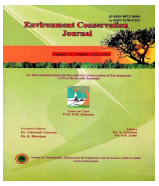
Environment Conservation Journal 24 (1):261-266, 2023

Journal homepage:https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)



Effect of exogenously administered thyroid hormones on gonadotropin, thyrotropin and deiodinases encoding genes in the catfish, *Heteropneustes fossilis*

Dinesh Raj Pant ✉
Fish Endocrinology Laboratory, Department of Zoology, University of Delhi, Delhi-110007 India.
Pooja Kumari
Fish Endocrinology Laboratory, Department of Zoology, University of Delhi, Delhi-110007 India.

ARTICLE INFO

Received : 28 August 2022
Revised : 27 October 2022
Accepted : 12 November 2022

Available online:

Key Words:
Deiodinase
Heteropneustes fossilis
Reproduction
Thyrotropin
Thyroid hormones

ABSTRACT

Thyroid hormones are known to regulate the basal metabolism rate of an organism. They are also known to regulate the seasonal reproduction of long-day breeding vertebrates in response to thyrotropin induced deiodinase enzymes switching in the brain. The current study attempted to investigate the effect of intraperitoneal administration of thyroxine (T4) and tri-iodothyronine (T3) hormones at various doses on gonadal recrudescence, plasma estradiol-17β and quantitative expression analysis of genes encoding for gonadotropin, thyrotropin, and deiodinases. The estradiol-17β levels were not affected by either thyroid hormone; however, the gonado-somatic index (GSI) and ovarian histology were varying. The gonadotropin releasing hormone 2 (*gnrh2*) and follicle stimulating hormone-β subunit (*fsh-b*) gene expressions correspond to the fish GSI and ovarian histology. The gene expressions show that T4 inhibits the expression of thyroid stimulating hormone-β subunit (*tsh-b*) and type 3 deiodinase (*dio3*), though it enhances the expression of type 2 deiodinase (*dio2*). T3, on the other hand, inhibits *tsh-b* and *dio2* expression while increasing *dio3* expression. In summary, the T4 appears to regulate gonadal recrudescence in *Heteropneustes fossilis* in a dose-dependent manner, whereas the T3 appears to have no effect on gonadal activity.

Introduction

Thyroid hormones, such as thyroxine (T4) and tri-iodothyronine (T3), are secreted from vertebrate thyroid follicles in response to thyroid stimulating hormone (TSH), released from pars distalis region of pituitary gland. Thyroid hormones are synthesized as a less potent pro-hormone T4, which undergoes an outer ring deiodination catalyzed by the DIO1 or DIO2 enzymes to get converted into active hormone T3. Thyroid hormones play an important role in physiology; their primary function is to maintain the basal metabolic rate in vertebrates it is also, involved in amphibian metamorphosis (Furlow and Neff, 2006) and fish metamorphosis (Power *et al.,* 2001). It has been reported in fish that thyroid hormone promotes gametogenesis and gonadal steroidogenesis (Cyr and Eales, 1996; Swapna *et al.,* 2006; Flood *et al.,* 2013; Tovo-Neto *et al.,* 2018). The thiourea (an antagonist of thyroid receptors), treatment depletes thyroid hormones; additionally, it reduces gonadal size, gonadal steroids, *gnrh2,* and *lh-b* expression in walking catfish (Swapna *et al.,* 2006). Hyperthyroidism, a condition of increased plasma thyroid level, stimulates the early gonadal recrudescence in pink salmon (Leatherland *et al.,* 1989), goldfish (Sohn *et al.,* 1999), cod (Norberg *et al.,* 2004) and stinging catfish (Biswas *et al.,* 2006). *Heteropneustes fossilis* is an annual breeding catfish, its annual ovarian cycle is divisible into four phases, *viz*., preparatory phase (February to April), pre-spawning phase (May-June), spawning phase (July-August) and post-spawning phase (September to January) (Sehgal and Sundararaj, 1970). The present

Corresponding author E-mail: *drpant@zoology.du.ac.in*
Doi:https://doi.org/10.36953/ECJ.17222519

study aims to identify the existence of the thyroid hormone-regulating genes, viz., thyroid stimulating hormone beta subunit (*tsh-b*), thyroid stimulating hormone receptor (*tsh-r*), type 2 deiodinase (*dio2*), type 3 deiodinase (*dio3*), along with gonadotropin releasing hormone 2 (*gnrh2*), follicle stimulating hormone-β subunit (*fsh-b*), and luteinizing hormone-β subunit (*lh-b*) and gonadal recrudescence after intraperitoneal administration of the thyroid hormones (T3 and T4), in the *H. fossilis*.

