growth (Devlin & Nagahama, 2002; Novitasari *et al*., 2022); appetite, reproduction, and food digestion processes (Cahyani *et al*., 2021; Nakamura *et al*., 1998; Novitasari *et al*., 2022). Oxygen is closely related to temperature (Novitasari *et al*., 2022; Priyono, 2013). As water temperature in-

creases, dissolved oxygen levels decrease, and vice versa (Arnu, 2020). A pH of 4 (acidic/low) or a pH of 11 (alkaline/high) can cause fish mortality (Siregar *et al.,* 2018).

Table 6. Water quality with different doses of coconut water

Parameter	Value	Tolerance Range
Temperature (°C)	27.00 ± 1	18 – 28 (Pethiyagoda *et al*., 2021)
pH	6.6 ± 0.14	5 – 9 (Pethiyagoda *et al*., 2021)
Dissolved Oxygen (mg/L)	4.3 – 4.6 ± 0.32	0.83 – 4.84 (Pethiyagoda *et al*., 2021)

Second Research: Masculinization using sterilized coconut water

Potassium Value of Coconut Water

This follow-up study aims to determine the potassium content in coconut water after sterilization by several methods. Result of potassium value in coconut water with different sterilization methods can be seen in Table 7 below.

Table 7. Potassium of *Cocos rubescens* with different sterilization methods

No	Sterizilation Procedure	Potassium Value(mg/L)
1	Without Sterilization	3,400 ± 0.00[b]
2	UV Light	1,100 ± 100.00[a]
3	Autoclave	1,566 ± 450.92[a]
4	Ozone	1,266 ± 503.32[a]

Notes: letters a and b indicate significant differences in each treatment result

Statistical analysis showed a significant higher in the potassium content in unsterilized coconut water compared to those sterilized using various methods (UV light, autoclaving, and ozone). Sterilization using autoclaving had the highest potassium content at 1,566 ± 450.92 among other sterilization methods (UV, autoclave, and ozon), significantly different from control (without sterilization at 3,400 ± 0.00), and had no different significant level with UV light (1,100 ± 100.00) or ozone (1,266 ± 503.32) method. Based on the analysis, there was a decrease in potassium content in coconut water after sterilization. However, literature references on sterilized coconut water are still limited, so additional research is needed to understand the reasons for the decrease in potassium after sterilization.

Guppy Sex Observation

As in the first study, there were differences both on morphologically and through histological staining in male and female guppies. Figure 2 below shows the results of guppy observations through morphological approaches and histological staining after autoklaving on coconut water. Male guppies have more varied colors than females (Figure 2a-b) and have purplish blue color based on histological staining using hematoxylin-eosin, while the female gonads are pink (Figure 2c-d).

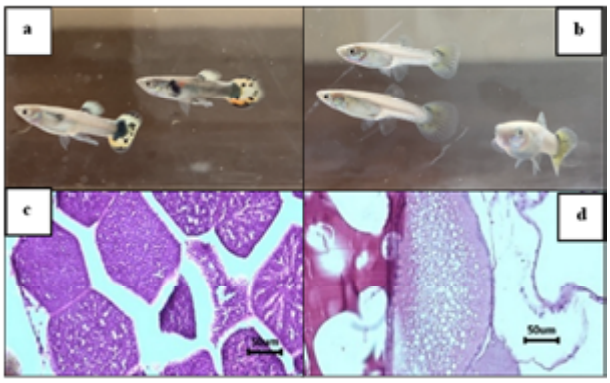

Figure 2. Sex differences based on visual morphology in guppies (a & b) at 45 days of and by hematoxylin-eosin histological staining (c & d) at 59 days.

