

STUDY ON THE MASCULINIZATION OF
FALSE CLOWNFISH (*Amphiprion ocellaris*) USING
17 α - METHYLTESTOSTERONE

MUHAMMAD ABDUH BIN YAZED

MASTER OF SCIENCE
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January 2010

Supervisor: *Prof. Dr. Yusoff Yusoff* (Ph.D.)

Co-supervisor: *Prof. Dr. Yusoff Yusoff* (Ph.D.)

Department: *Institute of Tropical Aquaculture*

MUHAMMAD ABDUH BIN YAZED

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**STUDY ON THE MASCULINIZATION OF FALSE CLOWNFISH
(*Amphiprion ocellaris*) USING 17 α -methyltestosterone.**

MUHAMMAD ABDUH BIN YAZED

January 2010

Chairperson : Associate Professor Abol Munafi Ambok Bolong, Ph.D.

Member : Prof Mohd Azmi Ambak, Ph.D.

Institute : Institute of Tropical Aquaculture

False clownfish (*Amphiprion ocellaris*) is the most common marine ornamental species that is popular among hobbyist. However, the production of this species in captivity is still low. Most of the broodstocks are wild-caught and the F1 generations failed to breed in captivity. Hormone treatment has been used widely to produce monosex population in fish species. The main objective of this project is to determine if hormone can be used to masculinize this fish. Therefore, in order to achieve this objective, several studies were conducted to study the effects of: (i) immersion and oral administration of 17 α -methyltestosterone in producing male fish, (ii) pairing and non-pairing of 17 α -methyltestosterone treated fish on gonad structure of *A. ocellaris*.

Juveniles of *A. ocellaris* (3.5cm-5.5cm) were immersed in different concentration of MT for 15 days and then kept for 2 months for further

observations. The concentrations were 1 ppm, 2 ppm and 4 ppm where for control the concentration is set at 0 ppm. Three fishes were sampled twice, at the end of the experiment and after 2 months for gonad histology. It is possible to achieve masculinization in this species when the androgen MT is applied using the immersion method at juveniles stages. The best concentration was 1 ppm due to the high survival rate and the existence of testicular cells even after 2 months post-MT exposure compared to other concentrations.

For the oral administration of MT, the fish were treated with 30, 60 and 120 mg/kg MT with one group of control which were fed with diet sprayed with only ethanol. Three fishes were sampled three times, at the end of the experiment, 1 month post treatment and 2 months post treatment for gonad histology. Oral administration of MT to *A. ocellaris* juveniles might give inconsistent results because factors of food consumption and the delivery of hormones. It is possible that the conversion of MT to 17 α -methyleneestradiol is the reason for the feminization of the fishes and estrogen synthesizing process done by aromatase enzyme to the androgen particle.

The third experiment was conducted to study the effect of stocking method on treated fish. The fish were treated using immersion method and treated with 1 ppm of MT. At the end of the experiment, 10 of them were separated into 10 different aquariums while the rest were paired and put into 5 aquariums. Sampling was done twice, 1 and 2 months post hormone treatment. After 1

month post-exposure to 15 days of MT, the ovotestes from the single fish (α) and the smaller from the paired fish (β), possessed all stages of spermatogenesis. Meanwhile, the bigger fish from the pair (γ) have no testicular cells in its ovotestes. The ovotestes were consisting mainly of pre-vitellogenic oocytes and some vitellogenic oocytes. After 2 months of rearing post-treatment, fish γ ovotestes possessed mostly developed ovarian cells. It becomes more aggressive and much bigger than their pair (fish β) which still has some testicular cells. Meanwhile fish α still possessed some testicular tissue though the amount starts to decreased. This may suggest that when paired, the more aggressive and much bigger fish might change sex to female which is the same condition with *A. ocellaris* in wild.

It can be concluded that immersion method was an effective mode of androgen administration compared to feeding method for *A. ocellaris*. This is due to consistence of amount of hormone throughout the hormone treatment in immersion method compared to feeding method. It can also be confirmed that MT treated fishes can change sex to female if the condition is right.

