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MOLECULAR CLONING OF VITELLOGENIN GENE IN THE HARD-LIPPED BARB (*Osteochillus hasseltii* C.V) AND THE EFFECT OF PHOTOPERIODS ON GENE EXPRESSION

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ABSTRACT

Fish reproduction is influenced by photoperiods through regulation on endocrine gland activities in producing hormones needed for gonadal growth and development, gametogenesis and reproductive cycles. This study was conducted to find out the effect of photoperiod on hard-lipped barb reproductive performance by manipulating photoperiod. Experiments were designed with three photoperiod treatments, namely 14L:10D (control), 8L:16D (short photoperiods) and 18L:6D (long photoperiods). Four aquaria consisting of nine fishes each were served as replicates. Fishes were kept under these photoperiods for 8 weeks. The observed variable was the liver activities evaluated by measuring gene expression of Vitellogenin. The normalized data were subjected to ANOVA followed by Tukey's multiple-comparison tests. The length of cDNA Vitellogenin was 1136 bp. The vitellogenin precursors encoded cDNA consisted of 378 amino acids. The average of vitellogenin gene in each experimental group significantly increased according to longer photoperiods ($P<0.05$), and the highest vitelogenin gene expression was in Long Photoperiods (LP, 18Light : 6 dark). These results indicated that photoperiods had a stimulatory effect in improving hard-lipped barb reproductive performance.

Keywords: vitellogenin, photoperiods, Hard-lipped barb, cDNA, amino acid.

INTRODUCTION

Reproductive activities in fish are regulated by several environmental and physiological factors (Bhattacaraya., 1992; Qingbo *et al*., 2005). Photoperiod exert it role on reproduction through brain that integrates and conveys input from external and internal cues to the pituitary via melatonin (Ekstrom and Meissl., 1997; Cassone., 1998; Miranda *et al*., 2008). Melatonin downregulating the synthesis and secretion of kiss gene and GnRH gene (Wang *et al*., 2013). GnRH control secretion GtHs (gonadothropins) such as GtH-I and GtH-II (Xiong and Hew., 1991). GtH-I and GtH-II regulate the two main activities of the gonads i.e. hormone and gamete production (Flack *et al*., 1994; Bayyari *et al*., 2004). Ovarian hormones especially estradiol and progesterone play an important role in maintaining and promoting gamete production (Biswas *et al*., 2005). Estradiol regulated vitellogenin secretion from liver. Vitellogenin play role in vitellogenesis process in ovarium (Sulistyo *et al*., 1998). Vitelogenesis process will increasing oocyt volume until maturation (Yaron., 1995; Utoh *et al*., 2003).

Photoperiod is one of important cues for the timing of spawning in many fish species as European sea bass, *Dicentrarchus labrax* (Rodriguez *et al*., 2004), Atlantic cod, *Gadus morhua L* (Skjæraasen *et al*., 2006), dan Chinook salmon, *Oncorhynchus tshawytscha* (Chyb *et al*., 1999). The majority of studies were conducted on temperate-zone fishes in which photoperiod strictly differ between seasons. Studies on influence of photoperiod on tropical fishes are still limited. The hard lipped barb (*Osteochilus hasselti* C.V) were tropical fish manipulated with longer

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photoperiods 18L:6D (18 hour light and 6 hour dark) were significantly decreased melatonin level and increased gene expression cGnRH-II (Prayogo *et al*., 2012). The level cGnRH-II and sGnRH genes altered increased GtH-Ia, GtH-IIa and GtH-IIb in hard lipped barb.

Hard-lipped barb is a synchronous batch spawner fish (Prayogo *et al*., 2016a) capable of spawning several times during the peak of the spawning period. Under a suitable environmental setting, this fish capable to spawn in 60 days after the previous spawning. Hard-lipped barb has been adapted to a photoperiod of 12L:12D to 14L:10D. The present study was examined the effect of different photoperiods on gene expression of vitellogenin of the hard-lipped barb. This study will inform photoperiods effect in increasing hard-lipped barb reproduction.