Notes: [a] Male [b] Female [c] Male [d] Female

Percentage of Male Guppies

Unsterillized coconut water had a significant effect on the results of sterilization using UV light, autoclave and ozone. Based on various sterilization methods, autoclaving (S3) showed significant differences from UV (S2) and ozone (S4). The results showed that different sterilization techniques on coconut water resulted in a decrease in potassium value and proportion of male guppies. The percentage of male fish obtained from coconut water without sterilization had the highest amount of 70.00 ± 1.41[c], which successively decreased in treatments with steriliza-

tion methods, namely 62.00 ± 1.15[a] (S2), 67.00 ± 0.81[b] (S3), and 64.00 ± 1.41[a] (S4). Different coconut water sterilization methods have a significant effect on the percentage of male guppies compared to those that do not use coconut water (Dwinanti *et al*., 2018; Malik *et al*., 2019; Perdana *et al*., 2022; Sulistyo *et al*., 2021). The sterilization method using autoclaving is proven to be able to extend the shelf life of coconut water and is the most appropriate method in increasing the percentage of guppies by 67%, the highest compared to other sterilization methods.

Table 8. Percentage of Male Guppies with different coconut water sterilizations

No	Sterilizations Procedure	Percentage of Male Guppies (%)
1	Without Sterilization	70.00 ± 1.41[c]
2	UV Light	62.00 ± 1.15[a]
3	Autoclave	67.00 ± 0.81[b]
4	Ozone	64.00 ± 1.41[a]

The decrease in the percentage of male guppies after coconut water was sterilized using different sterilization methods is suspected to be due to a reduction in potassium levels (Table 7). The decrease in potassium levels during sterilization using autoclave can be attributed to the effects of heat and water vapor pressure (Anggari, 2008), while the intensity of exposure to excess UV light and the length of exposure time can reduce potassium levels during sterilization (Triastarani, 2021), and the presence of unstable, highly reactive and easily decomposed compounds can cause a decrease in potassium levels with ozone sterilization (Rizqa, 2009). It is recommended to check the addition of external potassium sources, such as potassium hydroxide and potassium chloride, which can offset the reduction of potassium.

Survival Rate

There was no significant difference in the percentage survival of guppies with different methods of coconut water sterilization. These results indicate that masculinization through the addition of sterillized coconut water does not pose a risk to individuals, as well as the addition of different doses. Fish survival can be influenced by several factors such as feed and water quality. Good quality and quantity of feed, supported by environmental conditions, can increase fish survival (Renita, 2017). Feed plays an important role and is a crucial factor during larval rearing, namely the transition from a phase dependent on endogenous feeding to a phase of exogenous feeding (Taufiqurahman *et al*., 2017). In addition to feed, temperature factors in water quality affect the structure and function of proteins and other macromolecules in the fish body, affecting growth when it enters in turn (Devlin & Nagahama, 2002).

Table 9. Percentage of Guppies Survival with different coconut water sterilizations

No	Sterilizations Procedure	Percentage of Guppies Survival (%)
1	Without Sterilization	92.25 ± 2.50[a]
2	UV Light	91.50 ± 1.29[a]
3	Autoclave	90.75 ± 2.21[a]
4	Ozone	91.75 ± 2.21[a]

Notes: letters a and b indicate significant differences in each treatment result

Water Quality

The results of water quality measurements as shown in Table 10 are still within the tolerance limits to support the survival of guppies. The suitability of water quality to the needs of fish needs to be maintained to promote growth during rearing.

Temperature has a significant impact on important fish activities such as respiration, growth, reproduction, and metabolism. Temperature can affect oxygen levels which are also important in the growth process (Cahyani *et al*., 2021; Devlin & Nagahama, 2002; Nakamura *et al*., 1998; Novitasari *et al*., 2022; Priyono, 2013). (Soelistyowati *et al*., 2007) emphasized that fish need oxygen for their environmental needs and consumptive needs, which depend on metabolic conditions. Meanwhile, water that is too acidic (low pH) or alkaline (high pH) can cause fish death (Siregar *et al*., 2018).