Material and Methods

Ethical clearance: All experimental procedures were performed in conformity with animal care and use guidelines approved by the Institutional Animal Ethics Committee (IAEC), at Department of Zoology, University of Delhi, Delhi, under recommendation by the Committee for the Purpose of Control and Supervision of Experiments for Animals (CPCSEA), New Delhi, India (file no. DU/ZOOL/IAEC-A/01/2019).

Specimen collection and maintenance: *H. fossilis*, were procured from backwaters of the river Yamuna and its tributaries near Delhi region (Lat. 28°35'N and Long. 77°12'E) in February month. The fish were acclimatized to laboratory conditions at a temperature of 25±1°C and a photoperiod regimen of 12L: 12D for seven days prior to the initiation of experiments.

Thyroid dose preparation: 0.5 mg/mL stock solution of thyroid hormone (T4 and T3 separately) was prepared in 5% ethanol. The fish were divided into nine groups, group injected with vehicle served as control, four groups were administered with T3 (5 ng, 50 ng, 250 ng, and 500 ng) and four groups were administered with T4 (5 ng, 50 ng, 250 ng, and 500 ng) intraperitoneally on alternate days. Thereafter, fish were maintained under laboratory conditions with 25±1°C of water temperature and a photoperiod regime of (L:D::12:12) for 60 days.

Sample collection and evaluation: Fish from each experimental group were sampled at the onset of experiment to serve as initial control and again on 60th day, sample was collected. Fish were anesthetized with 2-phenoxyethanol (0.001%) and weighed. Blood was collected from the caudal artery with the help of a heparinized syringe for estradiol-17β estimation using the Cayman estradiol kit (Cat no. 501890), according to the manufacturer's protocol. Following that, fish were decapitated, ovaries were excised, and the GSI was calculated. A small piece of ovarian tissue was fixed in Bouin's fixative and processed for histological examination using hematoxylin-eosin staining. The brain with pituitary was excised and processed for total RNA isolation, followed by cDNA preparation as per our laboratory standard protocol (Kumari *et al.,* 2020) . The primer pairs for the genes *tsh-b*, *tsh-r*, *dio2*, *dio3*, *gnrh2*, *fsh-b* and *lh-b* used for the quantitative analysis are mentioned in the table 1. The *β-actin* gene was taken as an endogenous control and the relative gene expression ($2^{-\Delta\Delta Ct}$) was calculated, the RNA from initial control was used for calibration.

Table 1: The sequence of the primer pairs employed for qRT-PCR in the present study are as follows.

S.no.	Gene	Primer	Primer sequence for qPCR	Product size
1	*β-actin*	Forward	5'- CGA AGA CGA CAG GAT TTG CT -3'	105bp
		Reverse	5'- GTT TGA AGC GCT CGT CTC TC -3'	
2	*tsh-β*	Forward	5'- GCT GTA CCT ATC AGG ACG TG -3'	141bp
		Reverse	5'- TGT GGG CAC ACT CAT CAC TG -3'	
3	*tsh-r*	Forward	5'- TGC TGT AAT GCT CGG GGG TT -3'	74bp
		Reverse	5'- GGT AAC TGC TCA CCC CTA ACG -3'	
4	*dio2*	Forward	5'- GTT CCC GTT CGA GGT GAA GAA -3'	125bp
		Reverse	5'- CAT TGT TGT CCA TGC AGT CGG CC -3'	
5	*dio3*	Forward	5' GTA CCA GAT CCC GCG CC -3'	110bp
		Reverse	5' ACG AGT TGT CCA TGG TGT CC -3'	
6	*gnrh 2*	Forward	5'- TGT GAG GCA GGA GAA TGC TG -3'	132bp
		Reverse	5'- GCT ATG GTG CCG GGA TAT GT-3'	
7	*fsh-β*	Forward	5'- CAC ACA CGC CTA CTA CTG AAC-3'	128bp
		Reverse	5'-AAC CTG ATG GTA CGC AGT ATT T-3'	
8	*lh-β*	Forward	5'-AGC CCG TTC TCT TCC ATC TA-3'	125bp
		Reverse	5'-CAG CTT AGT GCG ACA GGA TAT G-3'	