MATERIALS AND METHODS

Treatment and sampling of fish

A total of 144 sexually mature female hard-lipped barb weighing of 100 g in average were maintained at Laboratory of Fisheries and Marine, Jenderal Soedirman University, Central Java, Indonesia. They were induced to spawn using ovaprim 0.5 ml/kg body weight. The day of spawning was designated as day zero post spawning period. The post-spawned females were divided into 4 groups. Each group consisted of 4 aquariums with 9 fish/50 L water.

In this study, three types of photoperiods namely 6L:18D (SP=short photoperiods), 14L:10D (C=control) and 18L:6D (LP=Long photoperiods). The aquaria were covered with light proof black polybag. The light source provided from 25 Watt (*Phillips*) bulb regulated by otomatic timers 24 hours cycles which were placed at the top of each aquarium. In control photoperiod (C) 14L:10D, light was turned on since 06.00 am until 08.00 pm and in short photoperiods (SP) 8L:16D light was turned on since 06.00 am until 02.00 pm and in lond photoperiods (LP) 18L:6D light was turned on since 06.00 am until 12.00 pm local time. Then, the fish were reared for 8 weeks at the laboratory of Aquaculture Department of Marine and Fisheries, University of Jenderal Soedirman. During the research, fish were fed on commercial pellet (protein 37% and fat 10%) as much as 3% of total body weight daily. The water was siphoned regularly to maintain water quality. The water temperature, dissolved oxygen, pH and carbon dioxide were monitored every 1 weeks. Every sampling time, the pituitary were collected from 3 fish of each group and were snap-frozen on liquid nitrogen for vitellogenin expression study. The expression of vitellogenin genes was evaluated using Real Time PCR applying primers derived from vitellogenin genes. Real Time PCR was conducted at the Research Laboratory Jenderal Soedirman University.

Total RNA Isolation and DNAse Treatment

Total RNA was extracted from whole liver using blue Sepasol R-RNA super-1 reagent, based on Ethanol-phenol-chloroform extraction method (Prayogo *et al*., 2016b). The integrity of the RNA was verified in a denaturing agarose gel, stained with ethidium bromide. The RNA samples were treatment with DNAse free RNAse (Takara). The quality and concentrations of total RNA were determined by agarose gel electrophoresis and optical density reading at 260 and 280 nm (Figure 1), the RNA was aliquoted in batches and frozen at -70°C.

RT-PCR

Total mRNA samples (1µl) were reverse transcript using cDNA synthesized kit (PrimeScriptTM Reverse Transcriptase, Takara Bio.Inc) using Random 6 mers (sequence pd (N)6, 50µM) primers and prime script R-tase with manufacture instruction.

cDNA Amplification

The Degenerated primer pairs, vitellogenin gene were designed from cDNA *cyprinidae* like *cyprinus carpio* and *carrasius auratus*. All sequence were aligned with multalin to identify the conserve region in ORF (Open Reading Frame) region. The primer to amplify the vitellogenin gene were designed using primer 3 software (Table 1).

Thirty five cycles of PCR for hard lipped barb vitellogenin were carried out using a thermal cycler (Robocycler, Stratagene) according to the following cycle, 95°C for 2 min, 35 cycles to 95°C for 30 s, 55°C for 30 s, 72°C for 60 s, followed by a

Table 1 The primer used to amplify the Vitellogenin genes and their PCR product

No	Name/Primer Code	DNA Sequence (primer)	Tm	PCR Product (bp)
1.	Forward VF (VF)	CGTGGATCHYTGMARTACGAGTT	62,81	1136 bp
2.	Reverse VR (VR)	ATGGTGGCRGCRTCATTGAT	62,83	
3.	Forward VF Real Time (FRT)	GACGCTCCACTCAAGTTTGTTCAG	61,23	150 bp
4.	Reverse VR Real Time (RRT)	GAGCCCAGATAGCCTCAATGTTC	62,34	
5.	Forward Actin (FA)	GAG CTA TGA GCT CCC TGA CGG	58,3	53 bp
6.	Reverse Actin (RA)	AAA CGC TCA TTG CCA ATG GT	55,6	

5 min extension at 72°C. After amplification, the PCR products was electrophoretically separated on a 1.5% agarose gel and stained with ethidium bromide.

