Thesis for the Degree of Master of Fisheries Science

Hormone effects on the scale, swim bladder and fin differentiation in larval zebrafish

by

Haliliy Marufchoni

The World Fisheries Graduate School

Pukyong National University

February 22, 2019

Hormone effects on the scale, swim bladder and fin differentiation in larval zebrafish

(제프라피쉬(Danio rerio) 미성어의 비늘, 아가미 그리고 부레의 분화에 미치는 호르몬의 영향)

Advisor: Prof. Christopher Lyon Brown

by

Haliliy Marufchoni

A thesis submitted in partial fulfillment of the requirements
for the degree of

Master of Fisheries Science

in the World Fisheries Graduate School,
Pukyong National University

February 2019

Hormone effects on the scale, swim bladder and fin differentiation in larval zebrafish

A thesis

by

Haliliy Marufchoni

Approved by:

_____

(Chairman) Md. Aminur Rahman

_____

(Member) Sang Go Lee

_____

(Member) Christopher L. Brown

February 22, 2019

Table of Contents

List of Figures

List of Table

Hormone effects on the scale, swim bladder and fin differentiation in larval zebrafish

Haliliy Marufchoni

The World Fisheries Graduate School,

Pukyong National University

Abstract

Bioactive compounds are critical regulator of general development and physiological functioning in zebrafish embryos. We have experimentally raised triiodothyronine (T_3) content of zebra fish eggs by immersion process with water. Exogenous thyroid hormone (TH) reportedly induces early stages of the zebrafish and observed the development of pectoral fin after 72 hpf, 5dpf and 7 dpf, which shows the differences between control and T_3 treatment. We also found the comparable difference in caudal fin length after 14 dpf, which increased after 14 dpf T_3 immersion than the control. We observed eyes differences after 3 dpf but no significant differences was found ($P>0.05$) after 10 pdf. The hormone treatment significantly accelerated the differentiation and

inflation of swim bladder. The inflation of swim bladder become higher in hormone treated group than in control after 5 days of treatment whereas it changes after 7 dpf. At the same time, we observed the color pigmentation in the body of hormone treated larvae while no significant color observed in control larvae. The pattern of larval mortality was an increase in larval death rate near the start of the 96 hpf. Treatment promoted survival significantly higher survival during the experiment ($P<0.05$). We interpret this with mean that there was a statistically significant relationship between untreated control and treatment. The possible reason of scale formation in the body of thyroid hormone treated larvae. This study extends the evolutionary relatedness of fish fin growth, eyes, scales, swim bladder and suggest that TH is required to complete the life cycle of a zebra fish.

1 Introduction

Zebrafish, *Danio rerio* is a small fresh water fish whose embryonic development is fundamentally completed within 3 dpf to 5 dpf at 24 to 32℃ temperatures (Kimmel et al., 1995). Newly hatched zebrafish embryos are extensively studied as vertebrate model organisms. Under laboratory conditions, the zebrafish is reared quickly in large numbers. Since it has a close phylogeny to humans, approximately 70% similarity with human, the organism is used often in biological and medical sciences such as for endocrine study. The embryonic to larval change marks a vital stage for the development study (Balon, 1990) due to a variety of major morphological changes which occurs in the yolk sac larvae from 3dpf to 5dpf period such as inflammation of the swim bladder, formation of the eyes, pectoral fin, caudal fin and pigmentation, and other organs (Pack et al., 1996). According to Chapman (1982), a remarkable feature of metamorphosis in fish is the replacement of fetus to larval whose development is controlled by another hormone. In some cases, triiodothyronine (T_3) treatment is reported to stimulate growth and organ development, thereby playing a significant role during the early

development stage of zebrafish (Lam 1985). Thyroid hormone supplementation in fish embryos and larvae is known to influence the early development although there exist a variety of responses which have been documented (Brown et al., 1988a). In recent times, there have been findings that indicate thyroid hormones stored in yolk may represent an additional source of vital compounds capable of influencing larval development (Brown et al., 1988b). It may be therefore be possible to reconcile some of the reported findings that indicate the different T_3 hormone involvement in the yolk mediated embryonic and larval development (Brown, 1997).

Generally, thyroid hormones are found in the organs of most fishes, notably in the circulatory system where there is a maternal release into the yolk of unfertilized eggs. The effect of thyroid stimulation in fish is more pronounced in species with marked TH- dependent pectoral fin variations. This could however be difficult to recognize in species where such variations are relatively insignificant. Most studies of this subject have focused on the readily recognized traits, and it is therefore likely that it is indirect but biologically significant. Zebrafish have occurred

TH dependent development mostly occurs in early ages (Parichy and Turner 2003). Thyroid hormone (TH) – dependent changes in external morphology have previously reported for zebrafish include in head during the embryo to larval transition and paired in differentiation pigmentation and scale development during metamorphosis (Brown, 1997). There are detailed reports that TH influences the development and discrimination of important organs in the embryo and larval processes in two primary and elevated vertebrate, aside playing active role in metamorphosis of larvae and nerve metabolism in adults (Oppenheimer et al., 1995). Thyroid hormone is operational in the early developmental stages of most fishes before metamorphosis occurs (Tanaka et al., 1995). Roh and Concha (2000), reports that the thyroid gland in zebrafish becomes active by 40hpf, albeit it could be influenced by the presence of iodine at 3 dpf (Brown, 1997). Large amounts of maternal 3, 5, 3'-L-triiodothyronine (T_3) which are released into the eggs of most freshwater fish species, are suggested to be responsible for growth and development of these embryos and larvae before endogenous production of TH (Lam, 1994).

