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Comparison of gonadotropin releasing hormone (GnRH), luteinizing hormone (LHb) and follicle stimulating hormone (FSHb) genes in Indian spiny loach (*Lepidocephalichthys thermalis*) at different stages of oogenesis and photoperiods

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Abstract
In *Lepidocephalichthys thermalis* the cDNA was synthesized from the ovary tissues and polymerase chain reaction were carried out for gonadotropin releasing hormone II, gonadotropin releasing hormone III, luteinizing hormone and follicle stimulating hormone genes; the PCR products sizes of the four genes were 270-bp, 250-bp, 290-bp and 260-bp respectively. The expressions of the four genes were compared at different stages of oogenesis and in the initial phase perinucleolus stage follicle, only gonadotropin releasing hormone II & III genes were expressed. In cortical alveolus stage, lipid droplet stage and mid vitellogenic stage except luteinizing hormone gene all other genes were expressed. In post vitellogenic stage follicle except follicle stimulating hormone genes all other genes were present. When the expressions of the four genes were compared at different photoperiods, it showed that the expression of genes were very less during 9:00 AM, moderate during 10:00 AM and was high during 12:00 noon.

Keywords: Follicle stimulating hormone, gonadotropin releasing hormone, *Lepidocephalichthys thermalis*, luteinizing hormone, oogenesis and photoperiods

Introduction
Lepidocephalichthys thermalis, was one of the commonly occurring loach in the extreme southwest of peninsular India. The loaches have more variation in their life histories and were of importance in both aquarium trade as well as aquaculture. The loach, for a long time, had been considered as traditional Chinese medicine in folk remedies for treatment of hepatitis, osteomyelitis, carbuncles, inflammation and cancer as well as for restoration to health in debilities caused by various pathogens and aging [1]. Apart from its economic value in East Asia, loach was also a promising model animal to study evolutionary biology for polyploidy and considered as an ideal model animal in genetics and toxicology [2, 3, 4]. Gonadotropin releasing hormone (GnRH) was a neuropeptide known to regulate the gonadotropin expression and secretion in vertebrates. To date, fourteen forms of GnRH decapeptides have been found in vertebrates, in which at least one unique form has been determined from a representative species of all vertebrate classes. Ten GnRH forms out of the fourteen were found in fish and six of them in teleosts: chicken GnRH II (cGnRH II) [5], mammalian GnRH (mGnRH) [6, 7], salmon GnRH (sGnRH) [8], catfish GnRH (cfGnRH) [9], sea bream GnRH (sbGnRH) [10], herring GnRH (hGnRH) [11] and Medaka GnRH (mdGnRH) [12]. cGnRH II was present in all vertebrate classes examined to date, and the mGnRH form was present in primitive non-teleostean bony fish, primitive teleosts (eels and others), amphibians and mammals. sGnRH, cfGnRH and sbGnRH were found in higher teleosts. This taxonomic distribution indicates that cGnRH II and mGnRH were ancient vertebrate forms that gave rise to other variants [13].
The first teleost leutinizing hormone (LHb) subunit gene to be isolated and sequenced was that of the Chinook salmon, *Onchorhynchus tshawytscha* (cs GtHIIb or cs LHb) by Xiong and Hew in 1991. This gene appears in the genome in a single copy and, like its mammalian homologs, contains three exons and two introns; the first introns were up-stream of the translational start site. This gene was seen to undergo alternative splicing to produce two transcripts varying by12 nucleotides, but differential functions of these isoforms have not been shown [14].

A single gene was identified for gold fish and two LHb genes were found in common carp, which encode the same protein. These genes share similar structure to the mammalian LHb genes, in regard to number and the position of introns and exons, and in the cDNA sequences, although the fish genes were generally shorter [15,16]. All four genes contain TATA box sequences, which were located 21 to 25 base pairs (bp) from the transcriptional start site. However the 5' flanging region of the cyprinids genes show little similarity with that of the csLHb gene [17].

