Sex determination in yellow perch: transcriptome profile of key sex related gene expression and effects of *cyp19a1* siRNA-silencing

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Abstract

In this study, we first sequenced the whole genome of the yellow perch (YP, *Perca favencens*) and screened transcripts of brain, liver, and gonadal tissues from fish of different sexes. By comparing known female sex-related genes in fish such as *cyp19a1* and *dmrt1* with YP genome, we obtained full-length sequences through genomic homology analysis. Then an experiment was conducted to evaluate the effects of *cyp19a1*-siRNA interference on gonadal development and sex determination in YP. Transcriptome analysis revealed that the gonadal tissue of female YP exhibited predominantly and differentially upregulated expression of genes than males, and *cyp19a1* was predominantly expressed in the gonads while *cyp19a2* showed predominant expression in the brain. The *cyp19a1*-siRNA interference led to an incomplete masculinization and hermaphroditism of all-female monosex YP. In addition, the *cyp19a1*-siRNA treatment group had significantly (P<0.05) lower body weight than the control groups, indicating that the interference with *cyp19a1* might have affected the expression of estrogen, leading to a trend similar to the growth of males.

Introduction

- Yellow perch is a native North American fish species with a phylogenetic relationship to the Eurasian perch
- It demonstrates female-biased size dimorphism, with females having larger body size and faster growth rates than males
- Identification of key genes involved in female sex determination and their functional mechanisms using RNA interference (RNAi) technology may provide novel insights into effective manipulation of the expression of these genes
- *cyp19a1* is primarily expressed in the gonads, playing a crucial role in sex differentiation and oocyte growth
- The objective of this study is to investigate the roles of *cyp19a1* in sex differentiation and determination in yellow perch through the in vivo injection of *cyp19a1*-siRNA for RNA interference

Methods and Materials

- All-female monosex yellow perch were used in this study
- At 44 dph, 320 random fish from each type were distributed to 32 flow-through 25-L round tanks including 8 experimental groups, each having 3 replicates and each tank containing 40 fish
- The eight groups were: blank control, PBS control with phosphate-buffered saline (PBS) treatment, MT positive control group L (MTL, fed with 20 mg MT/kg feed) and H (MTH, fed with 50 mg MT /kg feed), *cyp19a1* siRNA treatment group 1 (cT1), 2 (cT2), and the siRNA negative control group 1 (cNC1), 2 (cNC2), treated with negative siRNA (Fig. 1)

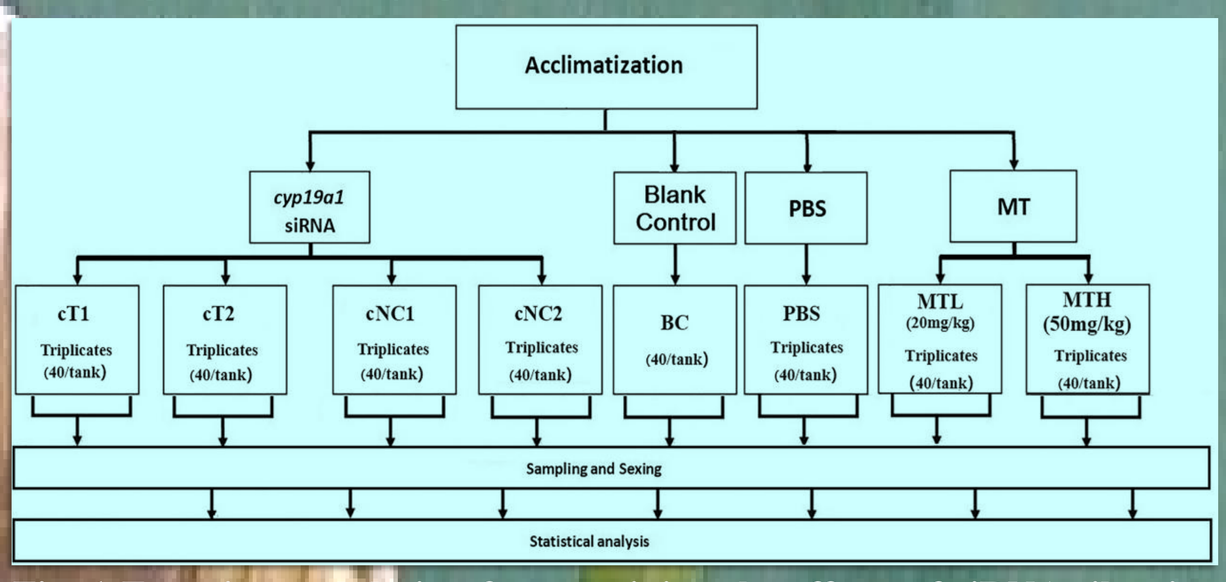


Fig. 1 Experimental design for examining the effects of siRNA silencing of *cyp19a1* on sex differentiation and determination in yellow perch

- Four rounds of the vivo injection in the PBS group, cT group, and cNC group were conducted every 20 days from 51 to 117 dph
- The siRNA injections were administered a dosage of 2 g/μl using the Micro Sample Syring and three duplicate samples were separately intraperitoneally injected on three different days

Results and Discussion

Transcriptome analysis and target gene expression

- The library was assembled using Trinity software with a Kmer value of 25.

