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Research Article

Masculinization of Banggai Cardinalfish (*Pterapogon kauderni*) using 17α-Methyltestosterone-enriched *Artemia* sp.

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ABSTRACT

Banggai Cardinalfish (*Pterapogon kauderni*) is one of the endemic fish from the waters of the Banggai Islands, Central Sulawesi Province. One factor challenging the production of *P. kauderni* larvae is the male fish will nurture the eggs and larvae for 30 days, hindering next spawning cycle. One effort to overcome this problem an increase of male fish number should be performed, for example through sex reversal. This study aimed to determine the effectiveness of the hormone 17α-methyltestosterone (17α-MT) through *Artemia* sp. to increase male sex percentage *P. kauderni*. This study used a completely randomized design by testing four treatments namely feeding *Artemia* sp. which has been soaked with 2.0 mg of the 17α-MT hormone for two- hours given to *P. kauderni* larvae for 10 days (A); 20 days (B); 30 days (C), and without hormone treatment 17α-MT (control). Each treatment included control was performed in triplicates. The percentage of male sex was 93.33 % at 30 days treatment and was higher ($P<0.05$) compared to controls (53.3 %). Additionally, the results showed that daily growth rate, biomass gain and survival rate of all treatments were not significantly different ($P>0.05$) with those of control. In conclusion, *Artemia* sp. contained 17α-methyltestosterone enhanced male percentage of *P. kaurderni*.

Keywords: growth, male percentage, *Artemia* sp., survival rate, *Pterapogon kauderni*

ABSTRAK

Banggai Cardinalfish (*Pterapogon kauderni*) merupakan salah satu ikan endemik yang berasal dari perairan Kepulauan Banggai, Provinsi Sulawesi Tengah. Salah satu faktor yang menghambat produksi larva *P. kauderni* adalah ikan jantan akan memelihara telur dan larva selama 30 hari, sehingga menghambat siklus pemijahan berikutnya. Salah satu upaya untuk mengatasi masalah tersebut adalah perlu dilakukan peningkatan jumlah ikan jantan, misalnya melalui sex reversal. Penelitian ini bertujuan untuk mengetahui efektivitas hormon 17α-methyltestosterone (17α-MT) melalui *Artemia* sp. dalam meningkatkan persentase jenis kelamin jantan *P. kauderni*. Penelitian ini menggunakan Rancangan Acak Lengkap dengan menguji empat perlakuan yaitu pemberian pakan *Artemia* sp. yang telah direndam dengan 2,0 mg hormon 17α-MT selama dua jam diberikan pada larva *P. kauderni* selama 10 hari (A); 20 hari (B); 30 hari (C), dan tanpa terapi hormon 17α-MT (kontrol). Setiap perlakuan termasuk kontrol dilakukan dalam tiga ulangan. Persentase jenis kelamin jantan adalah 93,33% pada perlakuan 30 hari dan lebih tinggi ($P<0,05$) dibandingkan dengan kontrol (53,3%). Selain itu, hasil penelitian menunjukkan bahwa laju pertumbuhan harian, perolehan biomassa dan laju kelangsungan hidup semua perlakuan tidak berbeda nyata ($P>0,05$) dengan kontrol. Kesimpulannya, *Artemia* sp. mengandung 17α-methyltestosterone meningkatkan persentase *P. kaurderni* jantan.

Kata kunci: Pertumbuhan, Persentase jantan, *Artemia* sp. Kelangsungan hidup, *Pterapogon kauderni*

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1. Introduction

Banggai Cardinalfish (*Pterapogon kauderni*) is one of the endemic fish of the waters of the Banggai Islands, Central Sulawesi Province (Hopkins et al., 2005; Vagelli, 2011; Safir et al., 2020). The body morphology and behavior that is classified as unique from *P. kauderni* (Ndobe et al., 2013b) make it one of the marine ornamental fish sought by ornamental fish lovers, in Indonesia and the other countries (Lunn and Moreau, 2004). This is an advantage for the people of Banggai Islands in providing *P. kauderni* on the market. But on the other hand it will be a threat to the preservation of *P. kauderni* if it still relies on catches from nature.

One effort that can be done to maintain availability of Banggai Cardinalfish in market is through artificial spawning for seed production. Successfully controlled *P. kauderni* spawning was carried out by Gunawan et al. (2010), by applying an equal male and female ratio (1:1), a density of 20 fish *P. kauderni* reared at 150 L^{-1} of water with a water quality temperature of 27-28 ºC, pH 7-8, dissolved oxygen ± 6 mg L^{-1}. Furthermore, the study also reported that during the spawning process *P. kauderni* always occupied in a quiet area together with its partner. In addition, after the spawning process, the male parent then incubates the eggs in the oral cavity until the larvae are released (ranging from 29 to 30 days) (Hopkins et al., 2005; Gunawan et al., 2010; Ndobe et al., 2013b).