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**KESAN PEMASKULINAN IKAN CLOWNFISH (*Amphiprion ocellaris*)
DENGAN MENGGUNAKAN 17 α -methyltestosterone.**

MUHAMMAD ABDUH BIN YAZED

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Pengerusi : Profesor Madya Abol Munafi Ambok Bolong, Ph.D.
Ahli : Prof Mohd. Azmi Ambak, Ph.D.
Institut : Institut Akuakultur Tropika

Ikan clownfish (*Amphiprion ocellaris*) adalah ikan yang marin yang paling digemari oleh pengumpul ikan hiasan. Walaubagaimanapun, penghasilan ikan ini di dalam kurungan masih rendah disebabkan oleh jumlah induk yang terhad dan tiadanya induk dihasilkan dari ikan F1. Rawatan hormon telah digunakan secara meluas di dalam akuakultur untuk menghasilkan populasi monoseks dalam spesies ikan. Objektif utama projek ini adalah untuk menentukan samaada hormon boleh digunakan untuk menjantankan ikan *A. ocellaris*. Untuk itu, tiga eksperimen telah dijalankan untuk mengkaji kesan: (i) rendaman dan pemberian makanan mengandungi 17 α -methyltestosterone untuk menghasilkan ikan jantan, dan (ii) pemasangan dan tak di pasangkan terhadap gonad ikan yang telah dirawat dengan 17 α -methyltestosterone.

Juvenil ikan *A. Ocellaris* (3.5 cm-5.5 cm) telah direndam kedalam kepekatan MT yang berbeza selama 15 hari dan kemudiannya di ternak selama 2 bulan. Kepekatan MT adalah 1 ppm, 2 ppm dan 4 ppm dan satu kumpulan kawalan dengan 0 ppm. Tiga ikan disampel sebanyak dua kali, iaitu di akhir eksperimen dan sekali lagi selepas 2 bulan eksperimen untuk histologi gonad. Terdapat kemungkinan untuk memperolehi kesan pemaskulinan untuk spesis ini bila MT digunakan dalam kaedah rendaman pada peringkat juvenil. Kepekatan yang terbaik adalah 1 ppm kerana kadar hidup yang tinggi dan kewujudan sel testis walaupun selepas dua bulan pendedahan terhadap MT berbanding kepekatan yang lain.

Bagi eksperimen pemberian makanan yang mengandungi MT pula, 3 kepekatan MT telah dipilih iaitu 30 mg/kg, 60 mg/kg dan 120 mg/kg dengan satu kumpulan kawalan yang makanannya cuma disembur dengan ethanol sahaja. Tiga ekor ikan telah di sampel pada akhir eksperimen, sebulan selepas eksperimen dan dua bulan selepas eksperimen. Kaedah pemberian makanan yang mengandungi MT terhadap juvenil ikan ini mungkin memberikan keputusan yang tidak stabil kerana faktor pengambilan makanan dan penyampaian hormon itu sendiri. Terdapat juga kemungkinan yang penukaran MT kepada 17α -methylestradiol dan adanya enzim aromatase dalam ikan yang menyebabkan kesan feminisasi terhadap ikan-ikan.

Eksperimen ketiga dilakukan untuk melihat kesan kaedah pengumpulan terhadap ikan yang telah dirawat dengan MT. Ikan-ikan ini direndam dalam MT berkepekatan 1 ppm. Di akhir eksperimen, 10 ekor ikan di masukkan kedalam 10 akuarium berbeza manakala 10 ekor lagi dipasangkan dan diletakkan kedalam 5 akuarium berbeza. Sebulan selepas eksperimen, sampel gonad dari ikan yang diletakkan bersendirian (α), dan ikan yang lebih kecil dalam ikan yang dipasangkan (β), mempunyai semua peringkat spermatogenesis. Manakala ikan yang lebih besar dalam ikan yang dipasangkan (γ) tidak mempunyai sel testis, sebaliknya mempunyai sel ovari di peringkat pra-vitellogenik dan vitellogenik. 2 bulan selepas rendaman, ikan γ mempunyai gonad yang dimonopoli oleh sel-sel ovari. Ianya menjadi lebih besar dan lebih agresif berbanding pasangannya (ikan β) yang masih mempunyai sel-sel testis. Manakala ikan α masih lagi mempunyai sel-sel testis tetapi jumlahnya semakin berkurangan. Ini menunjukkan bahawa ikan yang lebih agresif dan lebih besar cenderung untuk bertukar jantina yang mana keadaan ini sama dengan ikan *A. ocellaris* di lautan.

Sebagai kesimpulannya, kaedah rendaman adalah lebih berkesan daripada kaedah pemberian makanan dalam pemberian hormon. Ini adalah kerana jumlah hormon yang konsisten di sepanjang masa eksperimen dalam kaedah rendaman berbanding kaedah pemberian makanan. Juga boleh disimpulkan di sini bahawa, ikan yang telah di rawat dengan MT boleh menukar jantina ke betina sekiranya dalam keadaan yang bersesuaian.