Masculinization of betta fish (*Betta splendens*) through natural honey immersion with different concentration

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Abstract. Male betta fish is more popular and expensive compared to female betta fish because it has a more beautiful body shape and color. One effort to increase the population of male fish is the method of masculinization to reverse the sex of fish into males, so that the profit value is higher. Generally, sex directives or sex reversal usually use the steroid hormone: 17α-metilestosterone (MT) by the method: immersion, injection or orally through feed. However, this MT hormone has side effects which can cause pollution, liver damage in test animals and can cause mortality. This study was conducted to determine the survival rate of larvae and increase the percentage of male betta fish through natural honey immersion with different concentrations. The method used in this study was a laboratory experiment, using 3 treatments namely A (5ml/L), B (6ml/L), C (7ml/L) and 3 replications. Betta fish larvae as test animals are 7 days old, with natural honey immersion for 12 hours and then reared for 60 days. The results of this study indicate that the survival rate of betta fish larvae after honey immersion treatment (12 hours) averaged 100% and the percentage of survival of betta fish during rearing (60 days) ranged from 25.55 - 30.00%. And the average sex percentage of male sex larvae in the treatment of natural honey immersion is 5 ml / L (85.00%), 6 ml / L (95.24%) and 7 ml / L (100%). Thus it can be concluded that proper honey concentration plays an important role in the process of masculinization.

1. Introduction

One of the main commodities of ornamental fish that has the potential to be developed in Indonesia is Betta fish (*Betta splendens*) because it is a top 5 commodity in the world export market and is always experiencing quite rapid development from year to year [1]. Some betta fish have a price difference between male and female, generally male fish are higher in price than female fish. This is due to several advantages possessed by male fish both from the morphology and color that become aesthetic value. The problem found in betta fish culture is the sex ratio, where the number of male betta fish is always less than female betta, as reported by previous studies, only 40% male obtained while female 60%. One way to increase the population of male fish is to do masculinization that directs the sex of the fish into male, so that the profit value becomes higher [2].

The use of natural ingredients for masculinization to increase male fish population has been done either through feed or immersion. Several natural ingredients that have been used in the masculinization of Betta fish have been reported by several researchers where they used various methods, such as: Masculinization of Betta fish by immersing the embryo in purwoceng extracts was
successful in increasing the percentage of male fish 62.66% [3]. Then, masculinization of betta fish using extracts of sea cucumber (Holothuria scabra) succeeded in increasing the male sex ratio by 66.66% [2]. And also the use of purwoceng plants through artemia immersion succeeded in increasing the percentage of male betta sex ratio by up to 75% [3]. In addition, honey propolis which is used in masculinization of Betta fish with immersion time of 24 hours can produce a percentage of male fish up to 69.94% [4].

Natural honey contains chrysin which is known as a type of flavonoid and is recognized as an aromatase enzymes or better known as aromatase inhibitors. Besides that, natural honey is environmentally friendly and high mineral content, especially potassium. Potassium can function as a driver of fish sex differentiation through modulation of testosterone circulation, and control of androgen action [5]. Referring to previous studies which stated that the lethal limit of natural honey concentration in masculinization of betta fish was 7 ml /L for 12 hours of immersion for 5 days old larvae (D5), whereas in this study using betta fish larvae aged 7 days (D7) [6].

The purpose of this study was to determine the population of male betta fish in masculinization through natural honey immersion with different concentrations. This research was conducted from February to August 2019, and located in the Aquaculture laboratory, Faculty of Fisheries and Marine Sciences, Pattimura University, Ambon.

2. Material and methods
Betta fish larvae of 5 days (D5) as test animals were taken from freshwater fish hatcheries in Latuhalat, Ambon, taken to the cultivation laboratory, the Faculty of Fisheries and Marine Sciences, Pattimura University. The larvae were acclimatized in 50 L fresh water for 2 days in 2 glass aquariums (30x40x45 cm), equipped with aerators. The method used in this study was a laboratory experiment, using 3 treatments and 3 replications. The treatments are betta larvae immersion in natural honey with different concentrations, namely A (5ml/L), B (6ml/L), C (7ml/L) treatment and control (without natural honey).