Follicle stimulating hormone (FSHb) gene promoters have been published for gold fish and tilapia [18, 19]. Promoter regions of all of these sequences vary widely between species and even the "duplicated" gold fish FSHb genes were quiet divergent beyond 300 bp upstream of the transcriptional start site [19]. All of these genes contain large first introns, while the first exons are particular short: in the Chinook salmon (cs FSHb) the first introns over 6.2 kb while the first exon was just 27 bp. Although the csFSHb has just one transcriptional start site, the tilapia gene contains multiple start site [20]. Of the three species, only the gold fish proximal promoter sequences contain consensus TATA boxes, located at -26 bp. The tilapia FSHb TATA box was contained further upstream at -91bp, while the Chinook salmon FSH (csFSH) b gene proximal promoter contains only a TATA box in reverse orientation, at -43 bp. This indicates further the likelihood that divergent mechanisms regulate transcription of these homologous genes, possible relying considerably on other ubiquitous transcription factors to recruit the general transcription machinery [17]. *Lepidocephalichthys thermalis* forms a potential candidate species for aquaculture being a native species it also has high market demand with easy domestication procedure. Although loaches have an ornamental value and native or endemic status in India, very few studies have been carried out so far on their reproductive biology. In this article we discuss about the comparison of GnRH, FSH and LH genes at different stages of oogenesis and photoperiod which will provide an outline idea about the reproductive stages and conditions of *Lepidocephalichthys thermalis.*

Materials and Methods

The loaches for the experiments were collected from Chola fish farm, Vaduvoor, Thiruvarur District of Tamil Nadu, India. Total RNA was extracted from ovarian tissues of *Lepidocephalichthys thermalis* using Trizol™ reagent (Favourgen Biotech Corp., Taiwan) and *cDNA* was synthesized from total RNA(5µg) using Revertaid Reverse Transcriptase Enzyme (Thermo Fisher Scientific India Pvt. Ltd., Mumbai) as per the manufacturer's instructions. PCR (Eppendorf AG, Germany) was performed to amplify the desired cDNA fragments from the template. PCR amplification was performed in a total volume of 25 µl volume including 21 µl of *Taq* 2X PCR master mix red (1.5 U *Taq* DNA polymerase) with 1.5mM $MgCl_2$ (Ampliqon, Denmark), 1 µl forward primer, 1 µl of reverse primer and 2 µl of template cDNA. Primer used for amplifying GnRH II, GnRH III, LHb and FSHb genes were in Table1.The PCR amplification conditions are represented in Table 2. The PCR product sizes were determined by 2% agarose gel electrophoresis run along with DNA marker i.e., 100bp DNA ladder.

Table 1: Primers selected for amplification of GnRH II, GnRH III, LHb and FSHb cDNA.

S. No	Gene	Primers		Reference
1	GnRH II	Forward	ATGGTGCTGGTCTGCAGGCTG	Kuo *et al*, 2005
		Reverse	GTAGGAACTGCTGCAAATGGGT	
2	GnRH III	Forward	CACAGCAGTTTTAGCATGGAGTG	Kuo *et al*, 2005
		Reverse	ACACTCTTCCCCGTCTGTCGG	
3	LHb	Forward	CAAGAGCCCATTTTCCAC	So *et al*, 2005
		Reverse	AGGCTGCAGTCGACAGCT	
4	FSHb	Forward	CAGCTGTCGGCTCACCAATA	NCBI/Primer-BLAST(AB003583.1)
		Reverse	GCAAAGCAGTGTTTGGTTTCC	

Table 2: PCR protocol used for amplifying GnRH II, GnRH III, LHb and FSHb cDNA

Step	Process	Temperature	Duration	
1	Initial denaturation	94 °C	10 min	
2	Denaturation	94 °C	30 sec	35 cycles
3	Annealing	52 °C (GnRH II and GnRH III)	40 sec	
		53 °C (LHb)		
		51 °C (FSHb)		
4	Extension	72 °C	1 min	
5	Final extension	72 °C	10 min	

The gonads of the female fishes were taken and examined under different developmental stages. The five stages of development were adopted from Guzman *et al*. (2014) with slight modification. The samples for genomic analysis were taken under different photoperiods to analyze the expression of GnRH II, GnRH III, FSHb and LHb genes based on examinations of So *et al*. (2005) with slight modifications. Three different time periods were taken for examination, includes 9:00 AM, 10:00 AM and 12:00 noon respectively for the genomic analysis to analyze the expression of GnRH II, GnRH III, FSHb, LHb genes.

Results and Discussion

PCR product sizes were 270 for GnRH II, 250 for GnRH III, 290 LHb and 260 for FSHb. The expression of GnRH II, GnRH III, FSHb and LHb genes were analyzed and compared based on the different stages of oogenesis, it showed that in the initial phase perinucleolus stage follicle (Fig. 1) only GnRH II and GnRH III genes were expressed, FSHb and LHb genes were not expressed. In cortical alveolus stage follicle

(Fig. 2), lipid droplet stage follicle (Fig. 3) and mid vitellogenic stage follicle (Fig. 4) except LHb gene all the three genes were expressed. In the post vitellogenic stage follicle (Fig. 5) except FSHb gene all the three genes were observed.

