

THE DIGESTIVE TRACT DEVELOPMENT OF THE TIGER GROUPE *Epinephelus fuscoguttatus* LARVAE TREATED WITH THYROXINE HORMONE

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ABSTRACT

Thyroxin administration in tiger grouper larval rearing was conducted to determine its effect on development of digestive tract of the larvae. Newly hatched larvae were treated with 0 mg/L (control), 0.1 mg/L, and 0.2 mg/L of thyroxin for 2 hours in a 30 L tank before were transferred into a 1 m³ rearing tank. Samples were collected daily until 10 DAH and every 3 days until 40 DAH. Development of digestive tract was observed using histological method. For all treatments, the digestive tract was the same on 1 DAH and 2 DAH. The digestive tract of 1 DAH larvae was still closed and still in the form of a simple tube. The mouth, buccopharynx, liver, pancreas, and rectum were found on 2 DAH. On 3 DAH, the esophagus, stomach, intestines, and anus started to develop. At this stage, the digestive tract of larvae treated with and without thyroxin started to become distinguishable. Several goblet cells were noticed in the rectum of the treated larvae but not in the control. Digestive tract developed earlier in treated larvae compared to the control group, such as appearance of tongue, gill raker, fundic, and cardiac stomach development, formation of pharyngeal tooth, and gastric gland. Metamorphosis of larvae into juvenile on treated larvae occurred than that the control larvae earlier.

KEYWORDS: digestive tract, *Epinephelus fuscoguttatus* larvae, thyroxine hormone

INTRODUCTION

Groupers (family Serranidae) are widely distributed in the tropical and subtropical coastal waters. They are one of the most commercially important group of tropical marine fish. Grouper species that have high market value and demand such as *Epinephelus coioides*, *E. malabaricus*, *E. fuscoguttatus*, *E. lanceolatus*, *E. akaara*, *E. bleekeri*, *E. awoara*, *E. areolatus*, *E. amblycephalus*, *Plectropomus leopardus*, *P. maculatus*, and *Cromileptes altivelis* (Heemstra & Randall, 1993).

Marine fish larvae culture largely depends on successful first feeding and normal development and growth of fish larvae. Therefore is important to examine the structural and functional development of the endocrine system during early ontogeny of marine fish larvae. Growth and development in fish, as in all vertebrates, are governed through orderly release of hormones from the neuroendocrine system, which integrates with the environmental, physiological, and genetic information. Many of the developmental processes occurred

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during early larval stages of teleost fish, including growth and metamorphosis, are regulated by thyroid hormones, thyroxine or tetraiodothyronine (T_4) and triiodothyronine (T_3) (Eales, 1979; Power *et al.*, 2001; Blanton & Specker, 2007).

The information on the digestive tract developmental change associated with food assimilation process is crucial for understand the nutritional physiology of fish larvae. This information may help to identify the limiting factors during larval rearing, thus reducing the problem during the weaning process and synchronizing the stage of development with the rearing technology and feeding practices (Hamlin *et al.*, 2000).

The role of thyroxine in teleost larvae's early life and metamorphosis have been proven in the improvement of larvae development, such as *Chanos chanos* (Lam *et al.*, 1985), *Tilapia nilotica* (Nacario, 1983), Flounder *Paralichthys olivaceus* (Miwa & Inui, 1987). In the previous study, effect of thyroxine and thiorurea on metamorphosis of coral trout grouper *P. leopardus* has been studied by Trijuno *et al.* (2002).

Tiger grouper *E. fuscoguttatus* aquaculture was established during the past 10 years. Their seed production is still unpredictable and extensively. Average survival to fingerling stage (about 25 mm in length) was low, generally in the range 0%-10%. In addition, survival was highly irregular, one or two larval rearing 'runs' often resulted in reasonable (up to 10%) survival, while others had zero survival. Low average survival and unreliable hatchery production were major constraints to the commercialization of grouper larviculture technology (Rimmer, 1997; 1998; Rimmer *et al.*, 2000). Thus this study is to observe the effect of exogenous thyroxine on ontogeny of digestive tract in early life stage of *Epinephelus fuscoguttatus*.

