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Effect of Exogenous Thyroxine on Mophology and Development of Thyroid Gland in Marble Goby *Oxyeleotris marmoratus* Bleeker Larvae

A.B. Abol-Munafi, Asmanelli, A.M.W. Effendy and M. Awang Soh
University College of Science and Technology Malaysia, Mengabang Telipot,
21030 Kuala Terengganu, Malaysia

Abstract: This study was conducted to examine effect of the exogenous thyroxine (T_4) on ontogeny of thyroid gland in marble goby larvae and to found out whether the exogenous T_4 can alter the follicle structure and the level of T_4 in marble goby. The larvae were exposed for 1 h in T_4 solution at 0.1 ppm. Untreated larvae (control) were not immersed in T_4 hormone. It was found that T_4 levels in treated larvae were higher than control, but the development patterns in both treated larvae and control were similar in which occurred a decreasing of T_4 levels from 3-7 days after hatching (dAH), and then increased again from 10 dAH onwards. The histological evidences that the follicles structure in treated larvae were difference than control, in which suggested abundance of thyroid hormone. These findings suggested that thyroid hormones (THs) may play an important role during early larval's life and metamorphosis period. High total T_4 levels in the treated larvae suggested that exogenous T_4 has a predictable role to increase T_4 level, especially in early larval stage of marble goby.

Key words: exogenous thyroxine, marble goby, thyroid gland, structure

INTRODUCTION

The thyroid hormones (THs) consist of thyroxine (T_4) and triidothyronine (T_3) are product of the thyroid gland in all vertebrates. They have important role in early development and metamorphosis in teleost. Thyroxine (T_4) is the predominant hormone secreted, and quantify determination of thyroxine is easier. In freshwater fishes, the concentration of T_4 is generally higher than T_3 in both eggs and larvae[1].

The role of thyroxine in teleost larvae's early life and metamorphosis have been proven in the improvement of larva development[2-7]. Zairin *et al.*[5] has been proven that exogenous T_4 treatment, could accelerate eyespot formation, swim bladder and pigmentation of marble goby larvae. However, are better to know in advance when the thyroid hormone was functional and develop in marble goby larvae.

In the previous study, the presence of thyroid hormone in marble goby larvae have detected at hatching even the thyroid follicles were not yet developed. The decreasing of T_4 levels in early life stage are most likely caused the larvae used their own body hormone[1]. Therefore, the exogenous T_4 treatment in early life was hopefully could increase of T_4 levels in marble goby larvae. Based on above things, this study was conducted to examine the effects of exogenous T_4 on developmental changes of thyroid gland and also to found out whether the follicles structure would be altered by exogenous T_4.

MATERIALS AND METHODS

Larvae source: Groups of newly hatched larvae were stocked into the experimental tanks, and then exposed to exogenous thyroxine solution (T_4 0.1 ppm) by immersion for 1 h. All larvae were immersed in a bucket containing 20 L of water. The dosage of hormone was administered with identical conditions to the applications used in the previous study[5] The procedure for preparation of T_4 solution followed[2]. For control, larvae were not immersed in thyroxine hormone. After treatment, larvae were then reared in the fiberglass tanks with density 2000 larvae per tank, each contained 200 L of dechlorinated freshwater, midly aerated. The experimental conditions are followed to the applications in the previous study[8]. The experiment was terminated on day 30.

Sampling and analysis: Sampling was done prior to water change, daily for the first week and thereafter every 3-7 days for 3 subsequent weeks. Ten larvae were sampled for the purpose immunohistochemistry and histology analysis, were fixed in formalin solution and

Corresponding Author: Asmanelli, University College of Science and Technology Malaysia, Mengabang Telipot, 21030 Kuala Terengganu, Malaysia
Tel: +60 012 9654042/60 09 668 3253

Bouin's fluid, then Boin's fixed sample preserved in 70% ethanol and stored at room temperature. The tissues were embedded in paraffin and sectioned 4 or 5 μm.

For histology examination, sections were deparaffinized or dewaxed, dried and stained. The protocol following method as described by Kiernan[9] and Short and Meyer[10]. For immunohistochemistry, dewaxed sections were treated with 3% H_2O_2 in methanol for 10 min in order to block endogenous peroxidase. Blocking and all antibody dilutions were done in normal goat serum in PBS. As first antibody, used 1:400 rabbit anti-thyroxine (Biomeda, cat No. #A64). As a secondary antibody, was used a goat anti rabbit with ratio 1:200. The staining procedure was performed following protocols using the Vectastain elite ABC kit. (Vector Laboratories, Inc.USA).