Table 3: Comparison of the expression of GnRH II, GnRH III, FSHb and LHb genes of *Lepidocephalichthys thermalis* in relation to different stages of oogenesis

Oogenesis stages	GnRH II	GnRH III	LHb	FSHb
Perinucleolus stage follicle	Positive	Positive	Negative	Negative
Cortical alveolus stage follicle	Positive	Positive	Negative	Positive
Lipid droplet stage follicle	Positive	Positive	Negative	Positive
Mid vitellogenic stage follicle	Positive	Positive	Negative	Positive
Post vitellogenic stage follicle	Positive	Positive	Positive	Negative

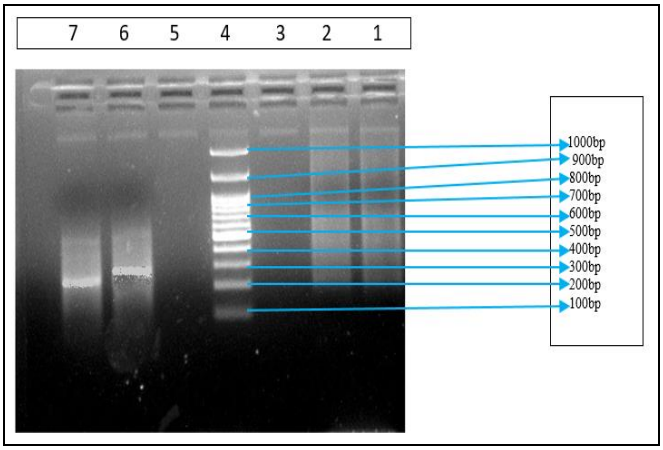

Fig 1: Perinucleolus stage follicles of *L. thermalis*; Lane 4: 100 bp ladder; Lane 6: GnRH III; Lane 7: GnRH II.

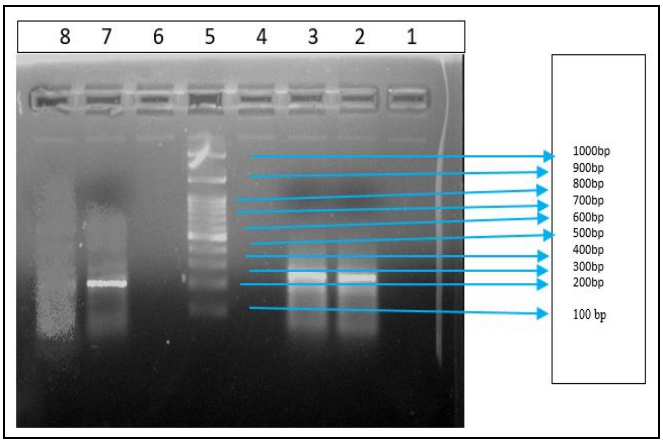

Fig 2: Cortical alveolus stage follicles of *L. thermalis*; Lane 5: 100 bp ladder; Lane 2: GnRH II; Lane 3: GnRH III; Lane 7: FSHb.

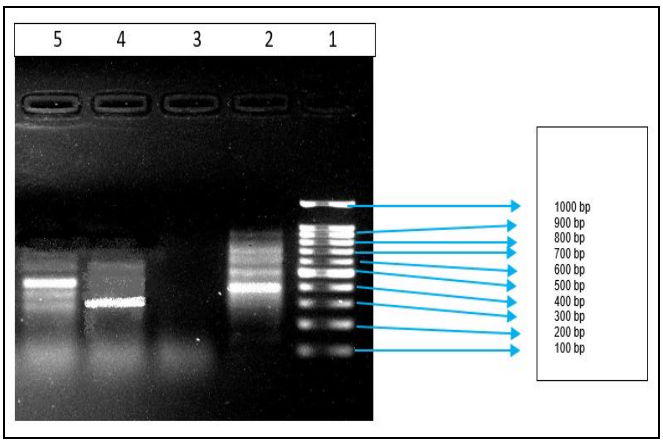

Fig 3: Lipid droplet stage follicles of *L. thermalis*; Lane 1: 100 bp ladder; Lane 2: FSHb; Lane 4: GnRH II; Lane 5: GnRH III.