METHODOLOGY

Larvae Rearing

Eggs of *E. fuscoguttatus* were obtained from natural spawning of a domesticated broodstock. Newly hatched larvae were immersed to exogenous thyroxine (treatment A = 0.1 mg/L T_4 and treatment B = 0.2 mg/L T_4) for 2 hours in a 30 L fiberglass tank. The T_4 solution was prepared following Lam (1980). For control (treatment C), larvae were not exposed

in thyroxine solution. After treatment, larvae were reared in 1 m³ rearing tanks with density 15,000/tank for 40 days rearing periods with gentle aeration. Phytoplankton *Nannochloropsis oculata* were added daily as water conditioner and as a food for rotifer. Larvae were fed with the rotifer for the first time at 2 DAH (days after hatch). An initial prey concentration is 5 rotifers/mL and progressively increases to 30 rotifers/mL at 30th DAH. Artificial feed were fed starting at 10 DAH and starting from 20 DAH the larvae were fed with *Artemia nauplii*. The wastes were siphoned out from the bottom of the rearing tank start from day 7 of the rearing.

Sampling and Analysis

Twenty samples were collected daily for 10 days and every 3 days until end of the experiment for histology analysis, samples were fixed in Bouin's solution before being preserved in 70% alcohol and stored at room temperature. Histological studies were then conducted following Kiernan (1990).

Samples for the analysis of total T_4 concentration were taken every 5 days until end of the experiment, approximately 50 mg wet weight of larvae (consist of hundreds of larvae) were pooled and frozen at -70°C until analysis. Larvae samples were then homogenized in ice cold 0.01 M Phosphate Buffered Saline (PBS). From this homogenate an aliquot of 25 μ L was analyzed using an Elisa Kit (Alpha Diagnostic Int. USA).

RESULT AND DISCUSSION

At 1 DAH, the digestive tract of tiger grouper larvae was still closed and just in a form of simple tube (Figure 1). The mouth, buccopharynx, liver, pancreas, and rectum begin to develop at 2 DAH (Figure 2). While the esophagus, stomach, intestines, and anus started to develop at 3 DAH. At this age, the digestive tract of larvae treated with and without thyroxin start to discriminate. Several goblet cells were observed in the rectum of treated larvae but absent in the control (Figure 3). Upper and lower teeth of treated larvae at the anterior of the mouth were also observed at this stage. At 4 DAH, the yolk completely disappears with gas bladder start to develop and the intestinal valve divided the intestine into midgut and hindgut. Upper teeth, lower teeth and several goblet cells in rectum were observed in control larvae.

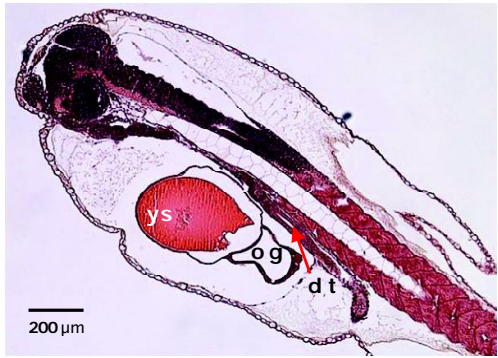


Figure 1. Sagittal section of digestive tract of tiger grouper larvae at 1 DAH. (magnification: 10x; staining: H&E; dt: digestive tube (arrow); og: oil globule; ys: yolk sac)

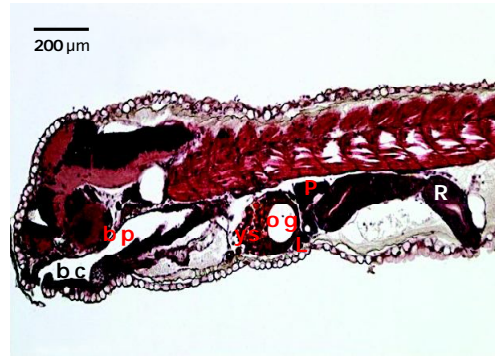
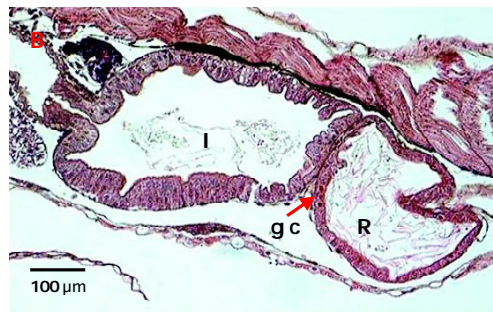
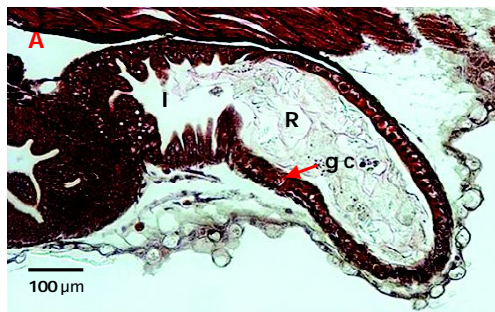