Samples for measuring of total T_4 concentration were taken from hatching until day 16 after hatching, approximately 50 mg wet weight of larvae (consisted of hundreds of fish) were then pooled and frozen at -70°C until analysis. Larvae samples were homogenized in 1.5 mL of ice cold 0.01 M Phosphate Buffered Saline (PBS) for 1 min using Ultra Turax homogenizer[11]. From this homogenate an aliquot of 25 μL each sample was taken for T_4 analysis. The amount of total T_4 in each sample was determined using an ELISA kit (Alpha Diagnostic, Int., USA). All experiment for immunohistochemistry were repeated at least three times independently, and for quantitative of total T_4 were repeated two times independently. The differences between means of T_4 levels were calculated and analyzed using Student's t-test.

RESULTS

T_4 treated larvae appeared the signal strength are stronger than untreated (control). Their shape is irregular but generally round or tube like in shape. Sometime the follicle of marble goby appear alone (single cell) but most of in loose aggregations along ventral aorta and not easy to counting.

The structure of thyroid follicles of marble goby showed different profile between control and treated larvae. Observed that untreated fry had the follicles contained a large amount colloid vacuolated (Fig. 1a and c), whereas in T_4 treated marble goby fry the colloid was less abundant, large follicles with squamous ephithelium, flat cell, peripherial vacuoles (Fig. 1b and d). The profile of follicle cells suggesting an overabundance of thyroid hormone.

This confirmation could be proved by use antibody against T_4, which give a strong signal especially on the follicular epithelium, it is confirming identity as thyroid follicles (Fig. 1e and f).

Detail of follicle structure is showing on the below picture (Fig. 2). At the apical outer surface of the follicular cells reveal the vesicle.

For exogenous T_4 treated larvae, concentration of total T_4 was higher than control about 4-5 fold higher (Fig. 3), but the thyroid hormone levels trend showed a similar decreasing pattern from 3 dAH to 7 dAH (4.2 to 3.1 μg dL^{-1}), whereas in the control was from (1.0 to 0.7 μg dL^{-1}). The profile of whole body T_4 development changes showed at t he below graphic (Fig 4).

From student's t test analysis, there are significant differences ($p<0.05$) between means of T_4 level in treated larvae and control (Table 1), in which concentration of total T_4 in treated larvae was higher than control. In treated larvae, the lowest means value (2.90±0.43 μg dL^{-1}) and the highest (6.10±0.21 μg dL^{-1}), whereas in the control groups were (0.65±0.11 μg dL^{-1}) and (1.62±0.2 μg dL^{-1}), respectively.

DISCUSSION

Generally, the first thyroid follicles appeared in first few days after hatching, specially in oviparous species seen at hatching[12]. In this study, the thyroid follicles were absent at hatching in both T_4 treated and control, although T_4 level already detected. In the beginning, the follicle was only recognized as anterior domain non follicular cells, and the first thyroid follicle in marble goby was found at 13dAH[13]. From serial sections, the follicles clustered around the ventral aorta in the gill region to the bulbus arteriosus. Early follicles had a small lumen containing colloid, and the staining intense was limited to the colloid of the follicle. In the latter stages, the follicles comprised of cuboidal thyrocytes, the follicle lumen was larger and vacuoles of the colloid was also greater.

Immersion larvae in T_4 solution distinct increased concentration of whole body T_4 and altered the structure of follicles. The morphology of the follicles remained irregular, some of round and tube like in shape. The structure of follicles in untreated larvae were lined by low cuboidal and contained a large amount of vacuolated colloid, whereas in the treated larvae the colloid was less

Table 1: The concentration of total T_4 in treated larvae and control

	Means of T_4 level	
	Lowest value (μg dL^{-1})	Highest value (μg dL^{-1})
T_4	2.90±0.43[a]	0.65±0.11[b]
Control	6.10±0.21[a]	1.62±0.20[b]

Values are the means±S.E.M of two times of the experiment, independently.
[ab] the different superscript letters in the same colum was significantly different in $p<0.05$