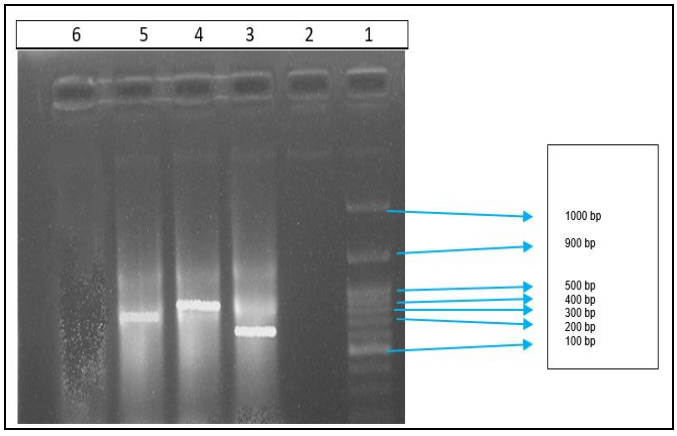

Fig 4: Mid vitellogenic stage follicles of *L. thermalis*; Lane 1: 100 bp ladder; Lane 3: GnRH II; Lane 4: GnRH III; Lane 5: FSHb.

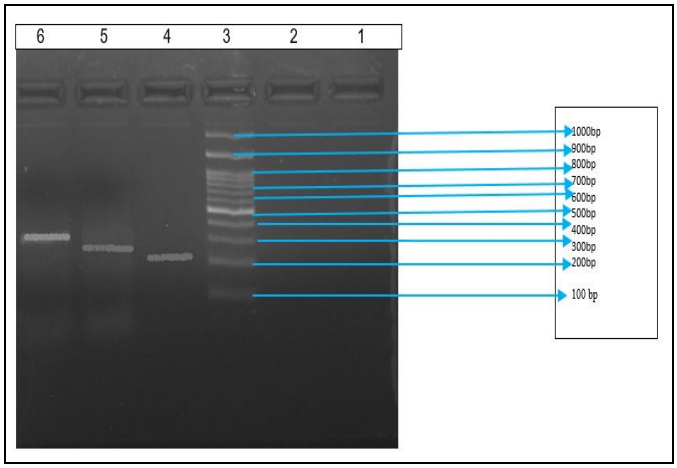

Fig 5: Post vitellogenic stage follicles of *L. thermalis*; Lane 3: 100 bp ladder; Lane 4: GnRH II; Lane 5: GnRH III; Lane 6: LHb.

When the expression of GnRH II, GnRH III, FSHb and LHb genes were compared at different photoperiods [22], it showed that the expression of genes were very less during morning 9:00 AM and moderate during 10:00 AM. The expression of genes was high during 12:00 noon

Gonadotropin-releasing hormone (GnRH) is well known for its role in moderating gonadotropin release from the pituitary. GnRH is a member of family of neuropeptides that play a key role in the development and maintenance of reproductive function in vertebrates. The midbrain neuron population in fish is believed to be exclusively GnRH-II-producing neurons and these cells are suggested to play a role in reproductive behavior [23]. The PCR amplified product size of GnRH II gene examined in this study was 270 bp whereas in hard-lipped barb (*Osteochilus hasselti*) Prayogo *et al.* (2011) amplified a product of 253 bp from brain cells and in catla

(*Catla catla*) Rather *et al*. (2015) amplified a product of 202 bp from brain cells. The brain cells were not used in this study because the size of the brain of *L.thermalis* is too small (insufficient in quantity) to carry out the genomic analysis, therefore the analysis was conducted on the ovarian tissue only.

GnRH III gene is expressed mainly in the olfactory system. GnRH III constitutes the main hypophysiotrophic factor governing the release of gonadotropins from the pituitary gonadotropes and these neurons innervate the pituitary [26]. The highest content of pituitary GnRH is the form that is found in the preoptic neurons [11, 27, 28]. The amplified PCR product of GnRH III in the present study was 250 bp from ovarian cells of *Lepidocephalichthys thermalis.* Prayogo *et al*. (2011) reported that in hard-lipped barb (*Osteochilus hasselti*), a product of 285 bp for GnRH-III from brain cells.

Gonadotropins (GTHs), follicle stimulating hormone (FSH) and luteinizing hormone (LH), are critical hormones in the regulation of reproduction in vertebrates, including fish [29]. FSH is involved in the initiation of gametogenesis and regulation of gonadal growth, whereas LH mainly regulates gonadal maturation, spermiation and ovulation. FSH and LH are complex heterodimeric glycoproteins, consisting of a common α subunit and a hormone-specific β subunit, encoded by different genes [30, 31]. Both subunits bind non-covalently into the gonadotropic cell, to form the biologically active dimeric hormone [32].