Statistical analysis: The statistical analysis one way ANOVA (P<0.05) was performed using IBM®SPSS 25.0 software. The results in bar and line graph were plotted as mean ± SEM. The significant difference was marked by using different superscripts.

Results and Discussion

Gonado-somatic index and estradiol-17β: The T3 treatment did not exhibit significant variation in GSI, nor did the plasma estradiol-17β levels. The GSI was significantly higher in the 50-ng and 250-ng T4-treated groups than in the two remaining T4-treated groups. However, the plasma estradiol-17β level among the T4 treated groups did not exhibit significant variation (Figure 1).

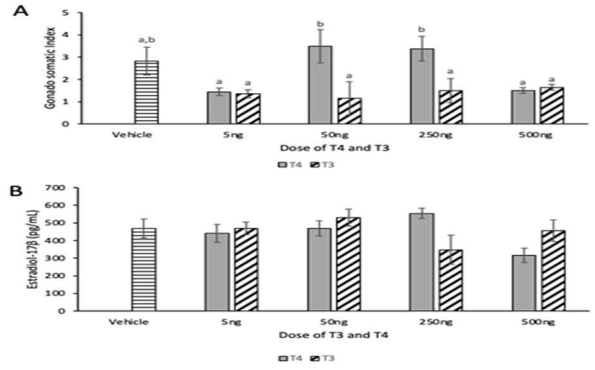


Figure 1: (A) Gonado-somatic index (GSI) and (B) Plasma estradiol-17β (pg/mL) estimation of intra-peritoneally T3 and T4 administered female *H. fossilis*. Values are presented as mean ± SEM (n=6). Bars with different superscripts indicate statistically significant differences and bars without superscript shows no variation (One way ANOVA followed by Tuckey test, p<0.05).

Gene expression analysis: The vehicle treated fish show the maximum expressions of *tsh-b* gene with significantly higher gene expression than 5 ng, 50 ng and 500 ng T3 and 5 ng and 250 ng T4 treated group, suggesting the inhibitory regulation of intraperitoneally administered T3 and T4 on brain *tsh-b* expressions (Figure 2a). The *dio2* expressions were increasing with the increasing dose of T4 with significant variation in expression between 500 ng T4 and 5 ng T4 treated group, suggesting the enhancement of expression of *dio2* by T4 administration in a dose dependent manner. On the contrary the T3 treatment reduces the *dio2* expression with increasing T3 dose, showing inhibitory effect (Figure 2c).

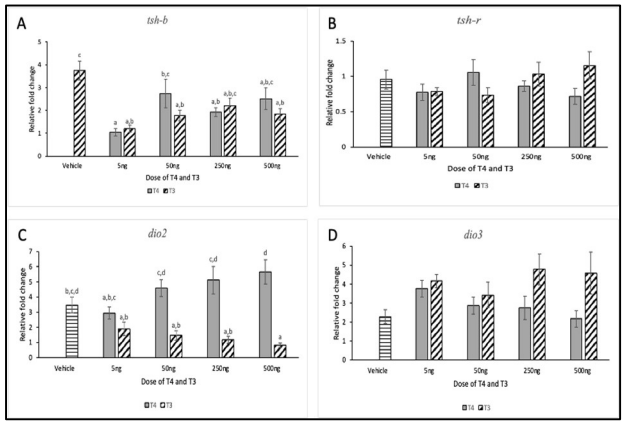


Figure 2: Quantitative expression analysis of (A) *tsh-b*, (B) *tsh-r*, (C) *dio-2*, (D) *dio-3* in the brain of intra-peritoneally T3 and T4 administered female *H. fossilis*. Values are presented as mean ± SEM (n=6). Bars with different superscripts indicate statistically significant differences and bars without superscript shows no variation (One way ANOVA followed by Tuckey test, p<0.05).