After acclimatization for 2 days, 7 days (D7) betta fish larvae, larvae are put into the treatment container, which is a plastic jar (vol. 5 L) that has been filled with 3 L treatment media with a larvae density of 30 ind./treatment, thus the number of larvae that are used in this study as many as 400 individuals including controls. Larvae immersion in natural honey for 12 hours, after that the larvae are reared for 60 days. At the beginning of larval rearing, egg yolks are fed as food, then artemia and mosquito larvae during reared, and given ad libitum. Siphoning and water exchanges were once a day in the morning before data sampling. The water was replaced 50% for the all treatments.

2.1. Data collection
The variables measured in this study were the survival rate and the percentage of male and female fish. Water quality data measured are temperature, DO and pH.

Survival Rate (SR). Survival is the percentage of fish that live at the end of rearing from the total number of fish larvae at the beginning of rearing in a container with the formula according to [7], as follow:

\[ SR(\%) = \frac{\text{Number of fish at the end research}}{\text{Number of fish at the beginning research}} \times 100 \]  \hspace{1cm} (1)

Percentage of male and female fish. To calculate the percentage of male and female fish can be done using the formula according to [4], as follows:

\[ \text{Male fish} (M)(\%) = \frac{\text{Number of male fish}}{\text{Number of samples}} \times 100 \]  \hspace{1cm} (2)

\[ \text{Female fish} (F)(\%) = \frac{\text{Number of female fish}}{\text{Number of samples}} \times 100 \]  \hspace{1cm} (3)
2.2. Data analysis
Data on survival rates (SR) and the number of male and female fish obtained were calculated and displayed in tables and graphs, then explained descriptively.

3. Result and discussion
3.1. Survival rate (SR) of Betta splendens larvae
The survival rate (%) of the early of betta fish larvae rearing after natural honey immersion show a good result (100%) for both treatments and controls (see Figure 1).

![Figure 1. Survival rate (%) of B. sendendens larvae in natural honey immersion treatment for 12 hours](image1)

The result shows that different treatment of honey concentration have no effect on the survival rate of betta fish larvae. While the survival rate of betta fish larvae during reared (60 days) ranges from 25.55 - 30% (see Figure 2).

![Figure 2. Survival of B. splendens larvae during larvae rearing (60 days)](image2)

It can be seen that survival rate of betta fish larvae in the controlled media (average 30%) is better than in the medium mix with natural honey of 5ml (mean=27.77%), 6ml (mean= 25.55%) and 7ml (28.88%). The average survival rate of betta fish larvae is less than 35%. The number of fish that survive in the control media is 9 fish and 21 mortality. The immersion larva in the media with the concentration of 5ml/L natural honey only 8 fish survived and 22 mortality; in. 6ml/L natural honey concentration only 7 fish survived and 23 mortality; and for 7ml/L natural honey concentration, only 9 fish alive while 21 mortality. The low survival rate post immersion of Betta fish larvae influenced by internal and external factors. Internal factors such the organ development of larvae and the gill maze formation resulting for their low survival rate. Some external factors that must be considered are natural feeding methods that are suitable with the size of mouth opening of fish larvae, food
availability in the growth media, routine siphoning of the faces and food for maintaining the clean water off the access food or faces.

3.2. Percentage of male and female Betta splendens fish
The immersion of betta fish larvae in the medium without natural honey (control) produces 25% males and 75% females (see Figure 3). The immersion of betta fish larvae in the 5ml/L media (treatment A) produces 85% males and 15% females (see Figure 4). The immersion of betta fish larvae in the 6 ml/L media (treatment B) produces 95.24% males and 4.76% females (see Figure 5). The immersion of betta fish larvae in the 7 ml/L media (treatment C) produces 100% males and 0% females (see Figure 6).

Figure 3. The sex percentage of Betta splendens larvae in the controlled media during rearing (60 days)

Figure 4. The sex percentage of Betta splendens larvae at 5ml/L media (treatment A) during rearing (60 days)
Figure 5. The sex percentage of *Betta splendens* larvae at 6ml/L media (treatment B) during rearing (60 days)

![Graph showing sex percentage of Betta splendens larvae at 6ml/L media (treatment B).]

Figure 6. The sex percentage of *Betta splendens* larvae at 7ml/L media (treatment C) during rearing (60 days)

![Graph showing sex percentage of Betta splendens larvae at 7ml/L media (treatment C).]