The first teleost LHb subunit gene to be isolated and sequenced was that of the Chinook salmon, *Onchorhynchus tshawytscha* (cs GtHIIb or cs LHb) by Xiong and Hew (1991). The product size of LHb reported by Rather *et al*. (2016) was 629 bp in catla (*Catla catla*) from brain cells. In the present study, the PCR product size of LHb was 290 bp from ovarian cells of *Lepidocephalichthys thermalis*. The LHb product size in Zebra fish was 958 bp from brain cells [22].

The PCR product size of FSHb gene examined in this study was 260 bp from ovarian cells of *Lepidocephalichthys thermalis*. In zebra fish, So *et al*. (2005) recorded 1038 bp size of FSH from brain cells. This implies that the expression of GnRH, FSHb and LHb genes are not confined to brain and pituitary, but also express in the ovarian tissues. The identification of GnRH, FSHb and LHb genes synthesied in gonads gives a relatively new direction about their function. The first evidence for expression of GnRH in gonads was in rat ovary [34]. The first report of GnRH gene expression in the gonads (ovary and testis) of adult midshipman *(Porichthys notatus)* was elucidated by Northern blotting [35]. A number of the GnRH forms are found in fish gonads of several fishes. For instance sGnRH, cGnRHII and sbGnRH, are reported in testis of the cichlid, *Haplochromis burtoni* [36] by PCR; sGnRH (mRNA-1 and mRNA-2) and cGnRH-II are found in ovary and testis of rainbow trout by sequencing [37]; sGnRH (mRNA-2) was detected in ovary and testis of sockeye salmon by sequencing; GnRH-I was found in adult sea lamprey testis but not in the ovary as examined by Northern blotting [38].

Sherwood and Adams (2005) stated that GnRH was best known in vertebrates for its expression in neurons and play a role in stimulating the release of gonadotropins from the pituitary. However, expression of GnRH, FSHb and LHb along with their receptors was not confined to the brain and pituitary but is widespread in peripheral tissues. Two sites of interest were the ovary and testis because they express both the genes (GnRH, FSHb and LHb) and their receptors. Therefore the result of the present study was in agreement with earlier studies [39].

Oogenesis was the developmental process by which an oogonium becomes a fully grown, mature, and fertilizable egg. As in other vertebrates, oogenesis in fishes is regulated primarily by gonadotropin releasing hormone (GnRH). Further regulation is controlled by pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), through interaction with their respective receptors, FSH receptor (FSHr) and luteinizing / choriogonadotropin receptor (LHcgr), in the ovary [40, 41]. Most studies of the biological activities of fish gonadotropins have focused on LH regulation of sex steroid biosynthesis and final oocyte maturation [42, 43] and FSH regulation of early gonadal recrudescence, whereas relatively few studies have investigated how FSH and LH may regulate oocyte growth and development during earlier stages of oogenesis [41, 44].

Guzman *et al*. (2014) stated that the expression of FSHb was at its peak during mid vitellogenic stage and expression of LHb was at its peak during post vitellogenic stage. In the present study, the expression of FSHb and LHb genes were compared at five different stages of oogenesis, namely, perinucleolus stage, cortical alveolus stage, lipid droplet stage, mid vitellogenic follicle stage and post vitellogenic stage. It showed that in the initial phase perinucleolus stage, FSHb and LHb genes were not expressed. In cortical alveolus stage, lipid droplet stage and mid vitellogenic stage FSHb gene was expressed but LHb gene was not expressed. In the post vitellogenic stage, LHb gene was expressed and FSHb gene was not expressed. Therefore the result of the present study was in agreement with that of Guzman *et al*. (2014) in respect of mid vitellogenic and post vitellogenic stages.

The expression of GnRH II and GnRH III genes was also compared at different stages of oogenesis. The results showed that both GnRH II and GnRH III genes were expressed in all the five stages (perinucleolus stage, cortical alveolus stage, lipid droplet stage, mid vitellogenic stage and post vitellogenic stage) of oogenesis. The presence of GnRH II and GnRH III in all five stages showed the importance of GnRH gene activities in the whole process of oogenesis. The presence of FSH gene in mid vitellogenic stage conforms its role in early oocyte maturation and presence of LH in post vitellogenic stage ensure its role in final gonadal maturation. It implies that a group of genes that expressed during secondary oocyte growth and suppressed during maturation, show a strong and positive correlation with ovarian FSH, LH and GnRH genes, and provides information on their involvement in the regulation of oogenesis process.