The development history of vertebrate fishes starts very early at the formative stages of the embryos, where body organs such as the eyes,

scales, swim bladder, heart, fin .etc. are formed. Recent embryonic and genetic studies in most bony fishes have provided new knowledge in the morphology and genetics of these fishes. In addition, there has been molecular advancement in determining eyes formation in these fishes (Chuang, 2002). The eye is a visual aiding organ of a fish and has complex composition and different degree of development in other vertebrates (Muller, 1975; Lamb et al., 2007 and Neuhauss, 2010) and due to this, a lot of research have been conducted in this area.

In the last decade, Bejarano et al., (2010) found it of a great interest to study the embryonic configuration of the eye of fish by THs hormone. Bohnsack & Kahana (2013) and Darras et al., (2015), reported that the growth of the eyes and craniofacial structure of vertebrates is quite complicated, albeit it is moderately influenced by thyroid hormones.

Thyroid hormone had proven to be vital in the growth and developmental processes of vertebrates, owing to the varied studies. However, thyroid hormones effects on the scale, swim bladder and fin differentiation in larval zebrafish during the early developmental stage is worth researching.

1.1 Aim

The aim of the study is to determine the effect of thyroid hormone on the growth and development of the scale, swim bladder and fin differentiation of zebrafish larvae reared under laboratory conditions

1.2 Objectives

(i) To identify the pectoral and caudal fins development mediated by thyroid hormone.

(ii) To monitor the impacts of thyroid hormone on eye development during embryogenesis.

(iii) To observe the TH treatment effects on the development of scale, swim bladder and body color in the zebrafish larvae after 14 dpf.

2 Materials and Methods

2.1 Study site

This experimental study was conducted at Pukyong National University

and the Korea inter-University Institute of Oceanography from July to November 2018. Zebrafish (*Danio rerio)* larvae were obtained by breeding commercially obtained broodstock animals in beaker set-ups in the laboratory, with closely monitored environmental conditions of temperature maintained at 25 ± 2 ℃. The first morning post-spawning, after the eggs were fertilized, were collected and moved to petri dishes for their respective incubation and hatching with light and good water quality over the ensuing 72 hours.

2.2 Experimental design

Two experimental treatments with three replicate each were established as Control and T_3 hormone based on baseline rate of development at 24℃ room temperature. Prior to the main experiment, Four treatments groups for the period was conducted from 1 to 14 dpf.

Table 1 Experimental treatments

Treatments	**T1**	**T3**
Status	Control group	Triiodothyronine group
Replicates	R1, R2, R3	R1, R2, R3

Number of replicates	3	3

2.3 Data collection

The length and morphological features of experimental larvae were recorded using "Active Measure" software with the help of a Stereo Microscope model SZX10 connected to the LCD screen. Digital picture of the organs of zebrafish larvae was taken with a wide zoom range of 1X magnification. The size at initial and final total length were taken in micron units (µm) and then converted to millimeter (mm), having sampled 5% of the experimental population.

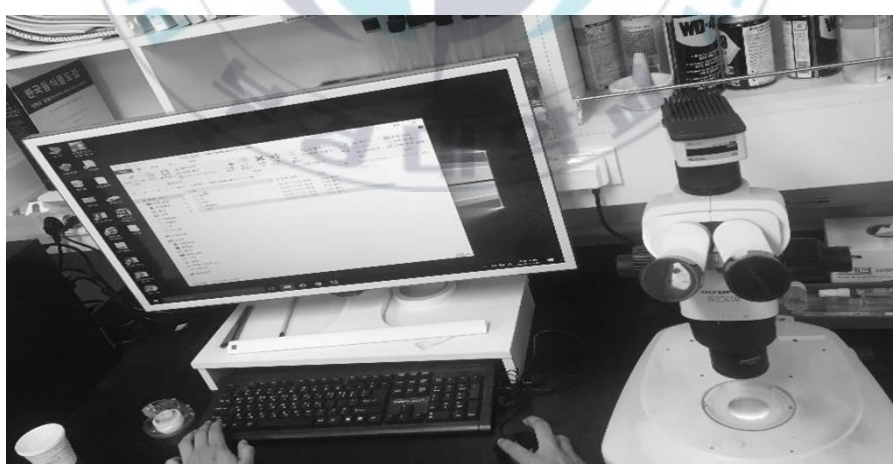

Figure 1 Microscope used for taking images

2.3.1 Preparation of T_3 hormone

Liquid Triiodothyronine Protein, measuring 50 Units or 0.55 ml was purchased from the commercial market at MYBiosource.com on the internet before the start of the experiment. This was stored below 4^0 C in refrigerator. To prepare the final hormone treatment for the experiment, 10µl stock per 10 ml culture water for immersion to [0.005912 ppm] was added to 3,5,39-L-triidothyronine (T_3) with water in laboratory (Brown et al., 2014). Finally, a microliter dilution 200µl x 5.92 = 1184µl triiodothyronine (T_3) was obtained. A mechanical pipette (100 ml) was used to mix the hormone with water into a standard Crystal Grade Polystyrene (sterile) petri dish measuring 90X15mm. This was then used in the respective treatments.

2.3.2 Pectoral fin measurement

The pectoral fin development of experimental larvae was monitored under the digital microscope from Control and T_3 treatments for 7 days (14dpf-21dpf) during the experimental period. It has done at regular timing of 24 hours intervals. After observation, the sampled experimental larvae were returned to their culture media, while at the same time maintaining stress to be barest minimum. Twenty sampled

larvae from each treatment had taken by using plastic pipette/ dropper and transfer to petri dish for microscope observations to note the developmental stages of the fins.