During the incubation process, the male did not consume food and causes shrinkage of body weight, as occurs in the brood stock female tilapia (Safir et al., 2017). This will certainly have an impact on the spawning cycle in the next stage and make the time longer because the male needs time to recover physiologically. While the female, after releasing an egg, will undergo a process of rematuration until its ready for spawning in the next period (Gunawan et al., 2010).

Naturally, after the male parent incubates and removes larvae from the mouth, the male will do the re-spawning process as early as five days after the release of larvae from the mouth (Hopkins et al., 2005). Spawning in these conditions, have an impact on the lower quality of seeds. On the contrary, the occurrence of spawning in a long period of time will cause less available seeds. Therefore, one of the efforts that can be done to accelerate spawning and continue to produce high quality of seeds is through providing high number of ready spawning male *P. kauderni*.

Sex reversal using the hormone 17α-methyltestosterone (17α-MT) is one way that can be done to produce male androgynous fish (Chakraborty et al., 2011; Safir et al., 2017). The mechanism of this hormone is to inhibit the activity of aromatase in the formation of androgen hormones into estrogen, so that androgen levels are higher and cause phenotypic changes in test fish so that it resembles males as occurs in tilapia (Zairin, 2002; Safir et al., 2017). One method that can be used in the application of the 17α-MT hormone is through artificial feed (Carman et al., 2008; Safir, 2018). However, studies related to the 17α-MT hormone in *P. kauderni* are relatively new. In addition, *P. kauderni* tends to be passive and in the larval phase prefers natural feed, such as rotifer crustacean larvae including rebon shrimp (*Acetes* sp.) and *Artemia* sp. (Vagelli and Erdmann, 2002; Ndobe et al., 2013a; Safir et al., 2020). Therefore, the reversal sex method that will be applied to *P. kauderni* larvae through natural food (*Artemia* sp.). As a first step in this study, effectiveness of the 17-MT hormone -enriched *Artemia* sp. with different dietary duration of time to produce male *P. kauderni* larvae was examined.

2. Materilas and Methods

2.1. Time and Place

The study was conducted in December 2019 until January 2020. The location of the research was in the LINI Foundation Field Laboratory, and the Fish Quarantine Office, Banggai Laut Regency, Central Sulawesi Province

2.2. Test Organisms

The test organism used was *P. kauderni* larvae aged three days after release from the mouth of a male parent (weight 0.023 ± 0.024 g; length 1.02 ± 0.13 cm). Larvae was obtained from brood stock eggs caught in nature. Furthermore, maintenance was done in a controlled tub until the parent releases the larvae perfectly.

2.3. The Experiment

The study was designed using a completely randomized design by applying four treatments. The treatment tested was feeding

Artemia sp. which has been soaked with 2.0 mg of the hormone 17α-MT for two-hours and given to *P. kauderni* larvae for 10 days (A); for 20 days (B); for 30 days (C), and without hormone treatment 17α-MT (control). Each treatment was given three replications.

2.4. Maintenance of the Fish Larvae

A total of 10 larvae *P. kauderni* seeds were included in each maintenance container that was equipped with a hose and aeration stone to supply oxygen and microhabitat of sea urchins (*Diadema setosum*). *Artemia* sp. which has been soaked with the 17-α-MT hormone, at each treatment time (for 10 days (A); for 20 days (B); for 30 days (C) and control), after the fish have been adapted to the environment and feed (*Artemia* sp.) for two days. Feeding time was done four times a day (morning, afternoon, evening and night) with *at satiation*. After the end of the treatment feeding period, continued with *Artemia* sp. without 17α-MT hormone immersion until the end of maintenance. To maintain the quality of water suitable for the maintenance of *P. kauderni*, a recirculation system was used and water was changed every two days as much as 50 % of the volume of maintenance media.

2.5. Observation Parameters

The parameters observed in the study included: daily growth rate measured every two weeks according to the method used by Safir et al., (2017). Furthermore, biomass gain and survival rate was measured at the beginning and end of maintenance by referring to the method used by Safir (2018). Whereas male sex ratio was measured by referring to the method used by Chakraborty et al. (2011) and Safir (2018). The sex samples observed were only five fish for each treatment because of endemic fish. Observations were carried out using the acetocarmine method (Chakraborty et al., 2011; Safir et al., 2017) and observed under a microscope (Primo star brand magnification 40x).

2.6. Data Analysis

All data obtained were analyzed using analysis of variance (ANOVA) at an error level of 5 %. If there was a different effect, then further tests was done using the Duncan test.

