APPLICATIONS OF THYROXINE FOR FISH LARVAL GROWTH AND DEVELOPMENT

Bhushan Nanasaheb Sanap
Ph.D. Research Scholar, Fish Nutrition, Biochemistry and Physiology Division,
ICAR-Central Institute of Fisheries Education, Versova, Mumbai, India
Rashmi Sateesh Ambulkar
Ph.D. Research Scholar, Aquaculture Division,
ICAR-Central Institute of Fisheries Education, Versova, Mumbai, India
Dr. Parimal Sardar
Principal Scientist, Fish Nutrition, Biochemistry and Physiology Division,
ICAR-Central Institute of Fisheries Education, Versova, Mumbai, India

Abstract: Thyroxine (T_4) plays a crucial role in physio-metabolic significance during the larval growth, survival and immune performances in the fishes. Usage of thyroxine during the fish larval culture for the improved natality of larvae has been elaborated in the present article. Demerits of utility of thyroxine through in-feed and injection treatment for the fish larvae incited the use of immersion treatment. Immersion treatment of thyroxine has been proved immensely beneficial especially in the larval growth of *Clarias batrachus* and *Anabas testudineus*. Different feeding strategies were used to maintain concentration of thyroxine in the larval culture media. It was concluded that thyroxine is extremely useful in the early larval development and can be used as prophylactic treatment so as to avoid mass mortality in the hatcheries also hatchery operators can easily use the given thyroxine concentrations in the various studies for the respective species. Outcomes of thyroxine usage at hatchery level are highly contributing to flourish the aquaculture industry.

Keywords: Thyroxine, Larvae, *Clarias Batrachus, Anabas Testudineus*, Growth.

Introduction: Thyroid hormones, thyroxine (T_4) and tri-iodothyronine (T_3), are products of the thyroid gland in all vertebrates (Power et. al., 2001). They are responsible to control the metamorphosis of amphibians and for the degradation of larval tissue and adult organogenesis (Shi et. al., 1986). Thyroxine plays a crucial role in the maintenance of a normal physiology in vertebrates and triggers the activity of a plenty of tissues and biological functions compared to any other hormones (Janz, 2000). It is also involved in the control of osmoregulation, metabolism, somatic growth, development, and post-hatching metamorphosis (Janz, 2000). When we compare both the thyroid hormones, thyroxine (T_4) and tri-iodothyronine (T_3), it has been assumed that the T_3 is more active on 'per mole' basis, however, T_4 is the pre-eminent secretion of the thyroid gland and more physiologically pertinent hormone as it allows the body cells to perform any appropriate deiodination (Boggazi et. al., 1997). Corroborative developmental phases are undergone in most of the teleost fishes (Power et. al., 2001). During the transition phase from larva to a juvenile in the Zebra fish, there occurs the transformation of fins to adult form, the formation of scales and development of the typical striped pattern of the adults (Brown, 1997). Noticeable results had been revealed in the Zebra fish that exogenous thyroid hormone induces the premature differentiation of the pectoral fins and also had a spurt on pelvic fin growth; however, on the contrary, use of goitrogen for the prohibition of thyroid hormone synthesis debilitated the transformation from larva to juveniles in the Zebra fish (Brown, 1997). In the marine fish larvae, thyroxine (T4) supplementation indicated that intact protein ingestion by pinocytosis in the rectal epithelium and fat absorption in the intestinal epithelium were augmented (Tanaka et. al., 1995). Taking into consideration the physio-metabolic significance of thyroxine (T4), in the larval growth, survival and immune performances present chapter aimed to elaborate the applications of thyroxine during the fish larval culture for the improved natality of larvae.