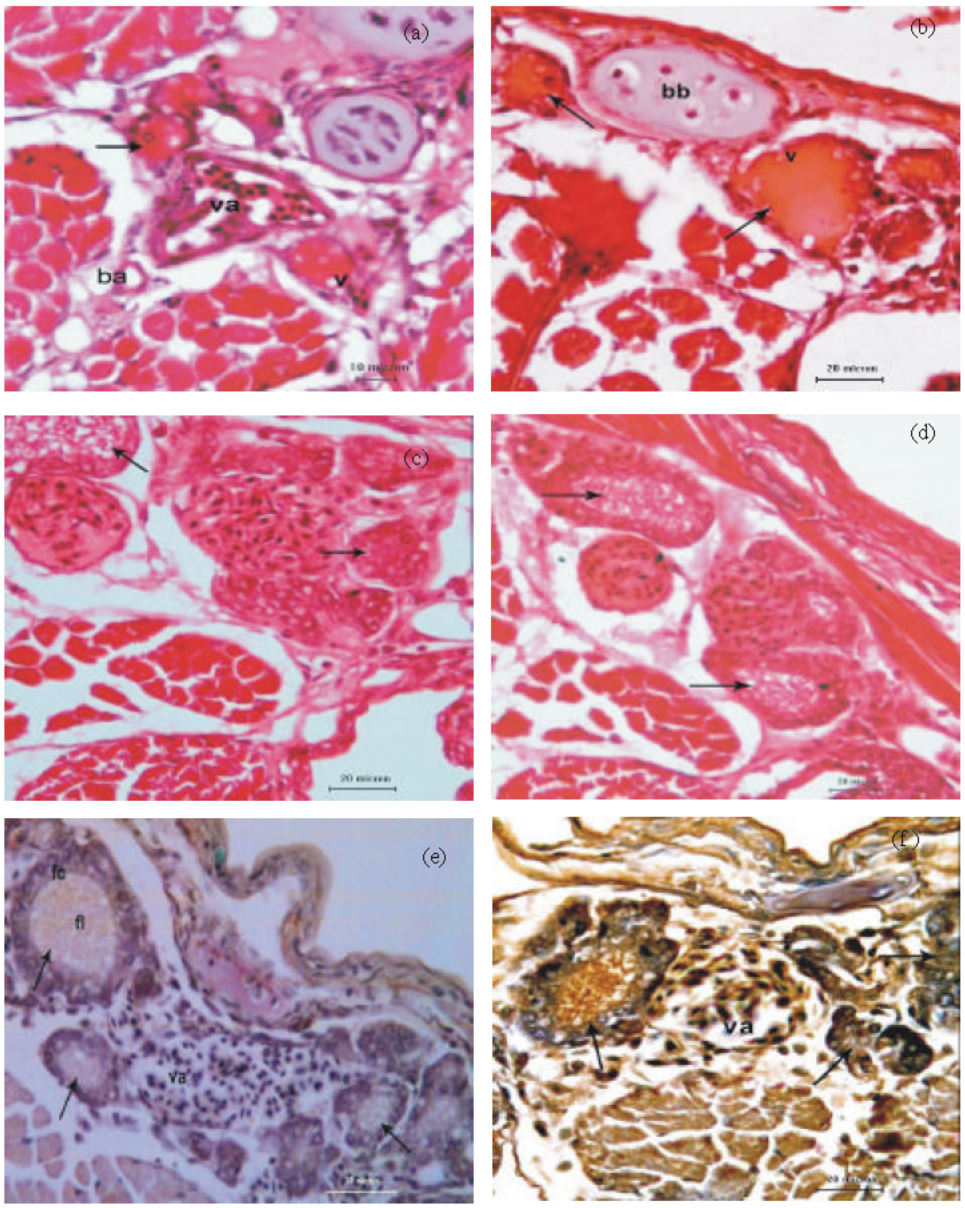

Fig. 1: Thyroid follicles profile of goby fry; a-b) 23 days old; c-f) 30 days old. Left side: untreated fry; Right side: T_4 treated fry; Magnification x 40. v, vacuole; va, ventral aorta; bb, basibranchial cartilage; fl, follicle lumen; fc, follicular cell; ba, bulbus arteriosus; arrow, thyroid follicle

abundance and peripheral vacuoles. These characteristics were similar with observed in T_4 treated tilapia *S. niloticus*[13] which suggested an over abundance of thyroid hormones. It could be proved by the T_4 immunostaining that appeared to be strong staining in the cytoplasma of some thyrocytes, which represent a thyroid hormone secretory phase[1,5]. From the above descriptions distinct suggested the positive effect of exogenous T_4 treatment in enhancement of T_4 level, particularly in early life stage of marble goby larvae. However, still needed the further studies to find out effect of exogenous hormone on growth and development of marble goby larvae.