2.3.3 Caudal Fin Observation

15 dpf experimental larvae were observed under the stereo microscope for caudal fin developments. Larvae was monitored at 24 hours intervals as 20 larvae were sampled from each petri dish for caudal fin observations for both control and T_3 treatments were monitored for developments. The stereo microscope was also used to take pictures of the rate of development of the caudal fins of sampled larvae and this was recorded.

2.3.4 Caudal Fin Observation

15 dpf experimental larvae were observed under the stereo microscope for caudal fin developments. Larvae was monitored at 24 hours intervals as 20 larvae were sampled from each petri dish for caudal fin observations for both control and T_3 treatments were monitored for developments. The stereo microscope was also used to take pictures of the rate of development of the caudal fins of sampled larvae and this was

recorded.

2.3.5 Eyes differentiation

At least ten sampled larvae in both treatments were used for eye differentiation. In determining this, two types of parameters were used; firstly, the length data of the full eye size, likeness and color of larvae preserved with T_3 and T_3 untreated (Control) were measured and recorded using the stereo microscope. Secondly, picturesque parameter was used to take photography of the developmental process of the eyes of sampled larvae. For each types of parameter, a ratio of Control and T_3 treatments were observed. Fifty self-regulating experiments were shown at 3 dpf and this number was used for subsequent analysis for exact eye resolution.

2.3.6 Swim bladder differentiation

Swim bladder developments were observed daily under the stereo microscope at 24 hours intervals. 20 sampled larvae from both control and T_3 treatments were from 1 dpf to 10 dpf. After taking images using the microscope, the total length of the sampled larvae was measured and recorded after which larvae were transferred back to their respective

petri dish holding facility. This was carefully done with the aim of reducing stress to the barest minimum.

2.3.7 Scale development

20 sampled larvae from both control and T_3 treatments were taken and observed under the stereo microscope on a daily basis at 24 hours intervals from 1 dpf to 20 dpf. Scale and muscle skeletal development of larvae were monitored in the experiment by observing physical changes on the fish. Observation were thus recorded.

3 Results and Discussion

3.1 Survival Rate

The impact of T_3 hormone was observed from the period of early development stage of the larvae (1dpf – 20dpf). Significant differences ($P<0.05$) in survival performances were found from both treatments. The higher survival rate was found in the T_3 treatment, which was (361±2.6) while the lower value was observed in control, were (265±6). These differences were observed throughout the experiment until 14 dpf. The pattern of larval mortality was observed until 96 hpf. T_3 treatment showed significant survival rate during experiment ($P<0.05$). This was interpreted to mean that there is a statistically significant relationship existing between untreated larvae (Control) and treated larvae (T_3 treatment). Ideally, it was not possible to quantify the daily mortality rate of the larvae. However, occasional mortalities were recorded from 3 dpf to 5 dpf. Larvae treatment (T_3) was observed to have less mortality rate (10%).

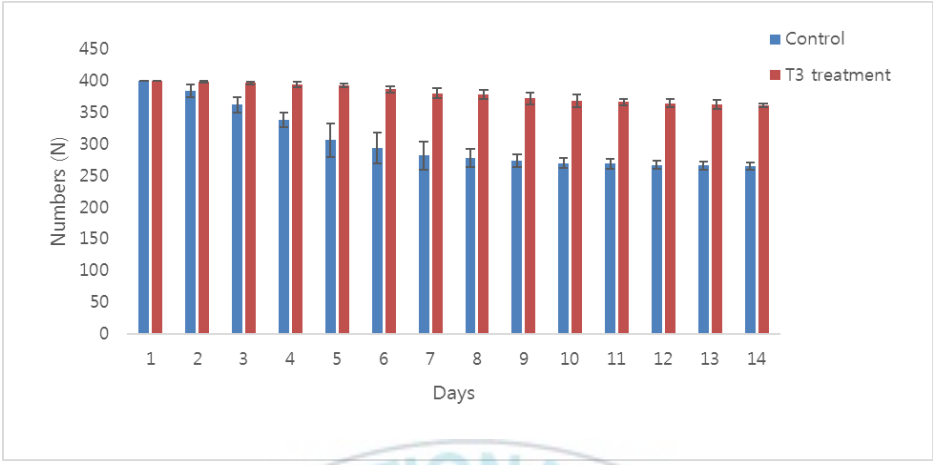

Figure 1 Survival rate of treatment at 14 dpf

3.2 Pectoral Fin Development

There were observed pectoral fins differentiation of experimental zebrafish larvae between treatments. In this experiment, T_3 hormone added to the culture water of zebra fish eggs until 14 dpf of larval growth exhibited visible effect on the pectoral fin development. It was observed that in the T_3 treatment, the pectoral fin budded at 24 hpf and at 72 hpf, the fins had already extended such that the shape of the fin become visible at hatching (Figure 3). In addition, T_3 treatment larvae appeared to show untimely differentiation of their pectoral fins than control larvae so that they began to develop like the appearance of fins. The pectoral fins of T_3 - treated larvae also grow larger than controls fin

at the same stage. The growth and development of the pectoral fins of the T_3 treatment larvae was relatively faster than the control larvae. At 5 dpf, the pectoral fin in T_3 treatment larvae were more pronounced than that of the control larvae (Figure 3.1); juxtaposed to a more pronounced one in control treatment at 7 dpf.