The temporal expression profiles of *FSHb, LHb,* and *cga* (common gonadotropic 'a' subunit) in the pituitary during the daily ovulatory cycle was investigated by So *et al*. (2005). Based on their observations, the germinal vesicle in the full-grown oocyte starts to migrate from the center to the periphery at around 01:00 AM, which seemed to be the time when the expression of all gonadotropic (GTH) subunits reached their peak levels. The expression levels appeared to remain high until 04:00 AM, when the germinal vesicle had already reached the periphery. Therefore, the expression of FSHb and LHb were at its peak during 1:00 AM, and declines till 7:00 AM and then again increases till 12:00 noon. In the present study**,** when the expression of GnRH II, GnRH III, FSHb and LHb genes were studied at different photoperiods viz., 9:00 AM, 10:00 AM and 12:00 noon. The results showed

that the expression of genes were very less during 9:00 AM and moderate at 10:00 AM and high during 12:00 noon. It implies that the secretion of GnRH II, GnRH III, FSHb and LHb genes is related to the photoperiods and increases with increasing photoperiod from morning 9:00 AM until 12:00 noon. Hence, the expression of the genes observed between 9:00 AM and 12:00 noon and it was found high at 12:00 noon in the present study.

This pattern of expression was understandable for LHb, because LH has been well known to be involved in inducing final oocyte maturation and ovulation. However, the interesting phenomenon is that the obvious concurrent elevation of FSHb expression at 9:00 AM, 10:00 AM and 12:00 noon, suggesting a functional role for FSH in the final stage of follicle development. The expression pattern of GnRH confirms the significant role of GnRH II and GnRH III in the teleost reproduction. The results obtained in this study, will provide a brief idea about the hormonal state of GnRH II, GnRH III, LHb and FSHb at three different photoperiods (9:00 AM, 10:00 AM and 12:00 noon).

Conclusion

In conclusion, the results from present study will serve as a primary data for further research and development in the reproductive genomic aspects of Indian spiny loach (*Lepidocephalichthys thermalis*). It provides the idea about maintenance of Indian spiny loach (*Lepidocephalichthys thermalis*) and the way of preparing the samples for maturation that helps in further analysis in the aspect of reproductive genomic study. The PCR products can be further analyzed and sequenced to get better knowledge about the gonadotropin-releasing hormone II (GnRH II), gonadotropin-releasing hormone III (GnRH III), luteinizing hormone (LH), follicle-stimulating hormone (FSH). The comparison of GnRH II, GnRH III, FSHb and LHb genes based on different stages of oogenesis provides better knowledge about the maturation of the females of Indian spiny loach (*Lepidocephalichthys thermalis*). The study on expression of GnRH II, GnRH III, FSHb and LHb genes with different photoperiod gives an idea about daily cycle of hormonal secretion in Indian spiny loach (*Lepidocephalichthys thermalis*). The present study forms the basis for further analysis of reproductive genomic aspects of Indian spiny loach (*Lepidocephalichthys thermalis*).