Number of Bacterial Colonies in Sterilized Coconut Water

Coconut water samples tested by total plate count (TPC) method were equal to the number of samples in the guppy broodstock, which were put into each sample bottle for each feeding. The results of TPC

Table 10. Water quality with different coconut water sterilizations

Parameter	Value	Tolerance Range
Temperature (°C)	27.00 ± 1	18 – 28 (Pethiyagoda *et al*., 2021)
pH	6.6 ± 0.14	5 – 9 (Pethiyagoda *et al*., 2021)
Dissolved Oxygen (mg/L)	4.3 – 4.6 ± 0.32	0.83 – 4.84 (Pethiyagoda *et al*., 2021)

tests taken for several types of sterilized coconut water can be seen in Tables 11 and 12.

Sterilization with proper storage methods can extend the shelf life of coconut water. Sterilized coconut water (using UV light, autoclaving and ozone) stored in tightly closed sample bottles at -1°C or -4°C without being opened daily, will remain fresh for up to 20 days. If the coconut water sample bottle is opened once a day for six days (table 6), it will only last for less than three days. Sterilization is necessary to maintain the quality and nutritional content when stored for a long time. Coconut water cannot be stored for a long period of time because of the rapid deterioration of quality after being extracted from the coconut without sterilization (Az-zahra *et al*., 2019; Tranggono & Sutardi, 1990). Storage at low temperatures can inhibit the growth of microorganisms, which is also closely related to pH conditions (Susanty & Yudistirani, 2019). The combination of low temperature and pH can extend shelf life because it inhibits the proliferation of microorganisms (Az-zahra *et al*., 2019; Karlida & Musfiroh, 2017; Langkong *et al*., 2018).

Table 11. TPC results comparison of coconut water sterilization on days 10 and 20

Bottle of unopened coconut water sample	TPC testing (colonies/g)	
	Day 10	Day 20
UV Light (Freezers)	< 1.0×10^1 (3.5×10^0)	5.8×10^1
UV Light (Refrigerator)	2.0×10^7	1.4×10^8
UV Light (Room Temperature)	5.2×10^7	2.7×10^6
Autoclaves (Freezers)	$<1.0 \times 10^0$	$<1.0 \times 10^0$
Autoclave (Refrigerator)	$<1.0 \times 10^1$ (2.0×10^0)	7.2×10^2
Autoclave (Room Temperature)	3.4×10^7	1.8×10^7
Ozone (Freezers)	7.5×10^2	5.0×10^2
Ozone (Refrigerator)	1.1×10^8	3.2×10^7
Ozone (Room Temperature)	1.6×10^8	1.5×10^8

Source: the Department of Food Science and Technology Laboratory, Bogor Agricultural University - West Java

Table 12. TPC results of coconut water sterilization on days 3 and 6

Treatment of coconut water sample bottles opened every day	TPC Testing (c/g)	
	Day 3	Day 6
UV Light (Freezers)	1.3×10^4	1.8×10^5
UV Light (Refrigerator)	2.0×10^6	TBUD
UV Light (Room Temperature)	2.5×10^8	TBUD
Autoclaves (Freezers)	4.4×10^3	5.2×10^3
Autoclave (Refrigerator)	1.5×10^4	2.1×10^5
Autoclave (Room Temperature)	1.6×10^7	TBUD
Ozone (Freezers)	6.6×10^3	TBUD
Ozone (Refrigerator)	1.6×10^7	7.8×10^4
Ozone (Room Temperature)	1.7×10^8	1.8×10^5

Source: the Department of Food Science and Technology Laboratory, Bogor Agricultural University - West Java

At the commercial level, packaging can be designed for single-use, with optimized sizes meeting specific requirements, resulting in increased efficiency. Packaged sterile coconut water can be maintained by storing it in a freezer. The maximum number of bacterial colonies in bottled coconut water is 1.0×10^2 colonies/ml (SNI, 1996). The maximum number of bacterial colonies found in isotonic drinks is 2.0×10^2 colonies/ml (SNI, 1998). While the maximum concentration of bacterial colonies in bottled drinking water is $1{,}0 \times 10^5$ colonies/ml (SNI, 2009). Based on the threshold datas of the number of bacterial colonies in the packaging, the total plate number required in relation to the masculinization process of fish, including guppies, has not been known and identified.