Table 2 Pectoral fin development between controls treated hormone larvae until 7 dpf

Days	Control (µm)	T_3 (µm)
1	0±0	0±0
2	0±0	40.564±5
3	30.762±6	91.872±4
4	88.231±5	204.671±7
5	149.102±8	348.602±9
6	208.764±8	509.463±10
7	356.233±9	798.184±7

The mentioned table shows the pectoral fin development in zebrafish larvae during our experiment by micro millimeter. There were not significant difference in first and second day. The biggest pectoral fin difference was observed in 7 dpf where 798.184±7 (µm) were T_3

treatment compared with only 356.233±9 (μm) in Control (Table 2). The length data of the experiment showed that the mean TL of T_3 treatment larvae (6.8mm) was relatively higher than that of the control treatment (5.8mm). This was influenced by development It is worthwhile to note that in this study, hatchability mostly occurred between 72 hpf to 96 hpf after which larvae was observed to have a total length (TL) of 2.9mm – 3.17mm. This was however influenced by temperature (Figure 3.2). It was also observed that the yolk sac could sustain larval zebrafish growth throughout the first 7 dpf of pectoral fin development.

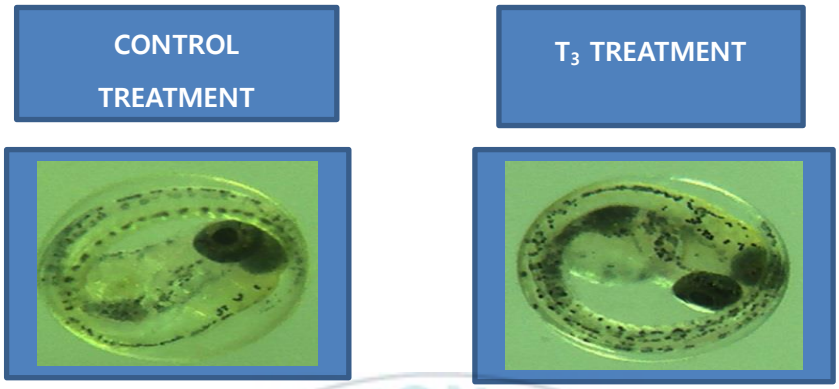

Figure 3 Pectoral fin development in between control and T_3 treatment after 72 hpf

Figure 4 Pectoral fin development in between control and T_3 treatment after 5 dpf

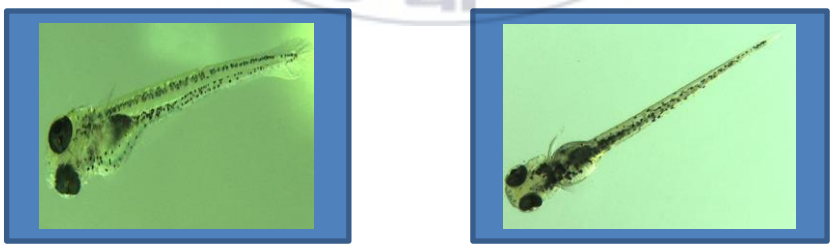

Figure 2 Pectoral fin development in between control and T_3 treatment after 7 dpf

3.3 Caudal Fin Development

Table 3 Caudal fin development between control and treated hormone larvae until 14 dpf.

Days	Control	T_3 Treatment
1	0±0	0±0
2	0±0	0±0
3	0±0	40±4
4	30.231±5	111.432±7
5	77.102±8	142.602±9
6	104.744±8	205.463±10
7	121.233±9	233.103±7
8	131.12±4	278.941±4
9	148.511±3	297.824±6
10	172.944±5	321±711±3
11	191.034±6	339.321±5
12	211.034±8	371.711±6
13	229.098±4	412.111±3
14	251.521±5	449.522±7

In this study, the caudal fin development was influenced by the presence of T_3 hormone. Initially, there were no caudal fin between Control and T_3 but it was visual from the inaugurating of 3rd day. The greatest caudal fin difference was observed in 6th days where 205.463±10 µm were T_3 treatment, compared with only 104.744±8 µm Control. Surprisingly, more control larvae took part in 8th day (131.12±4) µm whereas T_3 treated larvae were approximately doubled 278.941±4 µm. (Table. 3) This is evident in the T_3 treatment larvae such that T_3 hormone added to the induced the caudal fin development within 14 dpf. The result of this study showed a significant increase in the caudal inflammation (development) in T_3 treatment larvae after 10 dpf, whereas it was absent in control larvae (Figure. 6)