The expression of both *gnrh2* and *fsh-b* were significantly higher in the vehicle administered fish than all doses of T3 and 5 ng, 250 ng and 500 ng dose T4 treated groups. The expressions of both *gnrh2* and *fsh-b* are significantly lowest in T3 treated group, suggesting both T3 and T4 inhibits the expressions of *gnrh2* and *fsh-b* of which T3 is a potent inhibitor than T4 of these two genes (Figure 3a and 3b). The *tsh-r*, *dio3* and *lh-b* gene expression in the brain did not exhibit significant variation by intraperitoneal administration of T3 and T4 hormones (Figure 2b, 2d and 3c).

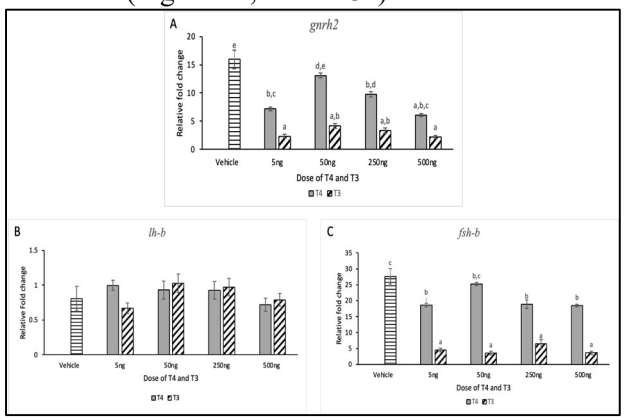


Figure 3: Quantitative expression analysis of (A) *gnrh2*, (B) *lh-b*, (C) *fsh-b* in the brain of intra-peritoneally T3 and T4 administered female *H. fossilis*. Values are presented as mean ± SEM (n=6). Bars with different superscripts indicate statistically significant differences and bars without superscript shows no variation (One way ANOVA followed by Tuckey test, p<0.05).

Ovarian histology: The catfish sacrificed at the commencement of the experiment had a regressed ovary (GSI ~0.7) containing stage 1 (non-yolky oocytes) and stage 2 (showing cortical alveoli) oocytes in equal proportions (Figs. 4a. At the end of the experiment, after 60 days, the ovarian histology showed the presence of vitellogenic oocytes in group injected with 50 ng dose of T4 followed by 250 ng dose of T4 (Figure 4).