The results of the total plate count test indicate that adding coconut water to the feeds is an effective method on a commercial scale. In addition, it was found that sterilized coconut water can last up to 20 days if stored in a freezer at -1°C to -4°C, further strengthening this conclusion. Therefore, for commercial purposes, the packaging can be customized to cater for single-use. This allows packaged sterilized coconut water to be mass-produced as it can be stored for up to 20 days.

The limitations of this study include storage duration, the need for specialized packaging, dependence on refrigeration facilities, effects on mass production scale, and the lack of sufficient data on the

long-term effects of adding sterilized coconut water to feed on fish health and environmental impacts on a larger scale. Therefore, although this method shows promise, further development is needed to address logistical limitations, packaging requirements, and cost efficiency.

CONCLUSION

Different doses of coconut water added to the feed had a significant impact on the male guppies percentage. The optimal dose of 0.15 ml/g feed was able to produce 71.75% male fish. At this dose after sterilization with various methods, there was significant lower male percentage compared to unsterilized treatment, which means that it is not appropriate to use it for masculinization in guppy. Suggestions for further research related to this study include a deeper investigation into the effectiveness of different sterilization methods for coconut water in maintaining the potassium content required for guppy masculinization. Future studies could focus on testing a wider range of coconut water doses, including those both below and above the optimal dose of 0.15 ml/g of feed, as well as examining the effects of storage methods and storage duration on the effectiveness of sterilized coconut water. Additionally, long-term research on the impact of adding sterilized coconut water to guppy health and its environmental effects should be conducted to better understand the implications for commercial-scale use.

REFERENCES

Angga S, M., Nurjanah, L., Nurkhasanah, A., Yusrina, W., & Purnama Rahayu, D. (2013). Coco Reverse: Application of Coconut Water in the Production of Male Monosex Populations of Red Tilapia. Institut Pertanian Bogor.

Anggari, C. P. (2008). *Journal of Autoclave Working Principle and Autoclave Components. A.H.M. Eisenga, CFD Prepared for the Process Industry, NPT Procestechnology*:5,19:5,1998, 35–37.

Arnu, F. (2020). The Effect of Immersion Duration of Guppy Fish (*Poecilia reticulata*) in Coconut Water (*Cocos nucifera L*) on the Effectiveness of Masculinization. *Jurnal Perikanan, 10, 175–182.*

Astuti, S., Wiadnya, D. G. R., & Sukandar, M. (2019). Histological Analysis Of Gonad Maturity Levels Of Females Kembung Fish (*Rastrelliger Brachysoma*, Bleeker 1851) In The Lekok Flow, Pasuruan. *Journal of Fisheries and Marine Research*, 3 (1), 8–21.

Ayuningtyas, S. Q., Junior, M. Z., & Soelistyowati, D. T. (2015). Sex Reversal of Male Nile Tilapia Using 17á-Methyltestosterone Through Feed and Increased Temperature. *Jurnal Akuakultur Indonesia,* 14(2), 159–163.

Az-zahra, N. I., Giyarto, G., & Maryanto, M. (2019). Characteristics of Coconut Water and Honey-based Isotonic Drinks in Cold Storage. *Agricultural Scientific Periodical*, 2(1), 1–5.

Barades, E., Hartono, D. P., Witoko, P., & Aziz, R. (2020). Increase in Male Nile Tilapia *(Oreochromis niloticus)* Ratio Using 17-Methyltestosterone Through Feed. *Jurnal Perikanan Urnam, 10(1), 50–54.*

Bhagya, D., Prema, L., & Rajamohan, T. (2012). Therapeutic effects of tender coconut water on oxidative stress in fructose fed insulin resistant hypertensive rats. *Asian Pacific Journal of Tropical Medicine*, 5(4), 270–276.