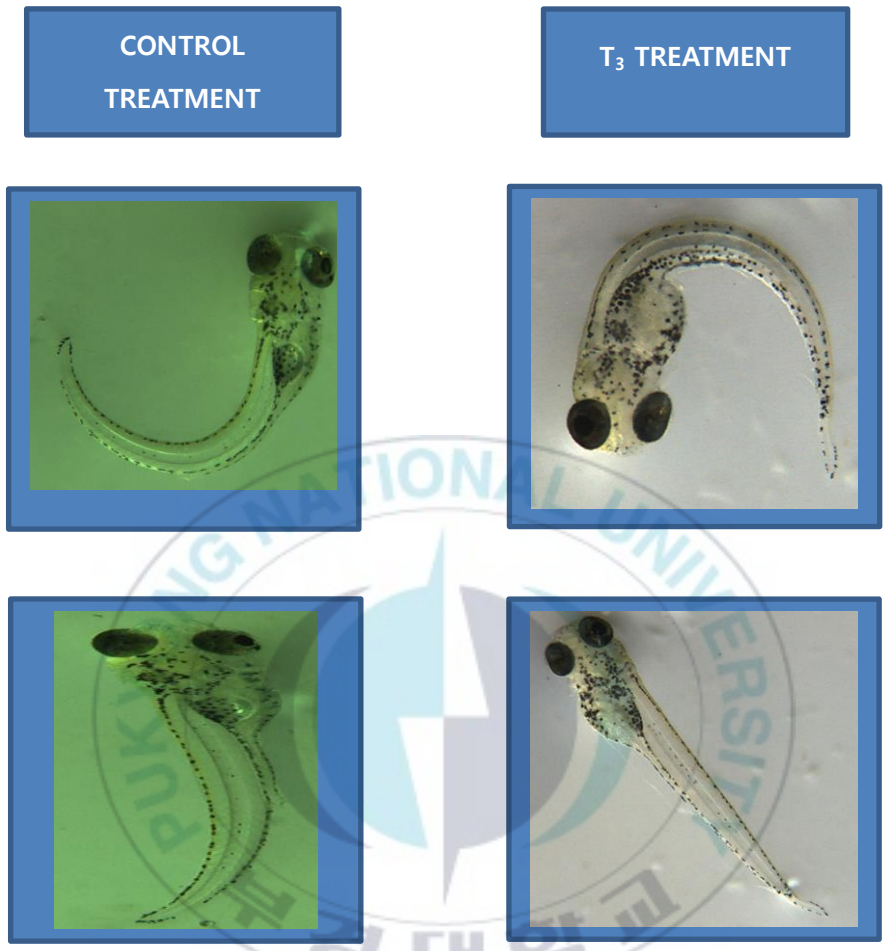

Figure 3 Caudal fin differences between control and T_3 treatment fish after 14 dpf

3.4 Eye differentiation

In this study, substantial differences in eye differentiation wasobser ved between control and T_3 hormone treated larvae after3 dpf (Fig ure 5). There was development of cornea in control treated larvae whereas T_3 treatment larvae, cornea had started to be pronounced. After 10 dpf, there was not specific difference between eye differe ntiation of both control and T_3 treatments (Figure 5.1). T_3 hormon e had significant effects on the retina layer in the eye of larvae.

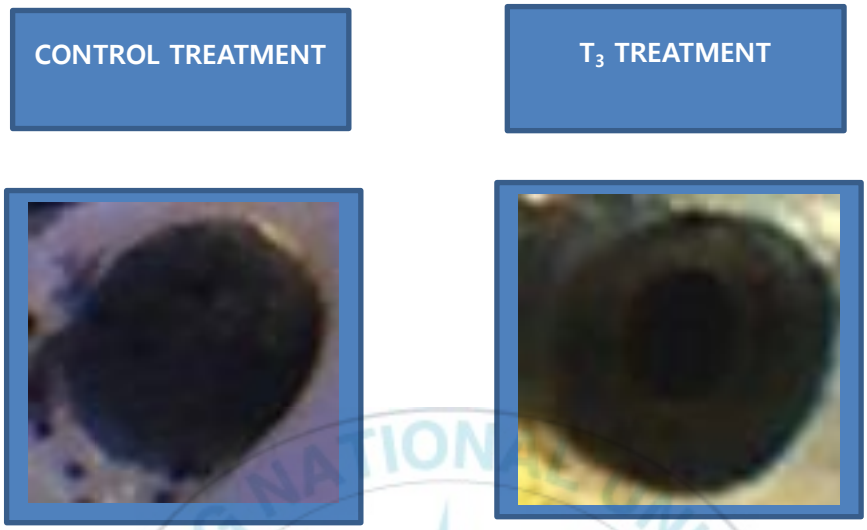

Figure 7 Eyes differences between control and T_3 treatment fish after 3 dpf

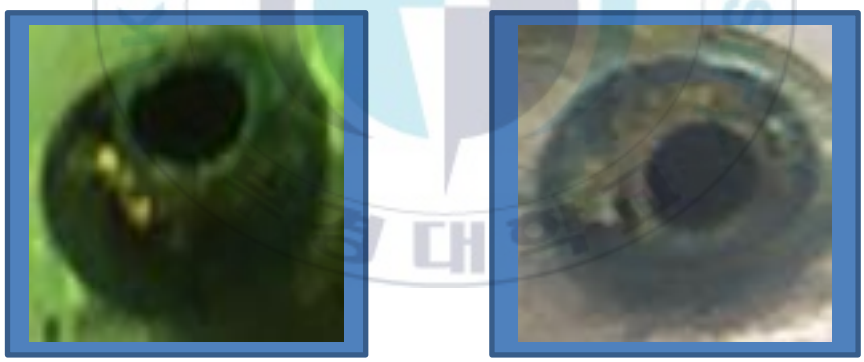

Figure 4 Eyes different between control and T_3 treatment fish after 10 dpf

Table 4 Eyes development between control and treated hormone Larvae until 9 dpf

Days	Control	T_3 Treatment
1	6.42±0.7	32.112±2
2	64.22±0.1	192.672±9
3	224.78±5	449.568±10
4	289.008±12	610.128±1
5	417.456±7	610.128±1
6	481.68±13	610.128±1
7	513.792±11	610.128±1
8	610.128±2	610.128±1
9	610.128±1	610.128±1

The given table gives the information about eyes development in term of different days reached by both control and treatment during our experiment. As far as seen, there are substantial differences was observed in both control and treatment at different days. The biggest pectoral fin differences observed in 4 dpf where 610.128±1 mm were in control, compared with control only 289.008±12 mm. Surprisingly, the length of eyes development was remained same at the age of 9 dpf where sized was 610.128±1 mm. (Table 3). It was also observed there was significant difference in the eye differentiation in all treatments until 7 dpf but no significant difference from 8 dpf - 9 dpf. A more pronounced eye onset was observed from 4 dpf in both control and T_3 treatment (Figure 6).