There are some different findings about exogenous T_4 treatment in period of early larvae development. Exogenous TH treatment of fish larvae has contradictory effects, both positive and negative. TH immersed larvae, can accelerate metabolism, growth, survival, gut function, yolk absorption, fin differentiation,

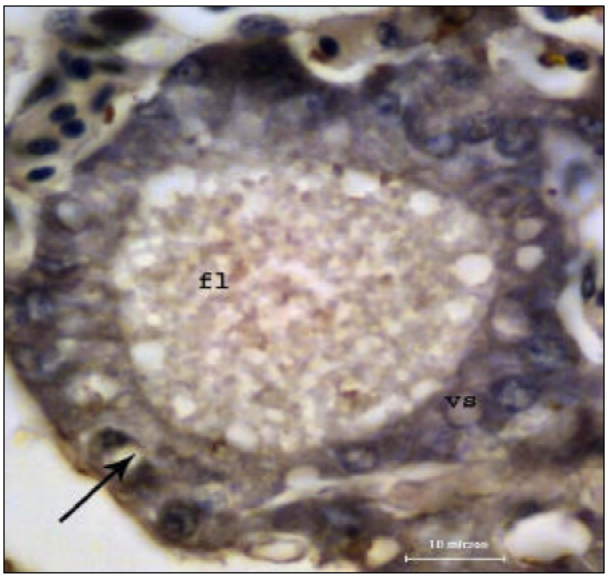

Fig. 2: Detail of thyroid follicle structure showing follicle lumen with the less colloid that characterized overabundance of thyroid hormone. IHC stain. Magnification x 100. fl, follicle lumen; vs, vesicle; arrow, follicular epithelium cell

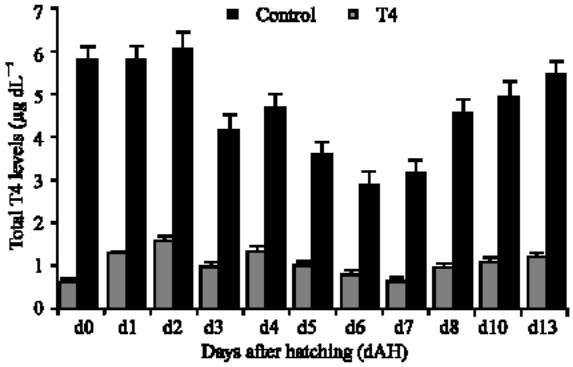

Fig. 3: Comparison of thyroid hormone levels in the control and treated marble goby larvae. Vertical bars indicate standard errors of the means, n = 2

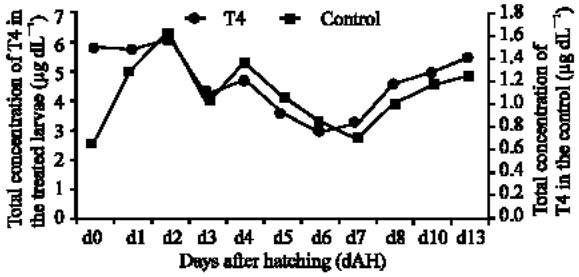

Fig. 4: Profile of thyroid hormone development for control and treated marble goby larvae

eye pigmentation[2-5,7,12,14,16]. Negatif effects only appear to be species spesific were characterized with skeletal deformity and reduce the viability of embryo and larvae[14,17,18] in which may be caused by high dose of hormones[14] and mode of administration of the THs used[1]. Furthermore, has been described by Eales[19] that exogenous T_4 treatment would increase T_4 levels in plasma, therefore decreased endogenous TSH secretion, and then resulting decrease in endogenous secretion.

However in this study, T_4 treated larvae indicative changes of hormone level were higher than control, although development pattern of T_4 was similar. There are significant differences between T_4 levels in treated and control ($p<0.05$). The levels of T_4 may reached 4-5 fold higher in treated than control.

The changes of hormone level are related to response and ability of the embryo or larvae to control the circulating TH levels[20]. Moreover, according to Lam[16] that in freshwater fishes the iodine uptake was easier compared to marine fishes, particularly in iodine less environment. It showed that marble goby has ability to control the circulating thyroid hormone levels. This response has also been demonstrated Zairin *et al.*[5] in acceleration of the eyespot, swimbladder and pigmentation of marble goby larvae. In the present study, from 10 dAH onwards, thyroid hormone levels is synchronized with the developmental stages of larvae. The increasing are related to direct development changes of marble goby from larvae to juvenile form. According to

Balon[21] the end of larvae stage when the fins are fully developed, the scales have appeared and most organs have been formed. In marble goby, larvae stage ends between 14-35 dAH, which all the fins are fully developed[22], gastric glands formed and larvae benthic habitat[8,23]. These evidences showed a important role of thyroid hormone, particularly in the early larval stage and during metamorphosis period[6,12,13,23]. However, still a lot of works are needed to asses its effect on the development, growth and survival of marble goby itself.

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