Cahyani, R., Serdiati, N., Tis'in, M., & Putra, A. E. (2021). Masculinization of Nile Tilapia *(Oreochromis niloticus)* Through Immersion in Coconut Water with Different Concentrations. *Agrisains*, 2, 89–97.

Chapman, F. A. (2000). *Culture of hybrid tilapia: A reference profile.* University of Florida.

Devlin, R. H., & Nagahama, Y. (2002). Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*, 208(3–4), 191–364.

Dikna, J. (2022). Morphology Of Worm Eggs Ascaris lumbricoides with Hematoxylin Eosin Stain. *Borneo Journal of Medical Laboratory Technology*, 5(1), 335–340.

Dwinanti, S.H., Putra, M. H., & Sasanti, A. D. (2018). Utilization of Coconut Water (*Cocos nucifera*) for Masculinization of Guppy Fish (*Poecilia reticulata*). *Jurnal Akuakultur Rawa Indonesia,* 6 (2), 117–122.

Falguera, V., Pagán, J., Garza, S., Garvín, A., & Ibarz, A. (2011). Ultraviolet processing of liquid food: A review: Part 2: Effects on microorganisms and on food components and properties. *Food Research International*, 44(6), 1580–1588.

Findayani, N., & Dina, M. (2022). Masculinization of Sangkuriang Catfish *(Clarias gariepinus)* Using Coconut Water with Different Soaking Durations. *Jurnal TROFISH*, 1(2), 79–84.

Ganong, W. F. (1983). Review of Medical Physiology, IIth ed. *Los Altos, CA: Lange Medical Publication.*

Hermana. (1991). Food Irradiation: A Method to Preserve and Enhance Food Safety. *Institut Teknologi Bandung*.

Hikmawandari, H., Ningsih, P., & Ratman, R. (2019). Determination of Potassium (K) in Green Coconut (*Cocos viridis*) from Dolo and Labuan areas

using Spectrophotometry. *Journal of Chemistry Academics*, 8(1), 34–37.

Himawan, A., Hastuti, S., & Yuniarti, T. (2018). The success of masculinization in rainbow fish (*Melanotaenia sp.*) at different stages through immersion in cow testis powder. *Journal of Aquaculture Management and Technology*, 7(1), 28–37.

Hunter, G. A., & Donaldson, E. M. (1983). 5 Hormonal sex control and its application to fish culture. *In Fish physiology* 9, 223–303.

Hutagalung, R. A. (2020). The Effect of Different *Sex Reversal* Methods Using Bull Testis Powder on Masculinization of Red Tilapia *(Oreochromis niloticus)*. *Manfish Journal*, 1(01), 9–14.

Ibrahim, S. (2020). The potential of young coconut water in increasing potassium levels. *Indonesian Journal of Nursing and Health Sciences*, *1*(1), 9–14.

Intan Kailaku, S., Nur Alam Syah, A., Risfaheri, R., Setiawan, B., & Sulaeman, A. (2015). Carbohydrate-electrolyte characteristics of coconut water from different varieties and its potential as natural isotonic drink. *International Journal on Advanced Science, Engineering and Information Technology*, 5(3), 174–177.

Islama., D., et., al. (2017). The Effect of Using Coconut Water with Different Concentrations on Masculinization and Survival Rate of Swordtail Platy Fish *(Xiphophorus helleri)*. *Jurnal Akuakultur Indonesia, 1–5.*

Jaya, R. S. (2015). Effect of Heating Temperature and Storage Duration on Changes in the Quality of Aren (*Arenga pinnata*). *Thesis.* Universitas Sumatera Utara.

Kailaku, S. I., Setiawan, B., & Sulaeman, A. (2016). Effects of Ultrafiltration and Ultraviolet Process on Nutritional Composition, Physicochemical and Organoleptic. *J. Littri*, 22 :43-51.

Kamala Devi, C. B., & Velayutham, M. (1978). Changes in the chemical composition of nut water and kernel during development of coconut. Proc First Annual Symp Plantation Crops, *PLACROSYM I, Nelliat EV (Ed), Indian Society for Plantation Crops, Kasaragod, Kerala,* 20–23.