Cloning and Sequencing of PCR Products

PCR products amplified from cDNA were separated by agarose gel electrophoresis, and the incised gels were purified using the DNA gel extraction procedure. The desired DNA fragments from mRNA vitellogenin were subcloned into T vector (10 ng) (Takara) and ligated with T4 ligase. The plasmid were transfected into *E. coli* and were spread into LB medium. The recombinant positive colonies were screened using ampicillin. Positive colonies were treated by scale plasmid preparation for sequencing. DNA sequences of these fragments were determined using the Big Dye version 3.1 sequencing method with specific primers (Table 1). The data were automatically collected on the ABI PRISM 3100 Genetic Analyzer (PE Applied Bio-systems).

Sequence analysis

The cDNA sequences for vitellogenin gene were checked using BLASTN searches (http://www.ncbi.nlm.nih.gov/ BLAST/) were performed with default settings on the complete, non redundant GenBank database nucleotide sequences.

Phylogenetic analysis

For phylogenetic analyses, hard lipped barb cDNA vitellogenin genes was compared to cDNA vitellogenin sequences from ten fish species. All sequences were retrieved from NCBI GenBank. The relationship between hard-lipped vitellogenin and other teleost vitellogenin was generated with CLUSTAL W with scoring method percent and the unrooted tree was generated using Treeview version 1.5.2.

Quantitative Real Time Analysis

The primers were designed based on vitellogenin (submit number: 1995561), using the Primer 3.0 software. For the actin used from hard lipped barb actin, used as endogenous control, was amplified by the following primers-actin forward 5-GAGCTATGAGCTCCCTGACGG-3, actin reverse 5- AAACGCTCATTGCCAAT GGT-3-and were used to normalize variations in RNA. After optimization, PCR reactions were performed in a 10 µl volume containing 2 µl cDNA, 5 µl SYBR mix (Applied Biosystem), 0.3 µl forward primer, 0.3 µl reverse primer and 2.4 µl DDW using the following condition: 95°C for 45 s, (45 cycles of 95°C for 15 s and 60°C for 1min),then 95°C for 15s, 60°C for 15s and 95°C for 15s. The results were analyzed using the standard curve mode, according to the manufacturer's recommendations (Applied Biosystems).

RESULTS AND DISCUSSION

Identification of Vitellogenin Genes in Hard Lipped Barb

The vitellogenin genes of the hard-lipped barb were successfully amplified from cDNA. The agarose gel electrophoresis of the cDNA

vitellogenin showed a specific band, approximately 1136 bp (submit number: 1995561). The corresponding cDNA sequences were called vitellogenin. The cDNA sequences were check with BLAST and we found there was not 100% similar identity with another vitellogenin gene. The nucleotide sequence identity of similar vitellogenin cDNA was 77% with carp (*cyprinus carpio* AB331884.1), 78 % with goldfish (*carrasius auratus*, FJ524335.1), 78 % mud crap (*Cirrhinus Molitorella*, GU324313.1), and 75% with zebra fish (*danio rerio*, NM001122610.3).

Gene Structure Vitellogenin

Vitellogenin gene share the same basic structure with another teleost. Amplification of vitellogenin gene in hard lipped barb showed cDNA fragment contained the open reading frame of vitellogenin. The cDNA fragment contained 1136 amino acid and identified as Mature peptide (figure. 1).