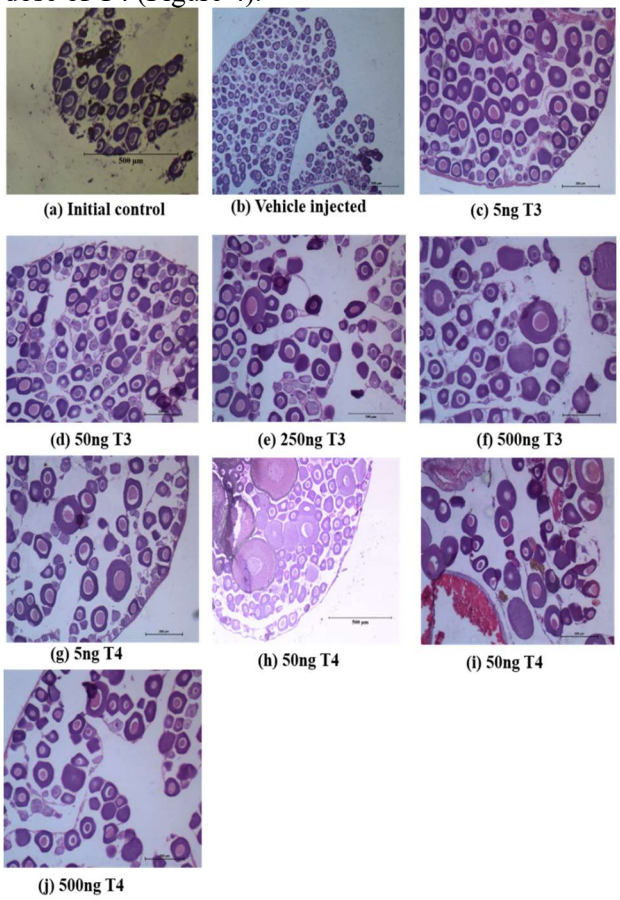


Figure 4: Transverse section of *H. fossilis* ovary, stained with hematoxylin-eosin; at the commencement of the experiment (a); vehicle administered (b); Tri-iodothyronine (T3) administered (c-f); Thyroxine (T4) administered (g-j).

The intra-peritoneal administration of thyroid hormones (T3 or T4) during the preparatory phase was studied in *H. fossilis*. The vehicle administered fish have highest *tsh-b* expression, followed by T4, and least in T3 administered in a dose dependent manner. The varying expression of *tsh-b* suggests that thyroid hormone downregulates *tsh-b* expression via a negative feedback loop. The *tsh-r*, *dio3* and *lh-b* gene expression in *H. fossilis* does not exhibit significant variation in gene expression after intra-peritoneal thyroid administration. The thyroid hormone is well known to be metabolized by deiodinases, and we found that T4 administration results in *dio*2 upregulation and *dio3* inhibition with increasing doses. On the contrary, T3 administration upregulates the *dio3* expression and inhibits the *dio2* expression in a dose dependent manner, however, both supporting and contradicting scenario have appeared in the existing literature (Coimbra *et al.,* 2005; Yamamura *et al.,* 2006; Gereben *et al.,* 2008; Hernandez *et al.,* 2010; Sabatino *et al.,* 2020). In a previous study, we found that the expression of *tsh-b*, *dio2*, and *dio3* in the brain varies seasonally and has a positive correlation with the GSI of *H. fossilis* (Pant *et al.,* 2023). The *gnrh2*, and *fsh-b* genes express maximally in the vehicle administered group, low expression in T4-administered fish and least in T3 administered group, suggesting an anti-gonadal effect of the thyroid hormone (T4 or T3).

The T3 treatment is also known to alter the glycoprotein alpha subunit (*cg-a*) expression based on sex and reproductive phase in *Carassius auratus* (Sohn *et al.,* 1999; Yoshiura *et al.,* 1999). The alteration in *cg-a* expression may further affect the hormone levels of TSH, LH, and FSH. The *tsh-b* gene expression and thyroid hormone have been shown to have an inverse relationship in several studies; *tsh-b* expression is inhibited by a high dose of T3 in *Oncorhynchus kisutch* (Larsen *et al.,* 1997) and *Carassius auratus* (Sohn *et al.,* 1999, Ma *et al.,* 2020) and T4 in *Carassius auratus* (Yoshiura *et al.,* 1999). T3 inhibits the *tsh-b* expressions in the goldfish pituitary under *in-vitro* conditions, though its impact can be altered by other factors *in-vivo* (Allan and Habibi, 2012). T3 is also known to reduce circulating LH level which implies gonadotropin inhibition by thyroid hormone in *Carassius auratus* (Allan and Habibi, 2012). In histological studies, T4 exposure reduces the size of gonadotropes and enhances the gonadotropin effect on ovarian development in *Carassius auratus* (Hurlburt, 1977). The thyroid hormone enhances Sertoli cells proliferation and spermatogonial cell maturation in *Danio rerio* (Morais *et al.,* 2013). Circulating T3 is known to induce vitellogenesis *via* upregulation of estrogen receptors in liver of *Carassius auratus* (Nelson and Habibi, 2016; Hur *et al.,* 2020).

In summary, the intra-peritoneal administration of T3 and T4 hormone inhibits the expressions of *tsh-b*, *gnrh2* and *fsh-b* genes in the brain. The *dio2* expressions were enhanced by T4 and inhibited by the T3 administration may infer the enhancement by substrate and inhibition by product. However, *tsh-r*, *dio3*, and *lh-b* expressions were not altered by either T3 or T4 treatment.