Karlida, I., & Musfiroh, I. (2017). Storage Temperature of Raw Materials and Pharmaceutical Products in Pharmaceutical Industry Warehouse. *Farmaka*, 15(4), 58–67.

KKP. (2014). Decree of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number 52/Kepmen-KP/2014 Concerning the Classification of Fish Drugs. *Ministry of Marine Affairs and Fisheries.*

Langkong, J., Sukendar, N. K., & Ihsan, Z. (2018). Study on the Preparation of Isotonic Drinks Made from Old Coconut Water (*Cocos nucifera* L) and Star Fruit Extract (*Avverhoa bilimbi* L) Using Non-Thermal Sterilization Method During Storage. *Canrea Journal: Food Technology, Nutritions, and Culinary Journal*, 1(1), 53–62. https://doi.org/10.20956/canrea.v1i1.22.

Malik, T., Syaifudin, M., & Amin, M. (2019). Masculinization of guppies (*Poecilia reticulata*) through the use of coconut water (*Cocos nucifera*) with different concentrations. *Jurnal Akuakultur Rawa Indonesia*, 7(1), 13 – 24.

Martin., CR. (1979). *Textbook of Endocrine Physiologi*. Oxford University Press.

Masprawidinatra, D., Helmizuryani, H., & Elfachmi, E. (2015). The Effect Coconut Water for Different Submirsion Between Masculinization of Parrot Fish (*Oreochromis niloticus*). *Fiseries*, 4(1), 13–16.

Muchlisin, Z. A. (2016). A preliminary observation on the effect of sperm extenders on the fertilization and hatching rates of seurukan fish *(Osteochilus vittatus)* eggs. *AACL Bioflux*, 9(2).

Nakamura, M., Kobayashi, T., Chang, X., & Nagahama, Y. (1998). Gonadal sex differentiation in teleost fish. *Journal of Experimental Zoology*, 281(5), 362–372.

Nakano, L. A., Leal Junior, W. F., Freitas, D. G. C., Cabral, L. M. C., Penha, E. M., Penteado, A. L., & Matta, V. M. (2011). Coconut water processing using ultrafiltration and pasteurization. *Embrapa Agricultura Digital.*

Novitasari, T. A., Hidayati, S., & Armando, E. (2022). Masculinization of Guppy Fish *(Poecilia reticulata)* Through Broodstock Soaking Method Using Coconut Water with Different Concentrations. *Indonesian Journal of Tropical Aquaculture*, 7(1), 11–18.

Nurlina, N., & Zulfikar, Z. (2016). Effect of soaking guppy *(Poecilia reticulate)* broodstock in honey on the sex reversal ratio of guppies. *Acta Aquatica: Aquatic Sciences Journal*, *3*(2), 75–80.

Penman, D. J. (2004). Aquaculture and fisheries biotechnology: Genetic approaches. *Journal of Fish Diseases*. 27(11), 677-677. https://doi.org/10.1111/j.1365-2761.2004.00584.x

Perdana, I. P., Johan, I., & Hasby, M. (2022). Effect Of Using Hybrida Water (*Cocos nucifera*) With Different Doses On Masculinization Of Guppy Fish Larves (*Poecilia reticulata*). *Agricultural Dynamics*,

38(3), 333–342.

Pethiyagoda, P., De Alwis, S., & De Silva, B. (2021). Effect of selected water quality parameters on the prevalence of *Poecilia reticulata* (Guppy) population in Sri Jayewardenepura canal system. *International Journal of Multidisciplinary Studies*, *8*(2), 41–58.

Phelps, R. P., & Popma, T. J. (2000). *Sex reversal* of tilapia. *Tilapia Aquaculture in the Americas*, *2*, 34–59.

Piferrer, F. (2001). Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture*, *197*(1–4), 229–281.