Figure 2. Sagittal section of digestive tract of tiger grouper larvae at 2 DAH. (magnification: 10x; staining: H&E; bc: buccal cavity; bp: bucopharynx; L: liver; P: pancreas; R: rectum; og: oil globule; ys: yolk sac)



Note:
 A. Larvae treated with 0.1 mg/L of thyroxine
 B. Larvae treated with 0.2 mg/L of thyroxine
 C. Larvae without thyroxine treatment (control)

Figure 3. Sagittal section of the rectum of tiger grouper larvae 3 at DAH. Note: Several goblet cells were observed in the rectum of treated larvae but absent in the control (magnification: 20x; staining: H&E; R: rectum; I: Intestine; gc: goblet cell (arrow))

Thyroxine administration on tiger grouper larvae has shown the effects on digestive system development. For larvae treated with 0.2 mg/L of T_4 , the tongue was observed at 5 DAH which is a day earlier compare to other treatment. Gill raker appeared at 6 DAH for larvae

treated with 0.2 mg/L of T_4 , at 7 DAH for larvae treated with 0.1 mg/L of T_4 and 8 DAH for control groups. Fundic and cardiac stomach were also started to develop at 6 DAH for larvae treated with T_4 a day earlier compared than control groups (Figure 4).

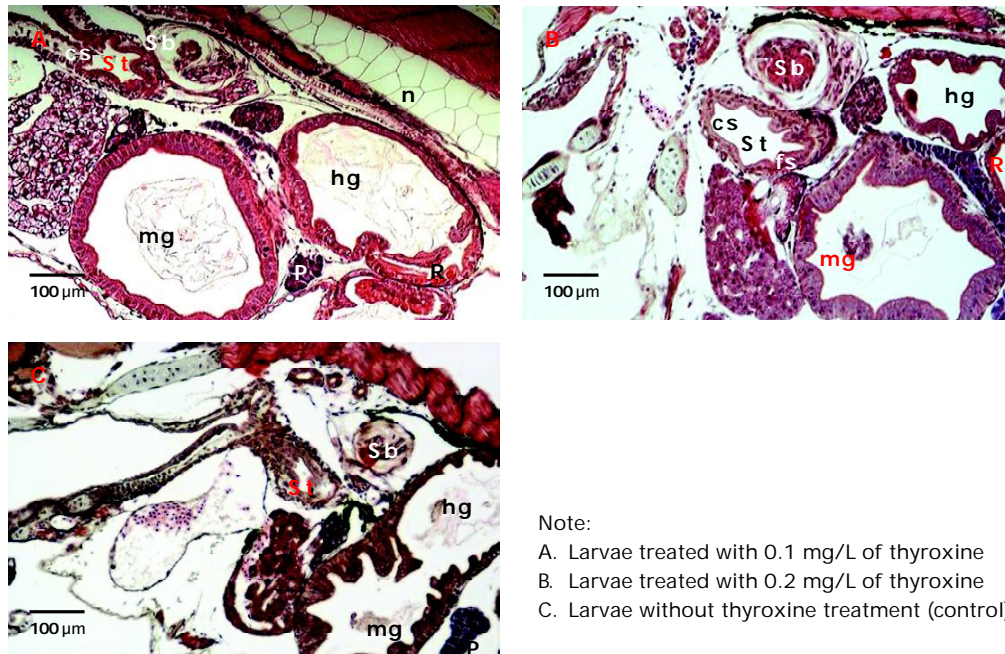


Figure 4. Sagittal section of digestive tract of tiger grouper larvae at 6 DAH. Note: Fundic and cardiac stomach started to develop at 6 DAH a day earlier for treated larvae with T_4 than control groups (magnification: 20x; staining: H&E; cs: cardiac stomach; fs: fundic stomach; hg: hindgut; mg: midgut; L: liver; P: pancreas; R: rectum; sb: swim bladder; St: stomach; n: notochord)