Conclusion

The *tsh-b, dio2,* and *dio3* expressions in the brain exhibit variation with thyroid hormone administration. The above-discussed results suggest thyroid hormones affect deiodinase expression via a negative feedback loop. The expression of *gnrh2*, *fsh-b,* and *lh-b* genes can be altered by the administration of thyroid hormones in a dose-dependent manner. The results of the experiment support the involvement of the thyroid hormones in altering the seasonal reproductive cycle in the female *H. fossilis*.

Acknowledgement

The authors are thankful to Prof. Neeta Sehgal, Department of Zoology, University of Delhi, for her guidance and providing the necessary facilities to conduct the experiments. The DRP is also thankful to CSIR for providing research fellowship.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Allan, E. R., & Habibi, H. R. (2012). Direct effects of triiodothyronine on production of anterior pituitary hormones and gonadal steroids in goldfish. *Molecular Reproduction and Development*, *79*(9), 592-602.

Biswas, A. K., Seoka, M., Tanaka, Y., Takii, K., & Kumai, H. (2006). Effect of photoperiod manipulation on the growth performance and stress response of juvenile red sea bream (*Pagrus major*). *Aquaculture*, *258*(1-4), 350-356.

Coimbra, A. M., Reis-Henriques, M. A., & Darras, V. M. (2005). Circulating thyroid hormone levels and iodothyronine deiodinase activities in Nile tilapia (*Oreochromis niloticus*) following dietary exposure to Endosulfan and Aroclor 1254. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *141*(1), 8-14.

Cyr, D. G., & Eales, J. G. (1996). Interrelationships between thyroidal and reproductive endocrine systems in fish. *Reviews in Fish Biology and Fisheries*, *6*(2), 165-200.

Flood, D. E., Fernandino, J. I., & Langlois, V. S. (2013). Thyroid hormones in male reproductive development: evidence for direct crosstalk between the androgen and thyroid hormone axes. *General and comparative endocrinology*, *192*, 2-14.

Furlow, J. D., & Neff, E. S. (2006). A developmental switch induced by thyroid hormone: *Xenopus laevis* metamorphosis. *Trends in Endocrinology & Metabolism*, *17*(2), 40-47.

Gereben, B., Zavacki, A. M., Ribich, S., Kim, B. W., Huang, S. A., Simonides, W. S., .Bianco, A. C. (2008). Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocrine reviews*, *29*(7), 898-938.

Hernandez, A., Quignodon, L., Martinez, M. E., Flamant, F., & St. Germain, D. L. (2010). Type 3 deiodinase deficiency causes spatial and temporal alterations in brain T3 signaling that are dissociated from serum thyroid hormone levels. *Endocrinology*, *151*(11), 5550-5558.

Hur, S. P., Mahardini, A., Takeuchi, Y., Imamura, S., Wambiji, N., Rizky, D., & Takemura, A. (2020). Expression profiles of types 2 and 3 iodothyronine deiodinase genes in relation to vitellogenesis in a tropical damselfish, *Chrysiptera cyanea*. *General and Comparative Endocrinology*, *285*, 113264.

Hurlburt, M. E. (1977). Role of the thyroid gland in ovarian maturation of the goldfish, *Carassius auratus* L. *Canadian journal of zoology*, *55*(11), 1906-1913.

Kumari, P., Kumar, M., Sehgal, N., & Aggarwal, N. (2020). In silico analysis of kiss2, expression studies and protein–protein interaction with gonadotropin-releasing hormone 2 (GnRH2) and luteinizing hormone beta (LHβ) in *Heteropneustes fossilis*. *Journal of Biomolecular Structure and Dynamics*, 1-15.

Larsen, D. A., Dickey, J. T., & Dickhoff, W. W. (1997). Quantification of salmon α-and thyrotropin (TSH) β-subunit messenger RNA by an RNase protection assay: Regulation by thyroid hormones. *General and Comparative Endocrinology*, *107*(1), 98-108.