3. Results

3.1. Daily Growth Rate

Daily growth rate (DGR) for all treatments is presented in Table 1. The average DGR for each treatment was 5.38 % (control; 0 days), 5.95 % (10 days), 5.56 % (20 days) and 5.97 % (30 days). Results of analysis of variance showed that DGR in all treatments did not show significant differences (P>0.05).

3.2. Biomass Gain

Biomass gain (BG) in *P. kauderni* feed *Artemia* sp. which has been soaked with the 17α MT hormone ranging from 0.93-1.17 g. The highest biomass growth was obtained at 10 days treatment feeding treatment (1.17 ± 0.36 g) and the lowest occurred at the control (0.93 ± 0.11 g). However, there was no statistically significant difference between all treatments (P>0.05) (Table 1).

3.3. Survival Rate

The survival rate of *P. kauderni* obtained at the end of maintenance for all treatments ranged from 73.33-86.67%. The treatments which had lowest to highest survival rate were control (73.33 ± 11.55), 20 days (76.67 ± 5.77), 10 days (83.33 ± 5.77) and 30 days (86.67 ± 5.77) feeding which has been soaked with the hormone 17α-MT. Based on the analysis of variance (ANOVA), the value did not show a significant difference (P>0.05) (Figure 1).

Table. 1. Daily growth rate (DGR) and biomass gain (BG), Banggai Cardinalfish (*P. kaurdeni*) feed at different duration time with *Artemia* sp. which soaked at 17α-MT hormone.

Parameters	Duration of feeding *Artemia* sp. which soaked in 17α-methyltestosterone (17α-MT) hormone			
	control (0 days)	10 days	20 days	30 days
DGR (%)	5.38±0.30	5.95±0.82	5.56±0.48	5.97±0.47
BG (g)	0.93±0.11	1.17±0.36	1.00±0.18	1.16±0.20

Explanation: values are expressed as mean ± standard deviation (n = 3). All treatments did not show a significant difference (P<0.05).

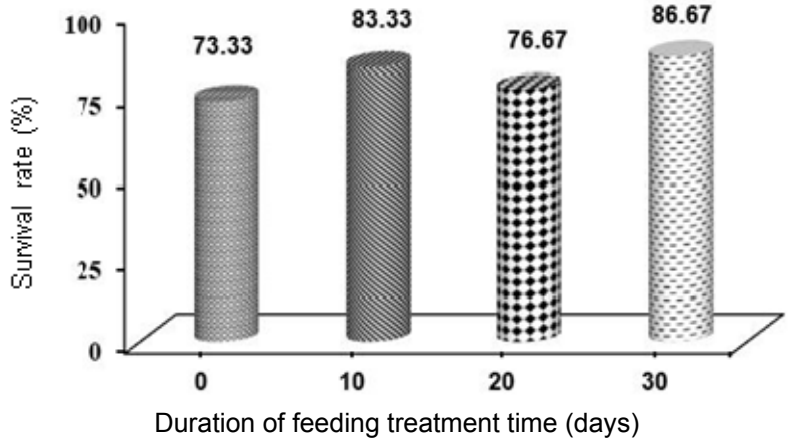

Figure 1. Survival rate of Banggai Cardinalfish (*P. kauderni*) at the end of maintenance

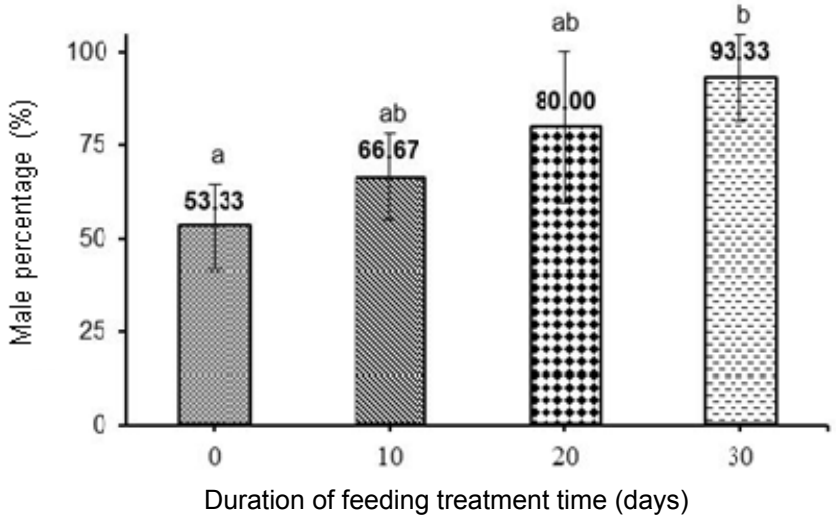

Figure 2. Percentage of male sex of Banggai Cardinalfish (*P. kauderni*) at the end of maintenance.