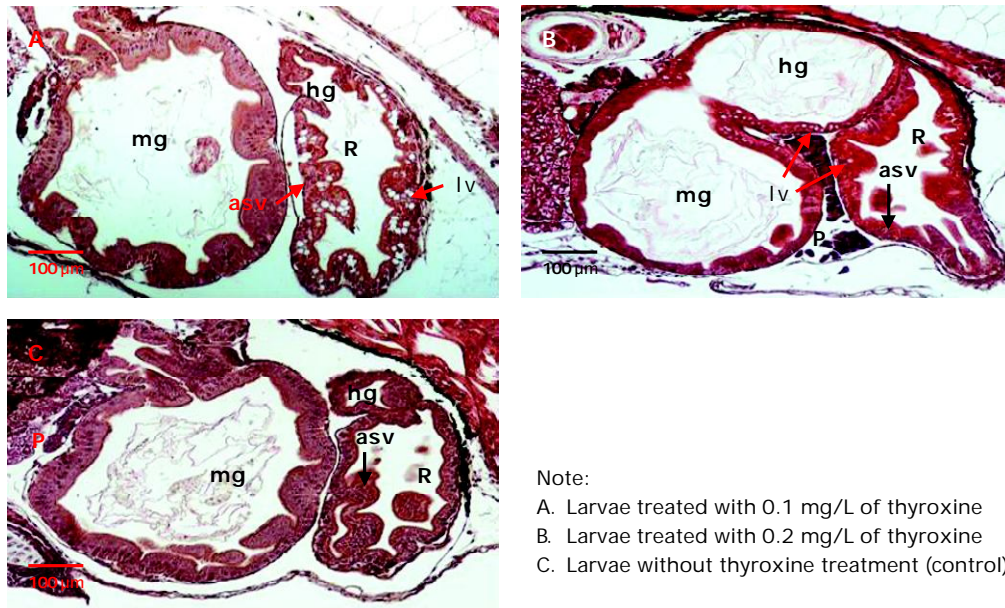
At 8th DAH, taste buds were visible at posterior pharynx in all larvae. At this age, acidophilic supranuclear, and lipid vacuoles observed more in the rectum of larvae treated with thyroxine compared than the control groups (Figure 5).

In larvae treated with exogenous thyroxine, a pharyngeal tooth was found at 10 DAH (Figure 6) and gastric gland had begun to appear at 25 DAH (Figure 7). While in control groups, pharyngeal tooth, and gastric gland was observed at 13 DAH and 28 DAH respectively.

Total T_4 concentration showed that larvae immersed with thyroxine have higher concentration than control especially after thyroxine administration and at the end of experiment. From ANOVAs, there are significant differences ($P < 0.05$) between treatment and control, but there are not significant differences between treatments.

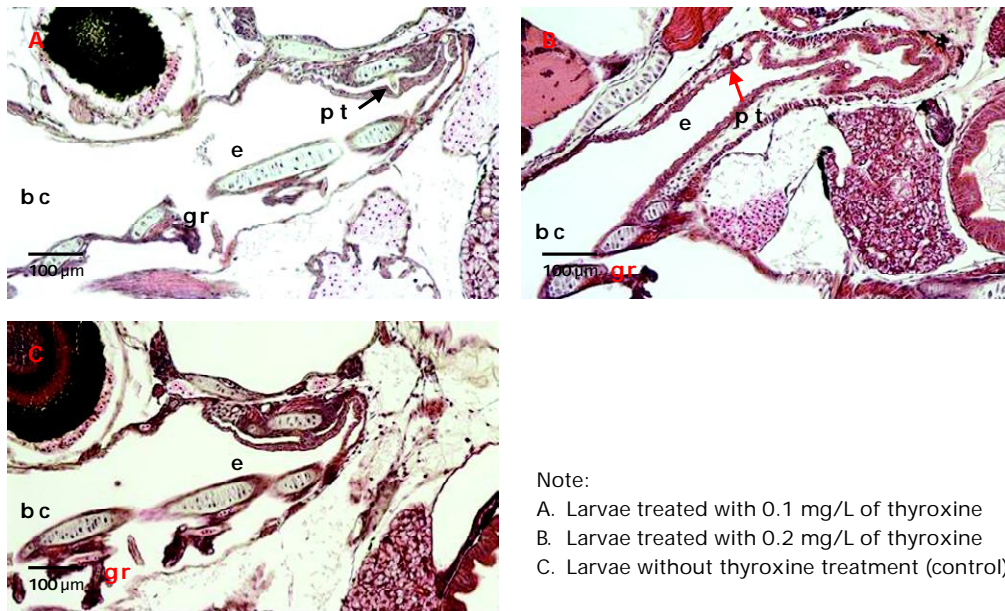
The influence of thyroxine treatment on the development of the digestive organs was observed histologically. At 1 DAH, the diges-