Leatherland, J. F., Lin, L., Down, N. E., & Donaldson, E. M. (1989). Thyroid hormone content of eggs and early developmental stages of five *Oncorhynchus species*. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*(12), 2140-2145.

Ma, Y., Ladisa, C., Chang, J. P., & Habibi, H. R. (2020). Seasonal related multifactorial control of pituitary gonadotropin and growth hormone in female goldfish:

Influences of neuropeptides and thyroid hormone. *Frontiers in endocrinology*, *11*, 175.

Morais, R. D. V. S., Nóbrega, R. H., Gómez-González, N. E., Schmidt, R., Bogerd, J., França, L. R., & Schulz, R. W. (2013). Thyroid hormone stimulates the proliferation of Sertoli cells and single type A spermatogonia in adult zebrafish (*Danio rerio*) testis. *Endocrinology*, *154*(11), 4365-4376.

Nelson, E. R., & Habibi, H. R. (2016). Thyroid hormone regulates vitellogenin by inducing estrogen receptor alpha in the goldfish liver. *Molecular and Cellular Endocrinology*, *436*, 259-267.

Norberg, B., Brown, C. L., Halldorsson, O., Stensland, K., & Björnsson, B. T. (2004). Photoperiod regulates the timing of sexual maturation, spawning, sex steroid and thyroid hormone profiles in the Atlantic cod (*Gadus morhua*). *Aquaculture*, *229*(1-4), 451-467.

Pant, D. R., Ila, K., Sahu, U.B., & Sehgal, N. (2023). Temporal expression of thyroid hormone regulating genes (*tsh-b*, *tsh-r*, *dio2* and *dio3*) and their correlation with annual reproductive cycle of the Indian freshwater catfish, *Heteropneustes fossilis* (Bloch). *Journal of Applied and Natural Science*, *15*(1).

Power, D. M., Llewellyn, L., Faustino, M., Nowell, M. A., Björnsson, B. T., Einarsdóttir, I. E., & Sweeney, G. E. (2001). Thyroid hormones in growth and development of fish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *130*(4), 447-459.

Sabatino, L., Kusmic, C., & Iervasi, G. (2020). Modification of cardiac thyroid hormone deiodinases expression in an ischemia/reperfusion rat model after T3 infusion. *Molecular and Cellular Biochemistry*, *475*(1), 205-214.

Sehgal, A., & Sundararaj, B. I. (1970). Effects of various photoperiodic regimens on the ovary of the catfish, *Heteropneustes fossilis* (Bloch) during the spawning and the postspawning periods. *Biology of Reproduction*, *2*(3), 425-434.

Sohn, Y. C., Yoshiura, Y., Kobayashi, M., & Aida, K. (1999). Seasonal changes in mRNA levels of gonadotropin and thyrotropin subunits in the goldfish, *Carassius auratus*. *General and comparative endocrinology*, *113*(3), 436-444.

Swapna, I., Rajasekhar, M., Supriya, A., Raghuveer, K., Sreenivasulu, G., Rasheeda, M. K., & Senthilkumaran, B. (2006). Thiourea-induced thyroid hormone depletion impairs testicular recrudescence in the air-breathing catfish, *Clarias gariepinus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *144*(1), 1-10.

Tovo-Neto, A., da Silva Rodrigues, M., Habibi, H. R., & Nóbrega, R. H. (2018). Thyroid hormone actions on male reproductive system of teleost fish. *General and comparative endocrinology*, *265*, 230-236.

Yamamura, T., Yasuo, S., Hirunagi, K., Ebihara, S., & Yoshimura, T. (2006). T_3 implantation mimics photoperiodically reduced encasement of nerve terminals by glial processes in the median eminence of Japanese quail. *Cell Tissue Res.,* **324**, 175–179.

Yoshiura, Y., Sohn, Y. C., Munakata, A., Kobayashi, M., & Aida, K. (1999). Molecular cloning of the cDNA encoding the β subunit of thyrotropin and regulation of its gene expression by thyroid hormones in the goldfish, *Carassius auratus*. *Fish Physiology and Biochemistry*, *21*(3), 201-210.