The vitellogenin sequence in hard lipped barb had a high similarity in the coding sequences with another teleost can be seen as the distance at the phylogenetic tree (Figure.2,3,4). The greatest differences within the preprohormone are within the GAP coding sequences. The striking contrast between conservation of the vitellogenin coding sequence and lack thereof in the GAP coding sequence is evidence of differential selective pressure within the gene. This is evident in cases where the identity and similarity of vitellogenin and GAP coding sequences have been compared for mRNAs of different vitellogenin genes within a species.

Phylogenetic analyses

Phylogenetic analyses were performed to establish an evolutionary context for the vitellogenin gene. Genetic distances (measured as substitutions per site) showed moderate low values, and the topology was well supported by

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gatgcaccagcccaggtagaattcatttaaaactaaaaaaaatctgaggccttttgaaatgc
  C  T  S  P  G  R  I  H  L  K  L  K  K  I  -  G  L  L  K  C
tgacgatcaacgttactccttcctagattattgaagttcttacgcaccttgttacaaacc
 -  R  S  T  L  L  L  P  R  L  L  K  F  L  R  T  L  L  Q  T
atgtggccatggtcgatgatgatgctcctctcatgtttattcagctcattcaactcctgc
 M  W  P  W  S  M  M  M  L  L  S  C  L  F  S  S  F  N  S  C
ctgttgccacccttgagaatgatgagtctatctgggctcactacaaggacaaaccagttc
 L  L  P  P  L  R  M  M  S  L  S  G  L  T  T  R  T  N  Q  F
acaggcgctggcttctggatgctcttcctgctgtgggcacaccagtaatggtgaaaatgt
 T  G  A  G  F  W  M  L  F  L  L  W  A  H  Q  -  W  -  K  C
acaaggagaaatgcctggctgtcgatcttacccttctgagatcattctgactgttgtgg
 T  R  R  N  A  W  L  S  I  L  P  F  L  R  S  F  -  L  L  W
gtgctctgcaaatggtgaccgctgttctaactatagtgccattcccatctagtttgggtt
 V  L  C  K  W  -  P  L  F  -  L  -  C  H  S  H  L  V  W  V
tgcacgcgaaaatctccccactccccctctgggttaagttgttatgctcggatatagtg
 C  T  R  K  S  P  H  S  P  L  W  V  K  L  L  C  S  D  I  V
ctaagaatggcgagcgcagtgttgctggtcccactcggtcttctgggctcctcaagccct
 L  R  M  A  S  A  V  L  L  V  P  L  G  L  L  G  S  S  S  P
gccatgggattgccgcagatgccattggtaagagcaaatgtcccgaaatccatttggctc
 A  M  G  L  P  Q  M  P  L  V  R  A  N  V  P  K  S  I  W  L
ttaaagttctgggaattgctggccttctcgctagttttaaacccatcatgaagttcctac
 L  K  F  W  E  L  L  A  F  S  L  V  L  N  P  S  -  S  S  Y
ctggattgggaattgaagttattgttttgcccttagggtccggttggaggccttttggg
 L  D  W  E  L  K  L  L  F  C  P  L  G  S  G  W  R  P  F  G
ccctgaggaccattgccaggaaagagcccaaattggttcagccagtggccattcagattt
 P  -  G  P  L  P  G  K  S  P  N  W  F  S  Q  W  P  F  R  F
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 A  K  H  -  G  V  L  F  P  I  L  G  G  P  -  N  L  E  L  T
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 V  L  P  P  V  A  G  A  A  N  V  A  I  K  L  M  S  R  K  L
gacaggcttagcttccatttcagcagagcctttcagactgacttctatcatacgtaagtc
 D  R  L  S  F  H  F  S  R  A  F  Q  T  D  F  Y  H  T  -  V
ttggaaatttctgctgagagagcttaattgtattttggagacgtttcttaaaat
 L  E  I  S  A  E  R  A  -  L  Y  F  G  D  V  S  -  N
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Fig 1 Vitellogenin nucleotide and amino acid sequence of Hard-lipped barb