tive tract of tiger grouper larvae was still closed and just in a form of simple tube. The mouth, buccopharynx, liver, pancreas, and rectum begin to develop at 2 DAH. While the esophagus, stomach, intestines, and anus started to develop at 3 DAH. These results were similar for all groups of larvae. Previous study on development digestive system in grouper larvae showed similar development, such as in kelp grouper *E. bruneus* (Kato *et al.*, 2004), dusky grouper *E. marginatus* (Glamuzina *et al.*, 1998), green grouper *E. coioides* (Quinitio *et al.*, 2004) and leopard grouper *Mycteroperca rosacea* (Martinez *et al.*, 2009). At 3 DAH, the digestive tract of larvae treated with and without thyroxin start to discriminate. Several goblet cells were observed in the rectum of treated larvae but absent in the control. Thyroxine administration had positive effects in tiger grouper larvae. Digestive tract develop earlier in treated larvae compared to control group, such as appearance of tongue, gill raker, fundic, and cardiac stomach development, formation of pharyngeal tooth and gastric gland. The pres-



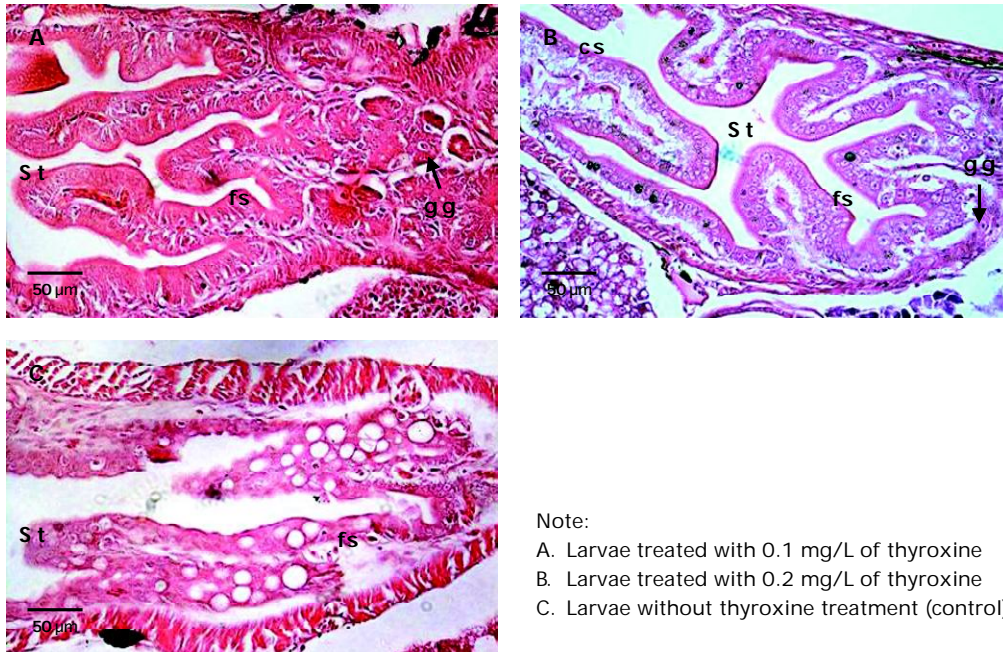
Note:
 A. Larvae treated with 0.1 mg/L of thyroxine
 B. Larvae treated with 0.2 mg/L of thyroxine
 C. Larvae without thyroxine treatment (control)

Figure 5. Sagittal section of digestive tract of tiger grouper larvae 8 DAH. Note: The rectal epithelium of treated larvae showing lipid vacuoles and acidophilic supranuclear vacuoles, while that of the control was appeared lesser. (magnification: 20x; staining: H&E; asv: acidophilic supranuclear vacuoles (arrow); hg: hindgut; lv: lipid vacuoles (arrow); mg: midgut; P: pancreas; R: rectum)



Note:
 A. Larvae treated with 0.1 mg/L of thyroxine
 B. Larvae treated with 0.2 mg/L of thyroxine
 C. Larvae without thyroxine treatment (control)