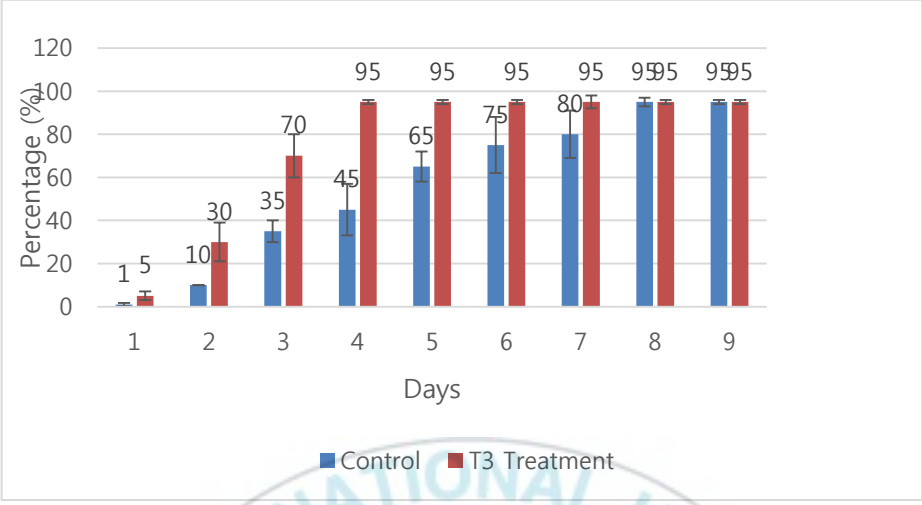

Figure 5 Eyes differentiation rate between treatments at 9 dpf

3.5 Swim bladder Development

The study assessed the effect of T_3 on swim bladder of the experimental larvae. The result showed that swim bladder was uninflated after 5 dpf in control whereas swim bladder was inflated in the T_3 treatment. Overall, there was a significant differentiation of swim bladder inflation in both treatments after 10 dpf. It was also observed that the improvement of swim bladder inflation has reduced larval mortality during early stages of development.

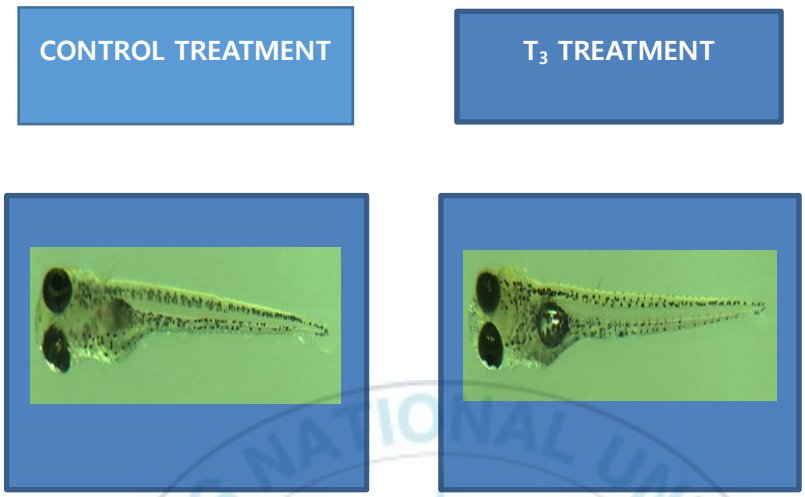

Figure 7 Swim bladder differentiate between control and T_3 larvae after 5 dpf

Figure 6 Swim bladder differentiate between control and T_3 larvae after 7 dpf

3.6 Scale Development

In this study, the scale and muscle skeleton were monitored for growth and development. 20 samples were taken and observed until 20 dpf during which time physical changes of scale, and muscle skeleton were captured for images recording. The result showed that there were significant observational variations in the scale and skeletal muscle of both treatments. T_3 hormone treated larvae showed profound scale and muscle skeleton development than the control treatment (Figure 8).

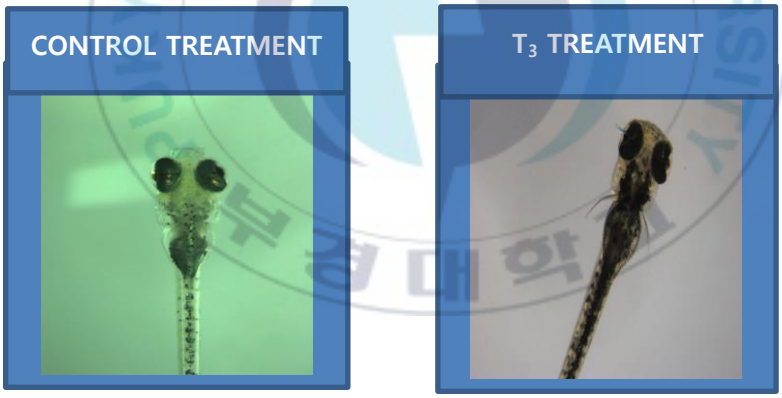

Figure 8 Scale and muscle skeleton development between control and T_3 treatment after 20 dpf

3.7 Pectoral Fin Development

During embryonic development in our experiment, the pectoral fin-bud formed at about 24 hpf and by 96 hours the fin extended in T_3 treatment. This was evident during swimming movement of the larvae where there was a visual variation in shape of the larvae. Similar result was found by Kimmel et al., (1995) who observed zebrafish eggs hatching most commonly between 48 -72 hpf when fish were about 2.9 – 3.17 mm in TL, although there was an assumption that the timing of cracking and body length depends on the ambient temperature. During embryonic development, the pectoral fin was formed at 24hpf and by 48hpf, and the fin elongated as fish skeleton begun to harden (Yelon et al., 2000).