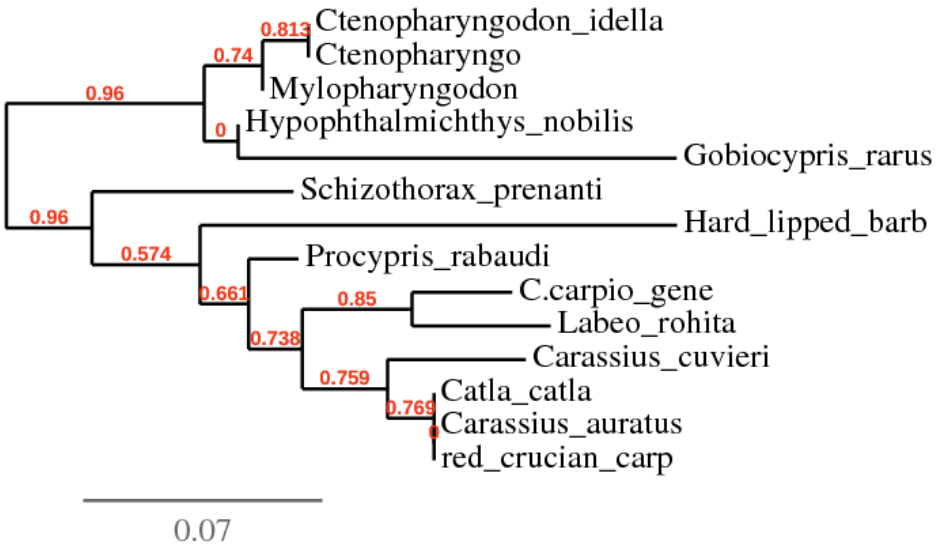

Figure 2 Phylogenetic relationship of precursors derived from known nucleotide encoding vitellogenin. The relationship was generated with CLUSTAL W and the unrooted tree was generated using Treeview version 1.5.2. The scale bar represents the estimated evolutionary distance as 0.1 amino acid substitutions per site.

strong bootstrap values. As expected, vitellogenin in hard-lipped barb was included within a sub-cluster of the *carp* (*Cyprinus carpio)* and goldfish *(Carrasius auratus*) with high bootstrap values (Figure 5).

This paper reports for the first time the clone vitellogenin gene from pituitary tissues of hard-lipped barb. Comparison the vitellogenin gene structure with previously reported gene structures of other fish species shows a high conservation. The nucleotide sequence identity of vitellogenin mRNA also very similar with another variant vitellogenin, from BLAST result showed 78% with carp (*Cyprinus carpio* AB331884.1), 78 % with goldfish (*Carrasius auratus*, FJ524335.1), 78 % mud crap (*Cirrhinus Molitorella*, GU324313.1), and 75% with zebra fish (*Danio rerio*, NM001122610.3) (Figure 3).

This result showed that hard lipped vitellogenin genes had a high similarity with that of carp and goldfish. The highest similarity was identified between hard-lipped barb and *Carrasius auratus* vitellogenin (78%). Based on this result we suggested that hard lipped barb had two forms of vitellogenin similar to goldfish (figure 3).

The vitellogenin precursor encoded by mRNA contained 378 amino acid residues. The vitellogenin precursor was composed of mature