Figure 6. Sagittal section of digestive tract of tiger grouper larvae at 10 DAH. Note: Pharyngeal tooth was observed at treated larvae but absent in control at 10 DAH, (magnification: 20x; staining: H&E; bc: buccal cavity; e: esophagus; gr: gill raker; pt: pharyngeal tooth (arrow))



Note:
 A. Larvae treated with 0.1 mg/L of thyroxine
 B. Larvae treated with 0.2 mg/L of thyroxine
 C. Larvae without thyroxine treatment (control)

Figure 7. Sagittal section of the stomach of tiger grouper larvae at 25 DAH. Note: The gastric gland of treated larvae was observed earlier than control, (magnification: 40x; staining: H&E; cs: cardiac stomach; fs: fundic stomach; gg: gastric gland (arrow); St: stomach)

ence of acidophilic supranuclear and lipid vacuoles observed more at the rectum of treated larvae; this indicated increasing of digestive activity in larvae treated with thyroxine than control. Tanaka *et al.*, 1995 demonstrated that treatment of larvae with thyroid hormones may be beneficial as T_4 appears to stimulate the uptake of both proteins and fats in the digestive tract. The most prominent means of nutrient uptake in larval fish appears to be intact protein ingestion by pinocytosis in the rectal epithelium (Iwai & Tanaka, 1968; Watanabe, 1984), which may be stimulated by exogenous T_4 . The presence of acidophilic supranuclear vacuoles was result of pinocytosis of protein and presence of lipid vacuoles confirmed fat absorption. Pynocitosis and intracellular digestion have been suggested as the main mechanism for protein absorption in teleost larvae during the absence of a functional stomach (Boulhic & Gabaudan, 1992; Gisbert *et al.*, 2004; Sarasquete *et al.*, 1995). Kim & Brown (1997) described the regulatory hormone (such as thyroid hormone) could precondition the digestive tract of larvae for increased digestion and nutrient absorption.

In treated larvae gastric gland had begun to appear at 25 DAH, while in control groups gastric gland was observed at 28 DAH. Miwa *et al.* (1992) demonstrated that thyroid hormone stimulates gastric development during metamorphosis of Japanese flounder. The end of larval stage when all fins are fully developed, the scales and most organs have formed (Balon, 1975). However, based on digestive organ development, the larvae stage end when gastric gland formed (Boulhic & Gabaudan, 1992; Bisbal & Bengston, 1995; Liem, 2001) and stomach was the last organ of digestive system to differentiate (Gisbert *et al.*, 2004). The first appearance of gastric gland among teleost is variable depend on fish species. In milkfish *Chanos chanos* it occurred at 42 DAH (Ferraris *et al.*, 1987). In dover sole *Solea solea*, it occurred at 22 DAH (Boulhic & Gabaudan, 1992). In Siberian sturgeon the appearance of gastric glands occurred earlier at 9 DAH (Gisbert *et al.*, 1998). The gastric gland differentiation indicated metamorphosis occurring, generally concurrent with the transition from larval to juvenile. Because the fully functional stomach is thought to increase digestive capacity of

Table 1. The concentration of total T₄ in treated larvae and control

	Means of T ₄ level (µg dL ⁻¹)
A. (0.1 mg/L)	1.54 ± 0.058 ^a
B. (0.2 mg/L)	1.53 ± 0.070 ^a
C. (0 mg/L)	1.49 ± 0.040 ^b

Note: ^{ab} the different superscript letters was significantly different in P<0.05

the gastrointestinal system and to be an important contributor to the high growth rate exhibited by newly metamorphosed juvenile (Tanaka, 1973 in Soffientino & Specker, 2003).

The measurement of total T₄ in tiger grouper larvae showed that larvae immersed with thyroxine have higher concentration than control especially after thyroxine administration and at the end of experiment. Abol-Munafi *et al.* (2005) suggested that the positive effect of exogenous thyroxine in enhancement of T₄ level, particularly in early life stage of marble goby *Oxyeleotris marmoratus*. Miwa & Inui (1985) also showed that T₄ administration has successfully increased plasma T₄ levels and caused body silvering of Amago Salmon (*Oncorhynchus rhodurus*). While Eales (1979) described that exogenous T₄ treatment would increase T₄ level in plasma, therefore decreased endogenous TSH secretion, and then resulting decrease in endogenous secretion. The changes of hormonal level are related to response and ability of the embryo or larvae to control the circulating TH levels (Balon, 1975). Moreover, in fresh water fishes the iodine uptake was easier compare to marine fishes, particularly in iodine less environment (Lam, 1995).

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