3.4. Percentage of Male

The percentage of male sex in *P. kauderni*, is increasing with the longer in the time of feeding *Artemia* sp. which has been soaked with the 17α-MT hormone. The percentage of male sex obtained at the end of maintenance is control treatment (0 days); 10 days; 20 days and 30 days in a row ie 53.5 ± 11.55 %; 66.67 ± 11.55 %; 80.00 ± 20.00 %, and 93.33 ± 11.55 %. The analysis showed that the duration of treatment feeding (control, 10 days and 20 days) did not show a significant difference (P>0.05). However, feeding treatment for 30 days showed a higher percentage (P<0.05) compared to other treatments (Figure 2).

4. Discussion

Daily growth rate of *P. kauderni* treated with *Artemia* sp. which had been soaked with the 17α-Methyltestosterone (17α-MT) hormone for all treatments was not significantly different (P>0.05) from the control (Table 1). Similar results have also been reported by Safir et al. (2017) using tilapia larvae aged 10 and 14 days soaked with the 17α-MT hormone for 4 hours and kept for six weeks. In another study, using the oral method on tilapia larvae aged 16 days with a duration of 28 days and kept for two months (Safir, 2018). Daily growth rate did not have different for all treatments with controls in this study was thought to be caused by the 17MT hormone in *P. kauderni* which did not have an anabolic effect. This is in accordance with what some researchers report that the anabolic effect (such as growth performance) from 17α-MT hormone treatment has not shown significant differences before fish experience gonad maturation (Celik et al., 2011, Safir et al., 2017; Safir, 2018). Those not different between all treatments, can directly affect the increase of

biomass gain. This is due, daily growth rate and biomass gain are positively correlated (Safir et al., 2017). Where the higher daily growth rate will directly increase the BG of fish treatment. This can be seen from the results of this study (Table 1), where the values of daily growth rate and biomass gain both show no significant difference from each treatment. Similar results have also been reported by Safir et al. (2017) and (Safir, 2018) in tilapia.

In addition, the results of this study also showed survival rate at the end of maintenance for all treatments did not show a significant difference (P>0.05) with control. Similar results have also been reported by Afpriyaningrum et al. (2016); Safir et al. (2017); Safir (2018), respectively in tilapia. From these results prove that the treatment of the hormone 17α-MT through immersion in natural food (*Artemia* sp.) In this study, did not affect the survival rate of test fish. However, the low survival in all treatments is thought to be caused by other technical factors such as poor handling during maintenance (sampling and water change), because these fish besides having slow movements also look sensitive. Syazili et al. (2012) and Safir (2018), revealed that one of the factors that caused the low survival of the treated fish was the poor handling of the fish, both during sampling and during water changes during the maintenance process.

The percentage of *P. kauderni* males shows a value that increases with increasing time of treatment feed (Figure 2). Increasing the percentage of males in treated fish strengthens the asumtion that 17α-MT hormone treatment can have androgenic effects on *P. kauderni*. Kwon et al. (2000) stated that the mechanism of the 17α-MT hormone in providing androgenic effects through increasing the percentage of male fish treated by inhibiting aromatase activity (Cyp19a1) in producing estrogen from androgens that occur in steroidogenic cells in the brain (larval phase), and in stromal cells near the virgin (phase when in the process of differentiation) (Kobayashi and Nagahama, 2009). These conditions cause higher androgen hormone levels and cause changes in phenotypic characters to be like a male. *P. kauderni* is suspected to occur in stromal cells in the area of blood vessels, because the relatively long differentiation period is illustrated by the percentage of males produced (93.33 %) in the 30-day treatment of 17α-MT hormone treatment. Similar results were reported by Safir (2018) on 16-day-old tilapia larvae treated with 17α-MT hormone through feed with a duration of 0, 14, 21 and 28 days and kept for six weeks yielding a percentage of males 47 %, 13 %; 71.01 %; 78.56 % and 81.00 %. Rosmaidar et al. (2014) added that one of the factors influencing the success of sex changes was the duration of hormone treatment because it was related to the sex differentiation period of test fish. The exact time of treatment with the time of differentiation will increase the percentage of males produced. This is illustrated in the 30 days treatment of treatment feed.

5. Conclusion

Reversal sex treatment using the hormone 17α-Methyltestosterone through *Artemia* sp. given to *P. kauderni* larvae is proven to increase the percentage of males by 93.33% (30 days treatment of administration). The daily growth rate, biomass growth and viability were not significantly different for all treatments. The shortcomings of this study, using the number of 10 fish test fish and observing the sex of 5 fish each of each repetition (related to endemicity).

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