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Cirrhinus molitorella vitellogenin B1 (Vtg-B1) mRNA, complete cds	651	651	87%	0.0	79%	GU324313.1
PREDICTED: Sinocyclocheilus grahami vitellogenin-like (LOC107563524), transcript variant X2, mRNA	617	617	87%	1e-172	78%	XM_016248425.1
PREDICTED: Sinocyclocheilus grahami vitellogenin-like (LOC107563524), transcript variant X1, mRNA	617	617	87%	1e-172	78%	XM_016248424.1
Petroleuciscus esfahani vitellogenin mRNA, complete cds	617	617	87%	1e-172	78%	KF766534.1
Carassius auratus ssp. 'Pengze' vitellogenin B variant 2 mRNA, partial cds	601	601	89%	1e-167	78%	KF373230.1
Carassius auratus vitellogenin mRNA, partial cds	595	595	89%	7e-166	78%	FJ524335.1
PREDICTED: Sinocyclocheilus rhinocerous vitellogenin-like (LOC107715697), mRNA	593	593	87%	2e-165	78%	XM_016521872.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107664867), mRNA	590	590	87%	3e-164	78%	XM_016455551.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107664873), mRNA	579	579	87%	7e-161	77%	XM_016455561.1
Gobiocypris rarus vitellogenin Ao1-like (vtgAo1) mRNA, complete sequence	573	573	87%	3e-159	77%	EU623080.1
Pimephales promelas vitellogenin precursor (Vtg) mRNA, complete cds	573	573	86%	3e-159	78%	AF130354.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107655402), transcript variant X3, mRNA	562	562	87%	7e-156	77%	XM_016442878.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107655402), transcript variant X2, mRNA	562	562	87%	7e-156	77%	XM_016442877.1
PREDICTED: Sinocyclocheilus anshuiensis vitellogenin-like (LOC107655402), transcript variant X1, mRNA	562	562	87%	7e-156	77%	XM_016442876.1
PREDICTED: Sinocyclocheilus grahami vitellogenin-like (LOC107570369), mRNA	562	562	87%	7e-156	77%	XM_016256599.1
Rhinichthys cataractae vitellogenin mRNA, partial cds	558	558	86%	9e-155	77%	EF202607.1
PREDICTED: Sinocyclocheilus grahami vitellogenin-like (LOC107570371), mRNA	556	556	87%	3e-154	77%	XM_016256602.1
Cyprinus carpio vg-B1 mRNA for vitellogenin B1, complete cds	556	556	87%	3e-154	77%	AB331884.1
Carassius auratus ssp. 'Pengze' vitellogenin B variant 1 mRNA, complete cds	553	553	86%	4e-153	77%	KF373229.1

Fig 3 Comparison between hard lipped barb vitellogenin nucleotide with other cyprinids