3.3. Percentage of male and female *Betta splendens* fish

The highest average percentage of betta fish larvae found in the immersion of larva in the medium that mixed with 7ml natural honey (33%), followed by 6 ml natural honey (31%), and then the treatment with 5ml natural honey (23%). The lowest percentage is found in the controlled media (8%) (see Figure 7). It shows that the higher the concentration of natural honey, the more male larve of betta fish is produced, because natural honey contains a chrysin compound that functions as a natural aromatase inhibitor and is gives an impact on the sex changes to become a male.

![Pie chart showing percentage of male sex from Betta splendens larvae for all treatments during observation.]

Figure 7. Percentage of male sex from *Betta splendens* larvae for all treatments during observation
Natural honey contains a chrysin compound that functions as a natural aromatase inhibitor [8]. In addition, aromatase inhibitors can cause a decrease in estrogen concentration which leads to inactivity in the transcription of the aromatase gene [9]. Decreased estrogen concentration, resulting in a large amount of the testosterone which will then direct genitals into males. Larvae that experience low aromatase activity will lead to the formation of the testicles, on the other hand larvae that experience high aromatase activity will lead to the formation of ovaries [8]. Thus, an effective dose is needed for the masculinization process, because the effective dose needed is not the same for all fish. High doses and too long immersion time will also be paradoxical that the results obtained are not an increase in the number of male fish, instead they will increase the number of female fish [9].

The high percentage of male betta fish obtained is thought to be due to the diffusion of chrysin contained in the natural honey solution into the fish body during immersion. The content of chrysin will also inhibit aromatase activities which results in more testosterone content compared to the estradiol hormone [10]. In the process of steroidogenesis in cells, the formation of estradiol from testosterone conversion due to the presence of the aromatase enzyme will be inhibited because of the presence of chrysin which acts as an aromatase inhibitor. Ultimately, the process of steroidogenesis ends in the formation of testosterone which will stimulate the growth of male genital organs and cause male secondary sex characteristics. In addition, Marti states that natural honey will enter by diffusion into the bloodstream and reach the target organ [11].

In the study, Betta fish larvae treated were 7 days after hatching and during immersion, the larvae movement is as active as before immersion. The survival rate of larvae is very influential on the success of changing the sex of the betta fish larvae. It is assumed that the survival rate of fish larvae is influenced by external factors such as space, competition, water quality, quantity of feed and improper handling especially when sampling. In this natural honey immersion method is expected to enter the fish's body through a diffusion process. Because in the larval phase, permeable walls of the larvae are generally still thin so it is expected that the process of diffusion of natural honey into the body will take place easily. Sex differentiation in fish is a relatively unstable process compared to higher vertebrates, and this condition allows for genital engineering. And to become male or female in the process of sex differentiation in vertebrate animals, depending on the presence or absence of the hormone testosterone in early development of the animal [12]. If there is testosterone, the gonads will differentiate into testicles and vice versa, if there is no testosterone, the gonads will develop into ovaries [13]. Increasing the percentage of male betta fish with natural honey immersion in this study shows that the material plays an important role in changing the direction of betta fish sex differentiation.

The success of natural honey as a natural material for masculinization that can produce 100% of male betta fish in the study occurred in immersion with 7 ml concentration. This is presumably because the concentration of natural honey given if the higher the betta fish larvae are 7 days will affect the sex changes of the male betta fish larvae. The use of natural honey as a natural and environmentally friendly material is able to produce high male betta fish population.

4. Conclusions
From the results of this study it can be concluded that the media of natural honey immersion which is good for producing high Betta splendens fish populations is a media with a concentration of 7ml/L and has a higher survival rate (28.88%) compared to other concentrations. The highest population of male Betta spendens produced from masculinization through natural honey immersion with a concentration 7ml/L for 12 hours with 7 days larvae is 100% compared to other media concentrations even though it has a survival rate of less than 50%. Further research should be done to focus on the functional (phenotype) of betta fish produced by the masculinization process using natural honey.
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Acknowledgements

The authors would like to acknowledge the support of Dean of Faculty of Fisheries and Marine Science, Prof. Dr. rer.nat. Ir. Abraham Samuel Khouw, M.Phil., and Head of Aquaculture Department Ir. Joice W. Loupatty, M.Si which has provided the opportunity for this research.