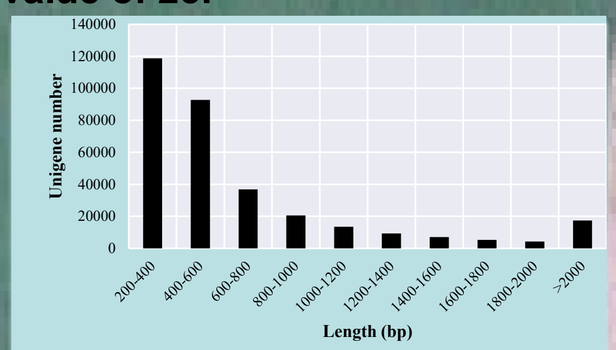


Fig. 2 The distribution of transcripts length in yellow perch

- CD-HIT-EST was employed to eliminate redundant sequences and a total of 325,637 transcripts were obtained.
- The results illustrated that the transcript length was predominantly distributed within the range of 200-400bp and 400-600bp (Fig.2).
- The original readings were filtered using the fastp program.
- Subsequently, the processed data was mapped to transcripts using the Bowtie2 program.
- The FPKM value of genes was calculated for each sample using the eXpress program.
- Differential expression analysis was performed to identify genes that showed significant expression differences using the DESeq software package.
- The differentially expressed genes between male and female gonads were compared using a volcano plot (Fig. 3) after the results of differentially expressed genes (DEGs) analysis.

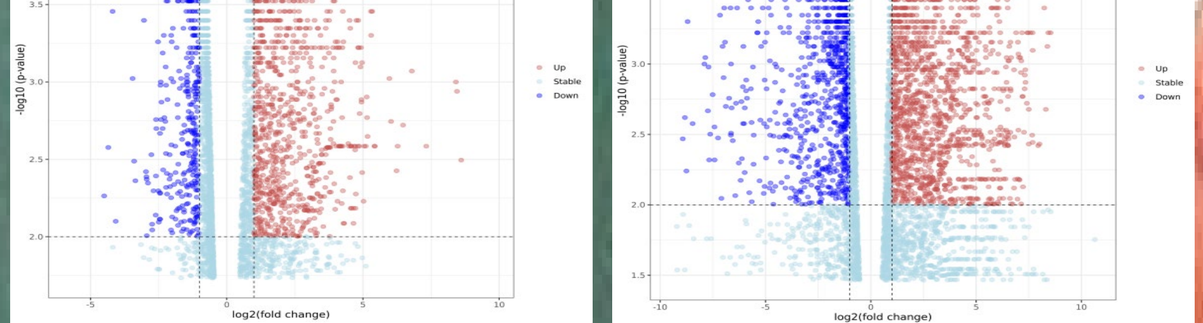
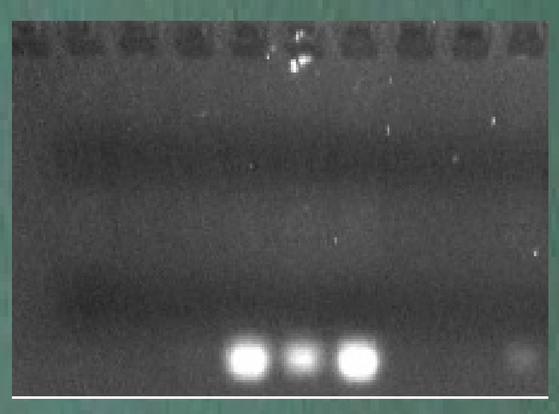


Fig. 3 Volcanic map of differential expression of genes of male and female gonads. F, ♀ gonad; M, ♂ gonad. Red, upregulation; Blue, downregulation



Fig, 4 *cyp19a1* sequence in yellow perch cDNA template validated through PCR analysis

- The full-length sequences of *cyp19a1* and *cyp19a2* in YP were obtained by comparing them with genomic homology confirming they belong to P450 family.
- *Cyp19a1* in YP was mainly expressed in the gonads, while *cyp19a2* showed predominant expression in the brain.
- PCR validation of the *cyp19a1* sequence showed three pairs of *cyp19a1* primers (Fig. 4).

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- The *cyp19a1* gene was mainly expressed in gonad tissue and the female groups displayed a significantly (P<0.01) higher expression than the male groups (Fig. 5A).
- The *cyp19a2* gene is mainly expressed in brain and the female groups displayed a significantly higher (P < 0.05) expression than the male groups (Fig. 5B). The expression of *cyp19a1 and cyp19a2* in liver could not be detected in all the samples.