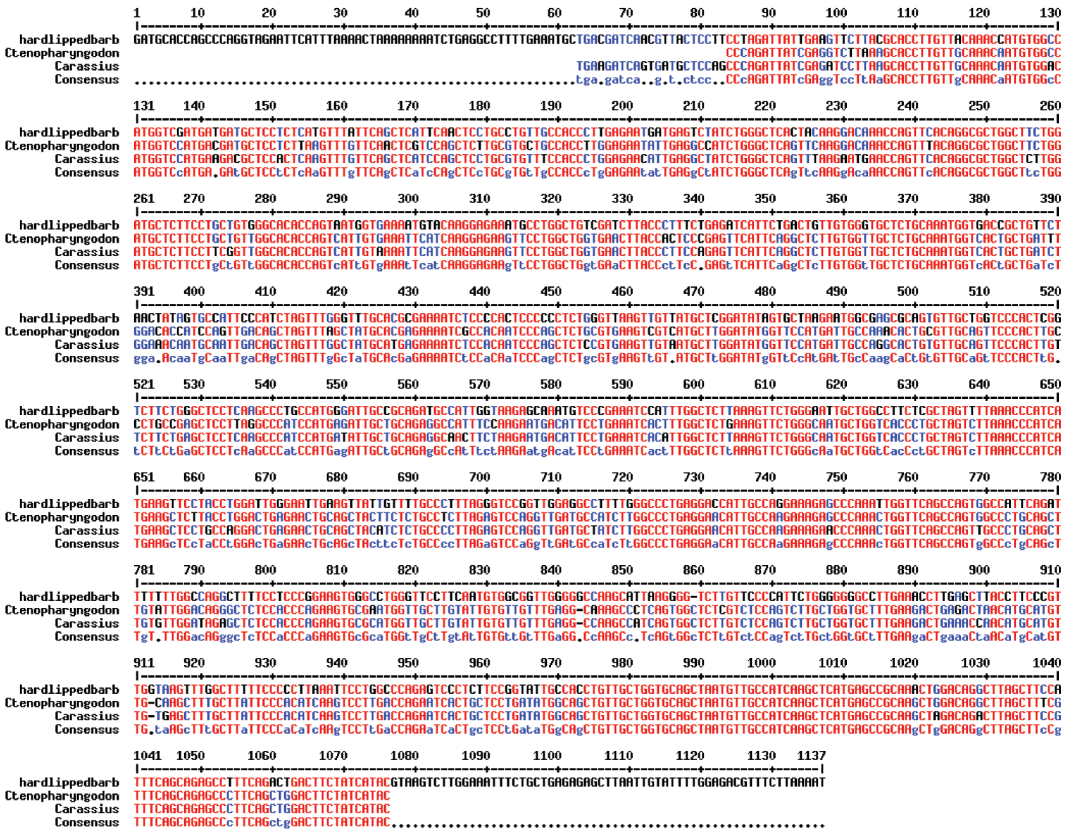

Fig 4 Multalin of Vitellogenin Sequence of Hard lipped barb with goldfish

peptide (Figure. 1). The amino acid sequences of hard lipped barb precursors encoded by mRNA were compared with some of previously identified vitellogenin precursors (figure. 3), such as the precursors of rohu (*Labeo rohita*), goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), rainbow trout (*Oncorhyncus mykiss*), and rock carp (*Procypris rabaudi*). The results showed that the amino acid homology of vitellogenin precursors within Cyprinoids was 65-77%. However from that alignment explained vitellogenin mature peptide between hard lipped barb compared with another teleost like rainbow trout and grass carp had different in amino acid 41(R) and 43 (N). Variation of vitellogenin GAP indicated function of GAP may different from each species.

The present study providing new evolutionary information on this vitellogenin gene family in the brain. The vitellogenin in hard lipped barb were grouped together with other teleost in the phylogenetic tree, suggesting a common ancestor for both groups of genes. Phylogenetic analysis shows that vitellogenin can be separated into 2 major groups. Subgroup I contains vitellogenin from balck carp until rutilus rutilus, sub group II from goldfish until carp. (Figure.4).

The mature peptide is a minimal structural requirement for delineated vitellogenin activity (Hiramatsu *et al.*, 2002). The vitellogenin mature peptide from hard lipped barb similar with carp, assumed that they had a same functional (Kang *et al.*, 2007). The comparison results of a sequences of vitellogenin precursors from different vertebrates showed that the mature vitellogenin were very conserved (figure. 4) A mature peptide is a peptide chain that coding sequence for the mature or final peptide or protein product following post-translational modification. So, vitellogenin were conserved during the process of evolution. The vitelogenin gene in hard lipped barb were similar with goldfish. From that result, it can be possible that vitellogenin in the hard lipped barb also has multiple vitellogenin genes (Kang *et al.*, 2007).