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Reference

1. Qin CG, Huang KX, Xu HB. Protective effect of polysaccharides from the loach on the *in vitro* and *in vivo* peroxidative damage of hepatocyte. J Nutr Biochem. 2002; 13:592-597. DOI:10.1016/S0955- 2863(02)00193-6
2. Khan MR, Arai K. Allozyme variation and genetic differentiation in the loach *Misgurnus anguillicaudatus*. Fish Sci. 2000; 66:211-222. DOI:10.1046/j.1444-2906.2000.00037.x
3. Shao J, Shi GQ, Jin XL, Song MY, Shi JB, Jiang GB. Development and validation of an enzymelinked immunosorbent assay for vitellogenin in Chinese loach (*Misgurnus anguillicaudatus*). Environ Int. 2005; 31:763-770. DOI:10.1016/j.envint.2005.03.001
4. Morishima K, Nakayama I, Arai K. Genetic linkage map of the loach *Misgurnus anguillicaudatus* (Teleostei: Cobitidae). Genetica. 2008; 132:227-241. DOI:10.1007/s10709-007-9165-2
5. Miyamoto K, Hasegawa Y, Nomura M, Igarashi M, Kangawa K, Matsuo H *et al*. Identification of the second gonadotropin-releasing hormone in chicken hypothalamus: evidence that gonadotropin secretion is probably controlled by two distinct gonadotropin-releasing hormones in avian species. Proceedings of the National Academy of Sciences. 1984; 81(12):3874-3878.
6. Matsuo H, Baba Y, Nair RMG *et al*. Structure of the porcine LH- and FSH-releasing hormone (I). The proposed amino acid sequence, Biochem. Biophys. Res. Commun. 1971; 43:1334-1339.
7. Burgus R, Butcher M, Amoss M, Ling N, Monahan M, Rivier J *et al*. Primary structure of the ovine hypothalamic luteinizing hormone-releasing factor (LRF). Proceedings of the National Academy of Sciences. 1972; 69(1):278-282.
8. Sherwood N, Eiden L, Brownstein M, Spiess J, Rivier J, Vale W. Characterization of a teleost gonadotropin-releasing hormone. Proceedings of the National Academy of Sciences. 1983; 80(9):2794-2798.
9. Ngamvongchon S, Lovejoy DA, Fischer WH, Craig AG, Nahorniak CS, Peter RE *et al*. Primary structures of two forms of gonadotropin-releasing hormone, one distinct and one conserved, from catfish brain. Molecular and Cellular Neuroscience. 1992; 3(1):17-22.
10. Powell JF, Zohar Y, Elizur A, Park M, Fischer WH, Craig AG *et al*. Three forms of gonadotropin-releasing hormone characterized from brains of one species. Proceedings of the National Academy of Sciences. 1994; 91(25):12081-12085.
11. Carolsfeld J, Powell JF, Park M, Fischer WH, Craig AG, Chang JP *et al*. Primary structure and function of three gonadotropin-releasing hormones, including a novel form, from an ancient teleost, herring. Endocrinology. 2000; 141(2):505-512.
12. Okubo K, Amano M, Yoshiura Y, Suetake H, Aida K. A novel form of gonadotropin-releasing hormone in the medaka, *Oryzias latipes*. Biochemical and Biophysical Research Communications. 2000; 276(1):298-303.
13. Zmora N, González-Martínez D, Munoz-Cueto JA, Madigou T, Mananos-Sanchez E, Doste SZ *et al*. The GnRH system in the European sea bass (*Dicentrarchus labrax*). Journal of Endocrinology. 2002; 172(1):105-116.
14. Xiong F, Hew CL. Chinook salmon (*Oncorhynchus tshawytscha*) gonadotropin IIβ subunit gene encodes multiple messenger ribonucleic acids. Canadian journal of zoology. 1991; 69(10):2572-2578.
15. Chang YS, Huang FL, Lo TB. Isolation and sequence analysis of carp gonadotropin beta-subunit gene. Molecular marine biology and biotechnology. 1992; 1(2):97-105.
16. Sohn YC, Yoshiura Y, Kobayashi M, Aida K. Seasonal changes in mRNA levels of gonadotropin and thyrotropin subunits in the goldfish, *Carassius auratus*. General and comparative endocrinology. 1999; 113(3):436-444.
17. Chong KL, Koh M, Melamed P. Molecular regulation of gonadotropin gene expression in teleosts. Hormones and their receptors in fish reproduction, 2005, pp. 76-104.
18. Rosenfeld H, Levavi-Sivan B, Melamed P, Yaron Z,