Priyono, E. (2013). Masculinization of gapi (*Poecilia reticulata*) through immersion of pregnant broodstock in honey solution with different immersion durations. *Jurnal Akuakultur Rawa Indonesia*, 1(1), 13–22.

Puspitha, S. M. M. T. P., Prasetia, I. N. D., & Wulandari, D. (2023). Test of Effectiveness of Coconut Water Immersion with Different Concentrations on Masculinization of Guppy Fish (*Poecilia reticulata*). *Juveniles: Scientific Journal of Marine and Fisheries*, 4(3), 167–174.

Rachmawati, D., Basuki, F., & Yuniarti, T. (2016). The effect of different doses of cow testis powder on the success of masculinization in betta fish *(Betta sp.)*. *Journal of Aquaculture Management and Technology*, 5(1), 130–136.

Rakocy, J. E., & McGinty, A. S. (1990). *Pond culture of tilapia*. United States Department of Agriculture.

Rana, B., Kaushik, R., Kaushal, K., Arora, S., Kaushal, A., Gupta, S., Upadhyay, N., Rani, P., & Kaushik, P. (2018). Physicochemical and electrochemical properties of zinc fortified milk. *Food Bioscience*, 21, 117–124.

Renita, R. (2017). Effect of Temperature on Hatching Time, Egg Hatchability and Larval Survival of Cupang Fish (*Betta splendens*). *Skripsi*. Universitas Muhammadiyah Pontianak.

Rethinam, P. (2006). Asian and Pacific Coconut Community activities, achievements and future outlook. Aciar Proceedings, 125, 15.

Rizqa, M. N. (2009). *Reduction of COD, TSS and Color Levels of Liquid Waste of Sasirangan Textile Industry by Ozonation Method.* STTL : Yogyakarta.

Rosmaidar, R. (2016). The effect of Nile tilapia (*Oreochromis niloticus)* larval age on the increase in masculinization using natural Methyl Testosterone (MT) hormone. *Jurnal Medika Veterinaria*, 10(2).

Sari, D. A., & Hadiyanto, H. (2013). Food storage technology and methods as an effort to extend shelf life. *Journal of Food Technology Applications*, 2(2), 52-59

Sarida, M., Putra, D. D., & Marsewi, H. S. Y. (2011). Production of male monosex guppies (*Poecilia reticulata*) by immersion of pregnant mothers and larvae in propolis at various dose levels. *Zoo Indonesia*, 20 (2).

Sary, R., & Zainuddin, R. E. (2017). Histological structure of female cork fish (*Channa striata*) gonads. *Journal of Veterinary Student Science*, 1(3), 334–342.

Sektiana, S. P., Rahardjo, S., & Zulendra, M. F. (2023). Improvement Of Guppy (*Poecilia reticulata*) Fish Production Using Coconut Water (*Cocos nucifera*). *Media Akuakultur*, 17(2), 53–58.

Siregar, A., Syaifudin, M., & Wijayanti, M. (2018). Masculinization of Cupang Fish (*Betta splendens*) Using Natural Honey Through Immersion Method. *Journal of Indonesian Swamp Aquaculture*, 6(2), 141–152.

SNI. (1996). *Bottled Coconut Water.* National Standardization Agency (BSN). Jakarta.

SNI. (1998). *Isotonic Drinks*. National Standardization Agency (BSN). Jakarta.

SNI. (2009). *Maximum limit of microbial contamination in food*. National Standardization Agency (BSN). Jakarta.

Soelistyowati, D. T., Martati, E., & Arfah, H. (2007). Effectiveness of honey on sex-direction of gapi (*Poecilia reticulata* Peters). *Indonesian Journal of Aquaculture* 6(2), 155–160.

Soelistyowati, D. T., Sudrajat, A. O., & Arfah, H. (2010). Masculinization on tilapia (*Oreochromis* sp.) by natural hormone steroid agent from bee resin through artificial diet. *Jurnal Akuakultur Indonesia*, 9(2), 178–183.