The T_3 treated larvae demonstrated faster growth and development of their pectoral fins than Control larvae such that it aided in their locomotion. This was similar to findings by Green et al., (2011, 2013) who reported that pectoral fin development affects locomotive movement of larval zebrafish. A similar finding by Van Der Boogart (1999) suggested that pectoral fins aid in respiratory processes in larvae.

3.8 Caudal Fin Development

Caudal fin plays a significant role in swimming, locomotion and growth of fish. In this study, it was found that there was a rapid growth in the caudal fin of T_3 treatment than control treatment. Elsewhere, it is stated that lack of caudal fin can cause a fall in the pool. However, the reduction of rudder liquids also leads to slowing down in the swimming process (Webb, 1973; Sinclair et al., 2011), with caudal fins that was damaged it makes it easier for each tail mil to finish the fish. In addition, in many fish species, shape and size of caudal fin plays a role in sexual dimorphism. Sometimes females tend to mate with males with bigger caudal fins, otherwise males contend with each other for females as a sexual behavior. Oftentimes, males with bigger caudal fins are successful in this light. (Warner and Schultz, 1992). This explains further that in some species of fish, the presence of caudal fins do not necessarily aid in swimming movement but rather aid in sexual behavior (Sinclair et al., 2011).

3.9 Eye Differentiation

The thyroid hormone plays a significant role in the eye differentiation of vertebrates (Darras et al., 2015). In spite of this, it is still not clear the impact of thyroid hormone on eye differentiation with respect to molecular changes. In this study, there was substantial diversity between the cornea (eyes) of the control and T_3 larvae treatments. Moreover, the practical effects of eye differentiation on the experimental larvae was not assessed. That notwithstanding, it is clear that undeveloped eyes obstruct vision and this could affect life processes of larvae such as locomotion, nutrition, escape from predation, survival .etc. Similarly, Tonyushkina (et al., 2013) signaled that hormone influences eye differentiation which in turn affects visual physiology and vision-related behavior of fish

3.10 Swim Bladder Development

In this study, it was observed that the swim bladder was still uninflated after 5 dpf in control treatment whereas substantial inflation was observed in the T_3 treatment. After 10 dpf, T_3 treatment has some significant difference whereas control larvae were inflated. Thyroid hormones increased swim bladder length during early stages but next

declined during later stages. It is established that such a function produces a reference point for hormonal functions of thyroid hormone in swim bladder inflation of fish (Chang et al., 2012).

In recent times, there is a substantial advancement in the larval raising skills, however a challenge still exist in growth which is typically influenced by early swim bladder inflation; a process which affect larval mortality. The findings of this study can show that various nourishing and abiotic factors could be operated in fin fish aquaculture to induce high rates of swim bladder inflation, thereby reducing possible distortion and early larva mortalities (Woolley et al., 2010).

3.11 Scale Development

The study showed that, the development of scales occurred after 20 dpf in T_3 treatment larvae compared to the control treatment. Age and size of the fish, but not neither age or size only (Sire et al., 1997a) influence scale development in fish.

4 Conclusion

The study revealed that the administration of thyroid hormone increases the morphological development of larval zebrafish. Thyroid hormone induced larvae showed positive impact in fin, scale and swim bladder and early eyes size development, which are vital for survival of larvae. In this way, decreasing early larval mortality and increasing early larval survival.

The present study revealed that thyroid hormone plays a key role in early larval development and fish production.

5 Bibliography

Balon, E.K. (1990) Epigenesis of an epigeneticist: the development of some alternative concepts on the early ontogeny and evolution of fishes. Guelph Ichthyol Rev 1:1–48.

Bejarano E. R; Blasco,M; Degrip, J.W.; Antonio , J. O.V; Martin-P, G. and Francisco-Morcillo, J.(2010). Eye development and retinal fish species, the Senegalese differentiation in an atricial sole (Solea senegalensis, kaup 1858). J.Exp. Zool.(314b):580- 605.

Bohnsack, B.L., Kahana, A., 2013. Thyroid hormone and retinoic acid interact to regulate zebrafish craniofacial neural crest development. Dev Biol 373, 300–309. doi: 0.1016/j.ydbio.2012.11.005

Brown, C. L., M. Cochran, S. Doroshov,and H. A. Bern. Enhanced survival in striped bass fingerlings after maternal triiodothyronine treatment. Fish Physiol. Biochem., 7(1–6): 295–299(1988a).

Brown, C. L., S. Doroshov, J. Núñez, C. Hadley, R. S. Nishioka, and H. A. Bern. Maternal triiodothyronine injections cause increases in swimbladder inflation and survival rates in larval striped bass, Morone saxatilis. J. Exp. Zool., 248: 168–176 (1988b)

Brown, D. D. The role of thyroid hormone in zebrafish and axolotl development. Proc. Natl. Acad. Sci. USA, 94: 13011–13016 (1997).