Application of Thyroxine in the Larval Culture Medium:

Application in Feed: In-feed treatment of thyroxine can be done, however, more than its advantages it possesses demerits. Thyroxine incorporated in the feed can be given to fry/juveniles but we think about newly hatched larvae or larvae just after their yolk sac absorption they need live organisms for their feeding. Live-feed enrichment can be a way for application but there are cases when larger and aggressive individuals consume more and are competitive in obtaining a large quantity of food compared to smaller ones. In the hatcheries, small quantities of feed are used for larvae and to provide accurate quantities of thyroxine additive dose to each and every larva is a bit difficult task. Surpassing all these demerits it is the fact that diseased fish cease feeding. In this case, in-feed treatment cannot be a successful one.

Injection Treatment: When injection treatment is considered for the larvae/fry/juveniles, a prerequisite is fish should be anesthetized otherwise fish can be injured leading to a death. However, injecting a fish which has just absorbed its yolk sac is a very difficult task and there high chances of injury and death. However, injection treatment can be applied with female brooders so that possibly it can exert its effect on oocytes and on the larvae in the early development. For the larvae, injection treatment of thyroxine can be applied using microinjections and other new technologies but they are expensive, carried out on experimental basis, time consuming and cannot be operated by farmers. This paves the way for immersion treatment which has been proved successful over both the treatments.

Immersion Treatment: Application of thyroxine through immersion treatment has proved successful and efficient for the 5 days old larvae. Larval culture water was prepared by adding thyroxine hormone (Eltroxine, GlaxoSmithkline, India) into the tubs of pre-settled clean natural water and stirred well for proper mixing. The same media was used for topping the water quantity so as to maintain the water quality and precise concentrations of thyroxine (T4). The culture medium was freshly prepared after every 3 days and fully changed in all the tubs. The normal water was used as the control. Proper aeration was provided in the control as well as in the treatments. Thyroxine application in the larval culture waters through immersion treatment was carried in *Clarias batrachus* and *Anabas testudineus* larvae (Srivatava et. al., 2013; Ambulkar et. al., 2016; Sanap et. al., 2017).

Feeding Strategies: Feeding strategy for the immersion treatment was applied carefully to maintain the precise concentration of thyroxine in the larval waters. Different feeding strategies were used in the various studies depending upon availability and suitability of feed to the respective species. In the thyroxine immersion treatment of 15g *Clarias batrachus* larvae were fed with freshly hatched brine shrimp larvae ad libitum for 15 days in three regimes 1 day (feeding once a day), 2 days (feeding twice a day), 3 days (feeding thrice a day). Dead nauplii, fecal matter, and accumulated debris were siphoned out daily to maintain the hygienic conditions and the thyroxine water was topped to maintain the concentrations (Srivastava et. al., 2013). When the thyroxine immersion treatment was used for *Clarias batrachus* 5 days old larvae were fed with egg-custard, newly hatched brine shrimp larvae, and plankton. The micropipette was used for application of egg-custard into the larval tubs. The micropipette was slowly immersed in the tubs so as to avoid turbidity and retain the water quality. Further, the larvae were allowed to feed only for 1 hour and then the remaining custard was pipetted out to maintain the water quality. Plankton was collected in the bucket at evening hours, plankton water was sieved and the net was washed in the treatment tubs so as to maintain the concentrations of T_4 and to avoid the addition of extra water along with plankton. Larvae were fed three times a day, in the early morning hours with baby artemia, in the afternoon with egg-custard and at night with plankton (Ambulkar et. al., 2016). Modified feeding strategy based upon the availability and suitability of feed was used when thyroxine was applied to the larval waters of *Anabas testudineus,* they were fed with mixed zooplankton comprising Brachionus sp., Moina sp. and Daphnia sp. for the experimental period of 15 days. Plankton was collected and filtered through fine-meshed plankton net (0.04mm) to obtain the quantitative estimates of plankton of 1ml (112.19± 17.52 zooplankton numbers ml^{-1}×10^3) and were counted using Sedgwick-Rafter (S-R) cell under a stereo microscope (Motic SMZ 4 MP) so as to maintain the concentrations of thyroxine in the larval waters (Sanap et. al., 2017).