Strüssmann, C. A., Karube, M., Miranda, L. A., Patiño, R., Somoza, G. M., Uchida, D., & Yamashita, M. (2005). Methods of sex control in fishes and an overview of novel hypotheses concerning the mechanisms of sex differentiation. *Fish Genetics and Aquaculture Biotechnology*, 65–79.

Sudrajat, A. O., & Sarida, M. (2006). Effectivity of aromatase inhibitor and 17á-metiltestosteron treatments in male production of fresh water prawn (*Macrobrachium rosenbergii* de Man). *Aquacultura Indonesiana*, 7 (1), 61–67.

Suharyono, A. S., K, M. E., & Kurniadi, M. (2009). Effect Of Ultra Violet Light And Duration Of Stor-

age On The Microbiological Properties And Rancidity Of Coconut Milk Cream. *Agritech,* 29(3), 174–178.

Sulistyo, D. G., Susilowati, T., & Windarto, S. (2021). The Effect of Immersion Doses of Hybrid Coconut Water on Guppy (*Poecillia reticulata*) to Increase the Male Larvae Percentage. *Indonesian Journal of Tropical Aquaculture,* 5 (1), 34-40.

Sulistyorinie, D. R., Andayani, S., & Suprastyani, H. (2020). Effect of Api-Pi (*Avicennia marina*) Leaf Coarse Extract on Liver Histopathology of Koi Fish (*Cyprinus carpio*) Infected with *Aeromonas hydrophilla* Bacteria. *Journal of Fisheries and Marine Research*, 4(2), 263–273.

Sulystyaningsih, N. D., Turmuzi, T., WSK, L. A. T. T., Muahiddah, N., & Sumahiradewi, L. G. (2022). The Effectiveness Of Coconut (*Cocos nucifera*) Soaking On The Masculinity Of Guppy Fish (*Poecilia reticulata*). *Agroqua Journal: Agronomy and Aquaculture Information Media*, 20 (2), 320–328.

Susanty, S., & Yudistirani, S. A. (2019). Efficacy of Coconut Water Vinegar as an Inhibitor of Microorganism Development in Indonesian Captured Fish. *Prosiding Semnastek*.

Takahashi, H. (1975). Rmctional Masculinization of Female Guppies, *Poecilia reticulata*, Influenced by Methyltestosterone before Birth. *Bulletin of the Japanese Society of Scientific Fisheries*, 41(5), 499–506.

Tatalede, P. A., Sinjal, H. J., Watung, J. C., Salindeho, I. R. N., & Kalesaran, O. J. (2019). Masculinization of Nile Tilapia *(Oreochromis niloticus)* through the Administration of Propolis Mixed in Artificial Feed. *E-Journal Budidaya Perairan*, 7(2).

Taufiqurahman, W., Yudha, I. G., & Damai, A. A. (2017). Effectiveness of different natural feeding on the growth of pond fish fry *Helostomma temminckii* (Cuvier, 1829). *E-Journal of Aquaculture Engineering and Technology*, 6(1), 669–674.

Tave, D. (1995). *Selective breeding programmes for medium-sized fish farms*.French : FAO.

Tranggono dan Sutardi. (1990). Biochemistry and Post-Harvest Technology. *Gajah Mada University Press.*

Triastarani, A. P. (2021). Effect of ultraviolet (UV) light exposure to minimize damage and antioxidant activity in salak fruit (*Salacca edulis* reinw). *Maulana Malik Ibrahim State Islamic University.*

Yamamoto, T.O. (1969). 3 Sex differentiation. *Fish Physiology*, 3, 117–175.

Yamazaki, F. (1983). Sex control and manipulation in fish. *Aquaculture*, 33(1–4), 329–354.

Yong, J. W. H., Ge, L., Ng, Y. F., & Tan, S. N. (2009). The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. *Molecules*, 14(12), 5144–5164.

Zairin, M. (2002). *Sex reversal : producing male or female fish*. Penebar Swadaya.