Chapman, R.F. (1982) the insects (Harvard Univ, Press, Cambridge, MA).

Chuang J.C., Raymond P.A. (2002). Embryonic origin of the eyes in teleost fish. 24 (6): 519-529.

Chang J., Minghua W., Gui, W., Zhao, Y., Yu, L. Zhu, G. (2012). Changes in Thyroid Hormone Levels during Zebrafish Development. Zoological Society of Japan: 181-184.

Darras, V.M., Houbrechts, A.M., Van Herck, S.L.J., 2015. Intracellular thyroid hormone metabolism as a local regulator of nuclear thyroid hormone receptor-mediated impact on vertebrate development.

Green Matthew H., Robert K. Ho, Melina E. Hale 2011, 2013. Movement and function of the pectoral fins of the larval zebrafish (Danio rerio) during slow swimming Journal of Experimental Biology 3111-3123; doi: 10.1242/jeb.057497

Hale E.M 2014. Developmental change in the function of movement systems: transition of the pectoral fins between respiratory and locomotor roles in zebrafish. Integr Comp Biol. 2014;54(2):238-49.

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B. and Schilling, T.F. (1995) Stages of embryonic development of the zebrafish. Dev Dyn 203:253–310.

Lam, T. J., J. V. Juario, and J. E. Banno. (1985). Effect of thyroxine on growth and development in post-yolk-sac larvae of milkfish, Chanos chanos. Aquaculture, 46(3): 179–184

Lam, T.J. (1994) Hormones and egg/larval quality in fish. J World

Aquaculture Society 25:2–12.

Lamb, D.L.; Collin, P.S. and Pugh, N.E (2007). Evolution of the vertebrate eye: opsins, photoreceptor, retina and eye cup.Nat.Rev.Neur. (8): 960-975.

Muller, W. (1975). Ueber die stam meent wiek lung des she organs der wirbelthieres .Aus der festschrift Zu Ludwig's Aus der festschrift Zuludwig,s tubilavm Leipzig .(cited by Al-Mosawai, 2008).

Neuhauss, CFS (2010). Zebra fish vision: structure and function of the zebra fish visual system. Fish Phsio. (29): 81-120.

Oppenheimer, J.H., Schwartz, H.L. and Strait, K.A. (1995) An integrated view of thyroid hormone actions in vivo. In: Weintraub, B.D. (ed) Molecular Endocrinology: Basic Concepts and Clinical Correlations Raven Press, New York, pp 249–268.

Osse, J. W. M. and van den Boogaart, J. G. M. (1999). Dynamic morphology of fish larvae, structural implications of friction forces in swimming, feeding and ventilation. J. Fish Biol. 55, 156-174.

Pack, M., Solnica-Krezel, L., Malicki, J., Neuhauss, S.C.F., Schier, A.F., Stemple, D.L., Driever, W. and Fishman, M.C. (1996) Mutations affecting development of zebrafish digestive organs. Development 123:321–328

Parichy, D. M., and J. M. Turner. 2003. Temporal and cellular requirements for Fms signaling during zebrafish adult pigment pattern development. Development 130:817-833.

Roh, K.B. and Concha, M.L. (2000) Expression of nk2.1a during early development of the thyroid gland in zebrafish. Mech Dev 95:267–270.

Sinclair, E. L. E., Ward, A. J. W. and Seebacher, F. (2011). Aggression-induced fin damage modulates trade-offs in burst and endurance swimming performance of mosquitofish. J. Zool. 283, 243-248

Sire, J.-Y., Allizard, F., Babiar, O., Bourguignon, J. And Quilhac, A. (1997a) Scale development in zebrafish (Danio rerio). J. Anat., 190: 545-561.

Tanaka, M., Tanangonan, J.B., Tagawa, M., de Jesus, E.G., Nishida,

H., Isaka, M., Kimura, R. and Hirano, T. (1995) Development of the pituitary, thyroid and interrenal glands and applications of endocrinology to the improved rearing of marine fish larvae. Aquaculture 135:111–126.

Tonyushkina KN, Shen MC, Ortiz-Toro T, Karlstrom RO. Embryonic exposure to excess thyroid hormone causes thyrotrope cell death. J Clin Invest. 2013;124 (1):321-7.

Warner, R. R. and Schultz, E. T (1992). Sexual selection and male characteristics in the bluehead wrasse, Thalassoma bifasciatum: mating site acquisition, mating site defense, and female choice. Evolution 46, 1421-1442.

Webb, P. W. (1973). Effects of partial caudal-fin amputation on the kinematics and metabolic rate of underyearling sockeye salmon (Oncorhynchus nerka) at steady swimming speeds. J. Exp.Biol. 59, 565-582.

Woolley, Lindsey & Qin, Jian. (2010). Swimbladder inflation and its implication to the culture of marine fish larvae. Reviews in Aquaculture. 2. 181 - 190. 10.1111/j.1753-5131.2010.01035x.

Yelon D, Ticho B, Halpern ME, Ruvinsky I, Ho RK, Silver LM, Stainier DYR. The bHLH transcription factor Hand2 plays parallel roles in zebrafish heart and pectoral fin development. Development.2000;27:2573–82

Youson, J.H. (1988) First metamorphosis. In: Hoar, W.S. and Randall, J.R. (eds) Fish Physiology. Academic Press, San Diego, Vol 11, Part B, pp 135–196.