Expression of vitellogenin mRNA under photoperiods manipulation

In female hard lipped barb, relative vitellogenin mRNA expression level in eight

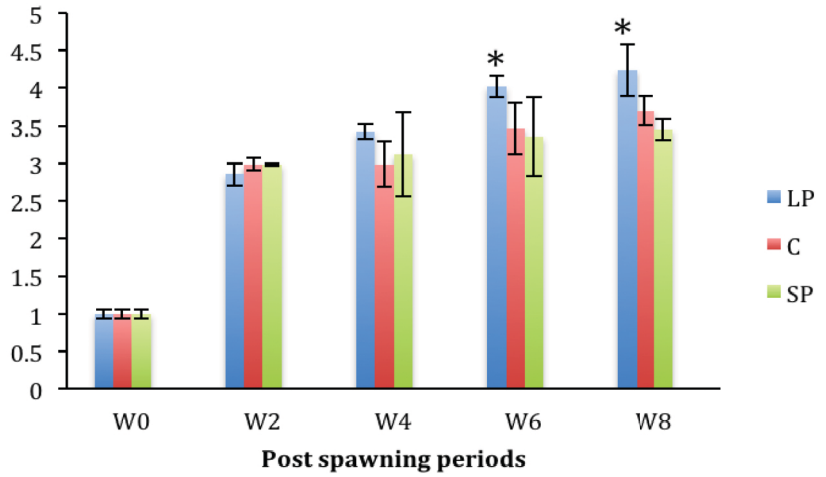

Figure 6 Vitellogenin gene expression of hard-lipped barb kept under different photoperiod for 8 weeks. LP=18L:6D, C=14L:10D, SP=6L:8D,). (*: Significantly different)

weeks were 1-4.35 (Figure 6). The highest vitellogenin mRNA expression (4.35) was observed LP group in 8 weeks significantly different ($P<0.05$). mRNA expression for 18L: 6D group increased with post spawning periods ($P<0.05$). The vitellogenin mRNA expression for other treatment photoperiods in 2 weeks and 4 weeks had non significantly different ($P>0.05$), but in 6 weeks and 8 weeks long photoperiods (LP) had higher gene vitellogenin than short photoperiods (SP) and control © ($P<0.05$).

In this study, changes in, gene expression of vitellogenin levels in hard lipped barb were analyzed to characterize the role of neuropeptides in the control of reproduction under photoperiods manipulation. This study confirms previous results from fisheries laboratory showing increased in vitellogenin genes levels during photoperiods manipulation in hard-lipped barb. In addition, we report for the first time, changes in the gene expression levels of vitellogenin genes in correlation with photoperiods manipulation. Although we are aware that mRNA levels do not always match with protein levels and/or the physiological effects of the protein products, the regulation of mRNA levels provides an indication of the activity of a particular peptide neuronal system.

In this study showed that vitellogenin level increased equivalent with the long photoperiod increased. This is proved that photoperiod exert it role on reproduction through hipotamaus-pituitary-gonad (Miranda *et al.*, 2008), that integrates and conveys input from external and internal cues to the pituitary organs (Cassone *et al.*, 1998; Bromage *et al.*, 2001). Photoperiods regulated melatonin production and melatonin mediated cyclical regulation of GnRH mRNA expression involve the protein kinase C and the extracellular signal-regulated kinase 1 and 2 pathways. Melatonin regulated act through membrane receptors to trigger the protein kinase C pathway and 12-O- tetradecanoyl phorbol-13-acetate (TPA), a modulator of this pathway, has been shown to suppress GnRH gene expression through the promoter (Qingbo *et al.*, 2005). GnRH binds to GnRH receptor and active G protein mediated phosphorilation to protein kinase C and synthesized Gonadotrophin (GtH-I and GtH-II)(Chyb *et al.*, 1999; Minniti *et al.*, 2007) . GtH-II secreted into blood vessel, to receptor in theca cell activated G protein and adenylate cyclase to phosphorilation cAMP and activation staR protein staR protein regulated cholesterol. Activity of receptor FSH and LH In gonad females promotes production estradiol-17h (E2) is the main sex steroid. E2 functions primarily to induce vitellogenin or yolk protein secretion by the liver. Generally, Estradiols peaks during the period of most active vitellogenesis, and returns to basal levels before ovulation. embryos are dependent on the egg-yolk for their nutritional requirements. The process of yolk deposition in oocytes - vitellogenesis - is a seasonal or cyclic phenomenon

CONCLUSIONS

In summary, the present work has reported for the first time sequence of vitellogenin an Hard-lipped barb. The phylogenetic results presented in this work support the idea that vitellogenin genes share the same basic structure. This meant that vitellogenin in Hard lipped barb very conserve, that assumed had a same function with another teleost. Photoperiod affected regulation of gene expression of vitellogenin, in the hard lipped barb. The longer photoperiod increased gene expression of vitellogenin, via HPG axis.

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