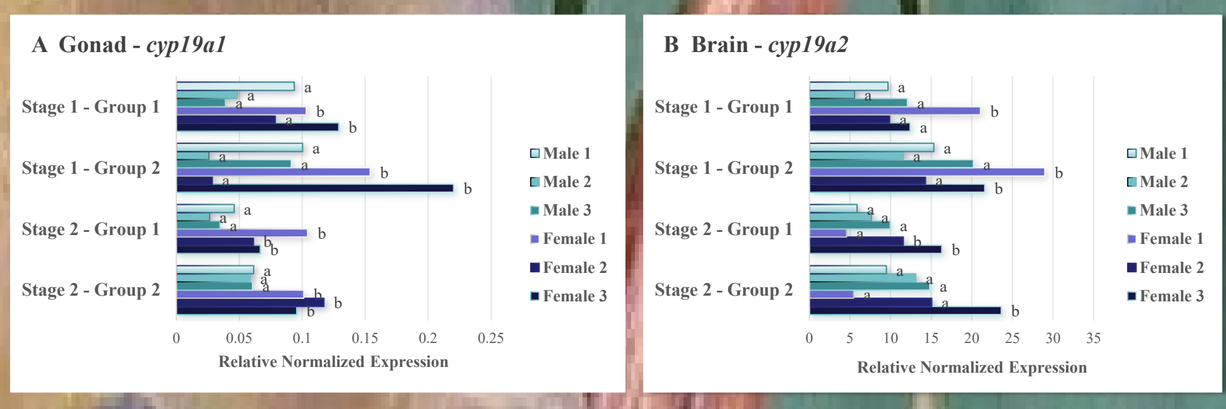


Fig.5 Gene expression of experimental cT, cNC and PBS groups. *represented significant; ** represented highly significant (P < 0.01)

- The expression level of the *dmrt1* gene in all the cT groups was significantly (P<0.05) upregulated compared to cNC and PBS groups, where showing significantly (P<0.05) lower-level expression of the *dmrt1* gene (Fig. 6)
- The cT group displayed a significantly (P<0.01) higher expression of the *cyp19a1* than the cNC and PBS groups. There were no significant differences (P>0.05) found in the expression of the *foxl2* and *hsp70* among the cT, cNC and PBS groups (Fig. 6)

Fig.6 The relative expression of experimental cT, cNC, and PBS groups. The data represent the mean ± SE. *represented significant; ** represented highly significant (P < 0.01)

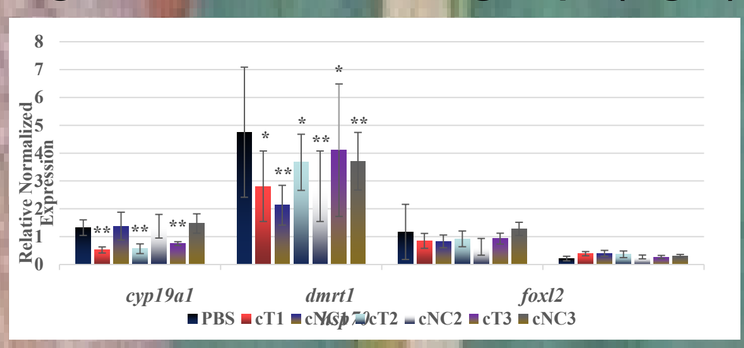
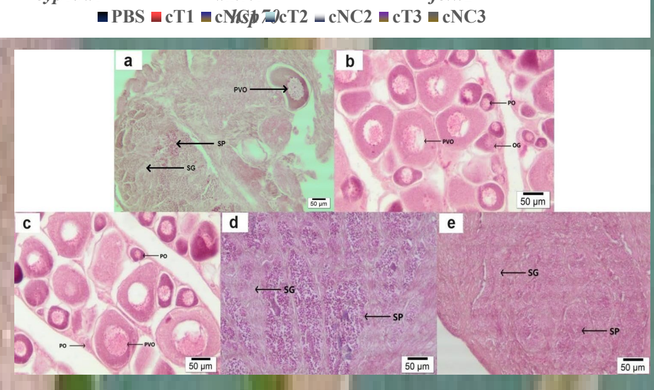


Fig. 7 Histological observation of gonads of cT group (a), cNC group (b), PBS group (c), MTH group (d), MTL group (e). OG, oogonium; PO, perinucleolar oocyte; PVO, previtellogenic oocyte; SG, spermatogonia; SP, sperm



Gonadal development and structure

- Some male germ cells were identified in the *cyp19a1* treatment group although it contained various stages of oocytes (Fig. 7a).
- The male germ cells identified were similar to those observed in the MTL group (Fig. 7d) and MTH group (Fig. 7e).
- In contrast, gonads in the PBS and cNC groups presented only regular oocytes (OG), perinuclear oocytes (PO), and pre-yolk oocytes (PVO) in the paraffin sections.
- No spermatogonia or sperm were observed in the cNC groups (Fig. 7b) and PBS groups (Fig. 7c)