Elizur A. The GTH β subunits of tilapia: gene cloning and expression. Fish Physiology and Biochemistry. 1997; 17(1-6):85-92.
19. Sohn YC, Suetake H, Yoshiura Y, Kobayashi M, Aida K. Structural and expression analyses of gonadotropin Iβ subunit genes in goldfish (*Carassius auratus*). Gene. 1998; 222(2):257-267.
20. Rosenfeld H, Levavi-Sivan B, Gur G, Melamed P, Meiri I, Yaron Z *et al*. Characterization of tilapia FSHβ gene and analysis of its 5′ flanking region. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 2001; 129(2-3):389-398.
21. Guzmán JM, Luckenbach JA, Yamamoto Y, Swanson P. Expression profiles of Fsh-regulated ovarian genes during oogenesis in coho salmon. PloS one. 2014; 9(12):e114176.
22. So WK, Kwok HF, Ge W. Zebrafish gonadotropins and their receptors: II. Cloning and characterization of zebrafish follicle-stimulating hormone and luteinizing hormone subunits—their spatial-temporal expression patterns and receptor specificity. Biology of reproduction. 2005; 72(6):1382-1396.
23. Sherwood NM, Adams BA. Gonadotropin-releasing hormone in fish: evolution, expression and regulation of the GnRH gene. Hormones and their receptors in fish reproduction. 2005; 4:1-39.
24. Prayogo NA, Wijayanti GE, Murwantoko Kawaichi M, Astuti P. Structure and phylogenetic of GnRH genes of Hard-lipped barb (*Osteochilus hasselti* CV). Middle-East Journal of Scientific Research. 2011; 10(3):332-341.
25. Rather MA, Bhat IA, Sharma R. Identification and characterization of chickenII-GnRH (cII-GnRH) gene of Catlacatla (Hamilton, 1822). Eur J Exper Biol. 2015; 5(2):53-56.
26. Kah O, Breton B, Dulka JG, Nunez-Rodriguez J, Peter RE, Corrigan A *et al*. A reinvestigation of the Gn-RH (gonadotrophin-releasing hormone) systems in the goldfish brain using antibodies to salmon Gn-RH. Cell and tissue research. 1986; 244(2):327-337.
27. Collins PM, O'Neill DF, Barron BR, Moore RK, Sherwood NM. Gonadotropin-releasing hormone content in the brain and pituitary of male and female grass rockfish (*Sebastes rastrelliger*) in relation to seasonal changes in reproductive status. Biology of reproduction. 2001; 65(1):173-179.
28. Amano M, Okubo K, Ikuta K, Kitamura S, Okuzawa K, Yamada H *et al*. Ontogenic origin of salmon GnRH neurons in the ventral telencephalon and the preoptic area in masu salmon. General and comparative endocrinology. 2002; 127(3):256-262.
29. Yaron Z. Endocryne control of gametogenesis and spawning induction in the carp. Aquaculture. 1995; 129:49-73.
30. Pierce JG, Parsons TF. Glycoprotein hormones. Structure and function. Ann. Rev. Biochem. 1981; 50:465-495.
31. Yoshiura Y, Suetake H, Aida K. Duality of gonadotropin in a primitive teleost, Japanese eel (*Anguilla japonica*). Gen. Comp. Endocrinol. 1999; 114:121-131.
32. Pierce JG. Gonadotropins: chemistry and biosynthesis. In: Knobil E, Neill J. (Eds.), the Physiology of Reproduction. Raven Press, New York, 1988, pp. 1335-1348.
33. Rather MA, Bhat IA, Sharma R. Identification, cDNA cloning, and characterization of luteinizing hormone beta subunit (lhb) gene in Catla. Animal biotechnology. 2016; 27(3):148-156.
34. Oikawa M, Dargan C, Ny T, Hsueh AJ. Expression of gonadotropin-releasing hormone and prothymosin-α messenger ribonucleic acid in the ovary. Endocrinology. 1990; 127(5):2350-2356.
35. Grober MS, Myers TR, Marchaterre MA, Bass AH, Myers DA. Structure, localization, and molecular phylogeny of a GnRH cDNA from a paracanthopterygian fish, the plainfin midshipman (*Porichthys notatus*). General and comparative endocrinology. 1995; 99(1):85-99.
36. White RB, Fernald RD. Genomic structure and expression sites of three gonadotropin-releasing hormone genes in one species. General and comparative endocrinology. 1998; 112(1):17-25.
37. Gray SL, Adams BA, Warby CM, von Schalburg KR, Sherwood NM. Transcription and translation of the salmon gonadotropin-releasing hormone genes in brain and gonads of sexually maturing rainbow trout (*Oncorhynchus mykiss*). Biology of reproduction. 2002; 67(5):1621-1627.
38. Suzuki K, Gamble RL, Sower SA. Multiple transcripts encoding lamprey gonadotropin-releasing hormone-I precursors. Journal of Molecular Endocrinology. 2002; 24(3):365-376.
39. Hamsavalli R, Jaculine Pereira J, Karal Marx K, Jeya Shakila R. Analysis of Gonadotropin Releasing Hormone (GnRH), Leutinizing Hormone (LHb) and Follicle Stimulating Hormone (FSHb) Genes in Indian Spiny Loach (*Lepidocephalichthys thermalis*). Int. J. Curr. Microbiol. App. Sci. 2019; 8(04):1004-1011. DOI: https://doi.org/10.20546/ijcmas.2019.804.116
40. Levavi-Sivan B, Bogerd J, Mañanós EL, Gómez A, Lareyre JJ. Perspectives on fish gonadotropins and their receptors. General and comparative endocrinology. 2010; 165(3):412-437.
41. Lubzens E, Young G, Bobe J, Cerdà J. Oogenesis in teleosts: how fish eggs are formed. General and comparative endocrinology. 2010; 165(3):367-389.
42. Nagahama Y, Yamashita M. Regulation of oocyte maturation in fish. Development, Growth & Differentiation. 2008; 50:S195-S219.
43. Zohar Y, Mylonas CC. Endocrine manipulations of spawning in cultured fish: from hormones to genes. In Reproductive Biotechnology in Finfish Aquaculture, 2001, pp. 99-136.
44. Patiño R, Sullivan CV. Ovarian follicle growth, maturation, and ovulaAtion in teleost fish. Fish Physiology and Biochemistry. 2002; 26(1):57-70.