Findings: Effects of water enrichment with thyroxine (T_4) on the growth and natality of *Anabas testudineus* larvae for the experimental period of 15 days revealed that 2.5 μg/L and 5.0 μg/L thyroxine exhibited significantly higher ($P<0.05$) length gain (10.50±0.23mm), weight gain (44.50±2.60 mg), percent weight gain (1294.90±75.60 %), specific growth rate (17.55±0.64 % per day), and cent percent survival. The growth of larvae was also confirmed by microscopic examination of a longitudinal section of larvae (Sanap et. al., 2017). This study was evident with corroborative research work in the 5 days old larvae of *Clarias batrachus* where, 2 μg/L of thyroxine immersion treatment exhibited 88±1.66% survival (Ambulkar et. al., 2016). However, there is a supportive study where cent percent survival has been obtained using 6.0 μgL^{-1} thyroxine immersion treatments for the growth and survival of 15 mg larvae of *Clarias batrachus* (Srivastava et. al., 2013). In one day old yolk-sac larvae of Tilapia (*Oreochromis mossambicus*), immersion treatment of 0.05 ppm T_4 or 0.01 ppm T_3 significantly accelerated the differentiation and growth of all the fins, particularly pectoral and tail fins which also accelerated yolk absorption and transition to free-swimming activity in the larvae (Reddy and Lam, 1992). The injection treatment of females of *Oreochromis niloticus* with L-thyroxine (T_4) (1 or 10 µg T4/g BW) greatly enhanced the development of the digestive tract and accessory glands of larvae and the subsequent effect on larval growth and survival were observed. The study concluded that exogenous T4 in maternal circulation might have been transferred into oocytes and larvae and thyroid hormone appears to play some role in the early development and growth of larvae Nile tilapia, *O. niloticus* (Khallil et. al., 2011). The growth of otolith in Nile Tilapia at least during the juvenile stage is regulated by thyroid hormones (Shiao et.al., 2008). Post-larval of *Sicyopterus lagocephalus* (Teleostei: Gobioidei) experience a true metamorphic stage especially the change in the position of the mouth is significantly accelerated under the control of thyroid hormones at the time of its recruitment into the river (Taillebois et. al., 2011). During critical phases of first feeding of *Heteropneustes fossilis* (Singhi) combined treatment of thyroxine and cortisol resulted in food utilization and promoted vital developmental processes which resulted in uniform growth, decreased mortality, better survival and transformation of larvae to juveniles (Nayak et. al., 2004).

Future Prospects: It is very well known that there are adverse effects of antibiotics and drugs. However, there is little area explored for the pernicious effects of hormones especially thyroxine. Though, there sterling results of thyroxine during the fish larval development, there are thrust areas unsealed for the research on the effect of thyroxine-treated fishes upon the human consumption. Thyroxine is a protein-based hormone (a modified form of amino acids i.e. Tyrosine) and not a lipid-based hormone, hence these may reduce the chances of storage into the body. However, ultimately aquaculture contributes to the human food consumption there is a need to explore the effects of thyroxine-treated fishes upon human consumption.

Acknowledgment: The authors are grateful to the Director, ICAR-Central Institute of Fisheries Education, Mumbai for providing the facilities to conduct research work and supporting the development in academic career.

References:

1. Ambulkar, Rashmi S., C. S. Chaturvedi, Gupta Subodh, Krishna Gopal, N. K. Chadha, Sanap Bhushan, and A. K. Pandey. "Supplementation of thyroxine and vitamin C for enhanced growth and survival in early larval stages of Clarias batrachus (Linnaeus, 1758)." Journal of Experimental Zoology, India 19. 2 (2016): 815-819.
2. Bogazzi, Fausto, Luigi Bartalena, Sandra Brogioni, Alessandro Burelli, Lucia Grasso, Enrica Dell'Unto, Luca Manetti, and Enio Martino. "L-thyroxine directly affects expression of thyroid hormone-sensitive genes: regulatory effect of RXRβ." Molecular and cellular endocrinology 134. 1 (1997): 23-31.
3. Brown, Donald D. "The role of thyroid hormone in zebrafish and axolotl development." Proceedings of the National Academy of Sciences 94. 24 (1997): 13011-13016.
4. Janz, D.M. "Endocrine system. In: The Laboratory Fish, G.K.Ostrander, ed., Chap. 25. Academic Press, San Diego. (2000). In: Blanton, Michael L., and Jennifer L. Specker. "The hypothalamic-pituitary-thyroid (HPT) axis in fish and its role in fish development and reproduction." Critical reviews in toxicology 37. 1-2 (2007): 97-115.
5. Khalil, Noha A., Hassan MM Khalaf Allah, and Mostafa A. Mousa. "The effect of maternal thyroxine injection on growth, survival and development of the digestive system of Nile tilapia, Oreochromis niloticus, larvae." Advances in Bioscience and Biotechnology 2. 05 (2011): 320.
6. Nayak, P. K., T. K. Mishra, J. Mishra, and A. K. Pandey. "Effect of combined thyroxine and cortisol treatment on hatching of eggs, post-embryonic growth and survival of larvae of Heteropneustes fossilis." Journal of the Indian Fisheries Association 31 (2004): 125-137.
7. Power, D. M., Lynda Llewellyn, M. Faustino, Mari Ann Nowell, B. Th Björnsson, Ingibjörg E. Einarsdóttir, Adelino VM Canario, and Glen E. Sweeney. "Thyroid hormones in growth and development of fish." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 130. 4 (2001): 447-459.
8. Reddy, P. K., and T. J. Lam. "Role of thyroid hormones in tilapia larvae (Oreochromis mossambicus): I. Effects of the hormones and an antithyroid drug on yolk absorption, growth and development." Fish Physiology and Biochemistry 9. 5 (1992): 473-485.
9. Sanap, B.N., Sanap. V.N., Ambulkar, R.S., Sardar, P., Srivastava, P.P., Borah, B.C., and Kamble, S. "Effects of thyroxine augmented ambient water on the growth and survival during early life stages of *Anabas*

testudineus (Bloch, 1792) larvae". Proceedings of the International Conference on Recent Trends in Agriculture, Veterinary & Life Sciences-(2017): *in press*.

10. Shi Y –B., Wong J., Puzianowska-Kuznicka M. and Stolow M. A. "Tadpole competence and tissue-specific temporal regulation of amphibian metamorphosis: roles of thyroid hormone and its receptors. Bioassays 18 (1996): 391-399.
11. Shiao, J.C., Wu, S.M., Hwang, Y.P., Wu, D.P. and Hwang, P.P., "Evaluation of thyroid-mediated otolith growth of larval and juvenile tilapia". Journal of Experimental Biology 211. 12 (2008): 1919-1926.
12. Srivastava, P.P., Chowdhary, S., Jena, J.K., Raizada, S., Singh, A.K. and Kumar, V., "Synergistic Effects of Thyroxine and Feeding Regimes on Early Survival and Biomass Gain in Asian Catfish, Magur (*Clarias batrachus,* Linn.)". National Academy Science Letters 36. 3 (2013): 265-270.
13. Taillebois, L., Keith, P., Valade, P., Torres, P., Baloche, S., Dufour, S. and Rousseau, K., Involvement of thyroid hormones in the control of larval metamorphosis in *Sicyopterus lagocephalus* (Teleostei: Gobioidei) at the time of river recruitment. General and Comparative Endocrinology 173. 2 (2011.): 281-288.
14. Tanaka, M., Tanangonan, J.B., Tagawa, M., De Jesus, E.G., Nishida, H., Isaka, M., Kimura, R. and Hirano, T., Development of the pituitary, thyroid and interrenal glands and applications of endocrinology to the improved rearing of marine fish larvae. Aquaculture 135. 1-3 (1995): 111-126.

***