Transcriptome profile of sex-related gene expression and effects of *cyp19a1* and *dmrt1* siRNA-silencing on sex differentiation and determination in yellow perch



Hong Yao, Ding-Kun Xie, Rafidah Othman, Paul O'Bryant, and Han-Ping Wang,
Ohio Center for Aquaculture Research and Development, The Ohio State University South Centers
1864 Shyville Road, Piketon, OH 45661, wang.900@osu.edu

THE OHIO STATE UNIVERSITY
CFAES

Introduction

- Yellow perch (*Perca flavescens*) is a native North American fish species with a phylogenetic relationship to the Eurasian perch
- It has an XX/XY sex determination system and exhibits female-biased growth.
- *dmrt1* is a conserved male-associated gene essential for testis development.
- *cyp19a1*, encoding gonadal aromatase, is critical for estrogen synthesis and ovarian differentiation.
- Disruption of either gene can alter gonadal development, sex ratios, and growth.
- In this study, in vivo, RNA interference (RNAi) was used to silence *dmrt1* and *cyp19a1* to clarify their roles in sex differentiation and evaluate their potential for sex control in yellow perch.

Methods and Materials

- Experiment had eight groups: 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)

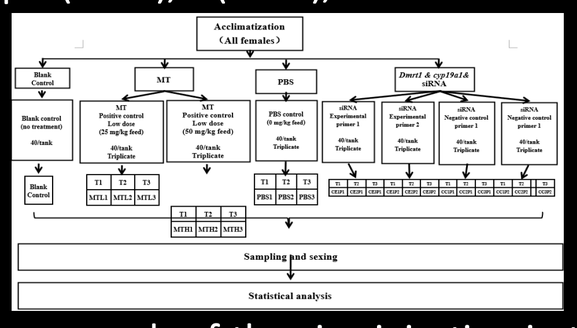


Fig. 1 Experimental design for examining the effects of siRNA silencing of dmrt1 and cyp19a1 on sex differentiation and determination in yellow perch

- 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.

Results and Discussion

dmrt1

Gene Expression

- PCR verification confirmed the presence of *dmrt1* in yellow perch. qPCR analysis revealed significant down-regulation of *dmrt1* and up-regulation of *cyp19a1* in *dmrt1* siRNA groups compared to controls, indicating successful gene interference (Fig.2).

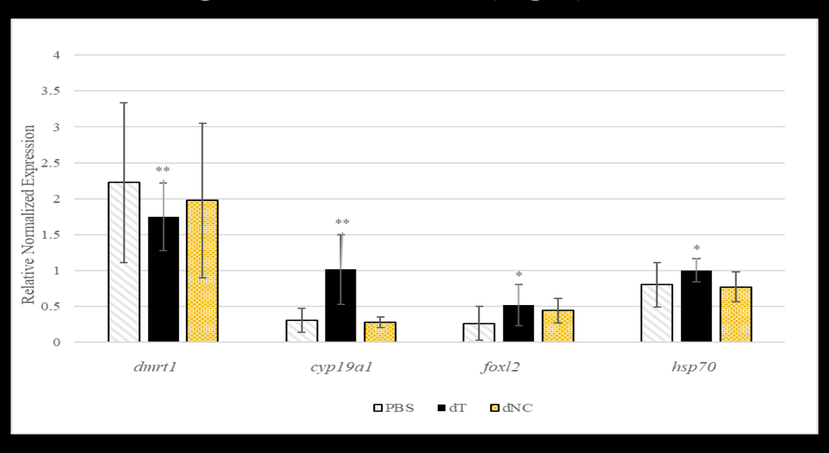


*Fig. 2 The relative expression of experimental dT, dNC, and PBS groups in double gonad tissues. The data represent the mean±SE. *represented significant; ** represented highly significant (p < 0.01).*

Gonadal Development

- Histological analysis showed distinct male and female gonadal structures in controls. siRNA treatment resulted in altered gonadal morphology, with the dT group exhibiting enlarged gonads compared to controls (Fig. 3 & 4).

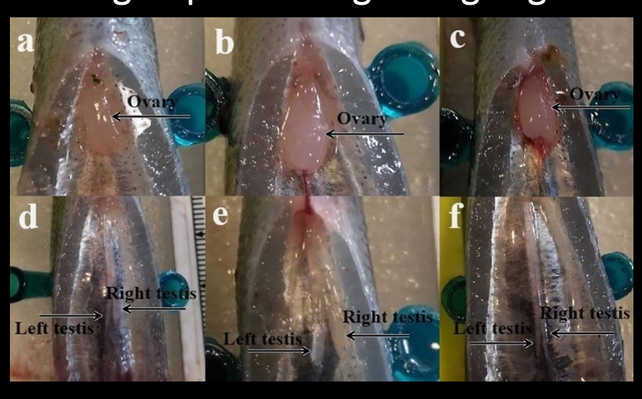


Fig. 3 Morphological observation of female and male gonads of MT group (a), dT group (b), and dNC group (c), and male gonads of MT group (d), dT group (e), and dNC group (f).



Fig. 4 Visual observation of single gonad (a) of a female and double gonad (b) of a male in control group.

Histological observation results



Fig. 5 Histological observation of single gonads (♂) of PBS (a,b), dT group (c,d), dNC group (e,f). OG, oogonium; PO, perinucleolar oocyte; PVO, previtellogenic oocyte.

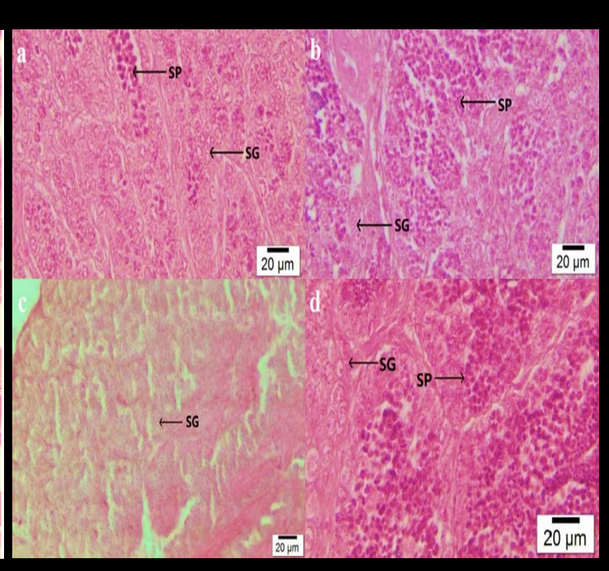


Fig. 6 Histological observation of double gonads (♂) of MTL group(a), MTH group(b), dT group (c), dNC group (d). SG, spermatogonia; SP, sperm.

Sex Ratios

- *The* full-length sequences of *cyp19a1* and *cyp19a2* in YP were obtained by comparing them with genomic homology confirming they belong to P450 family. MT treatment achieved 100% male reversal. siRNA-treated groups showed increased female ratios (dT1: 71.4%, dT2: 67.6%, dT1+dT2: 71.1%) compared to controls (46.9%-51.3%), demonstrating effective sex reversal.

Physiological Indices

- siRNA groups exhibited significant differences in gonadosomatic and hepatosomatic indices compared to PBS and negative controls. The dT group displayed higher liver function (HSI) and reduced gonadal maturation (GSI).

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cyp19a1

Gene expression

- The differentially expressed genes between male and female gonads were compared using a volcano plot (Fig. 7) after the results of differentially expressed genes (DEGs) analysis.

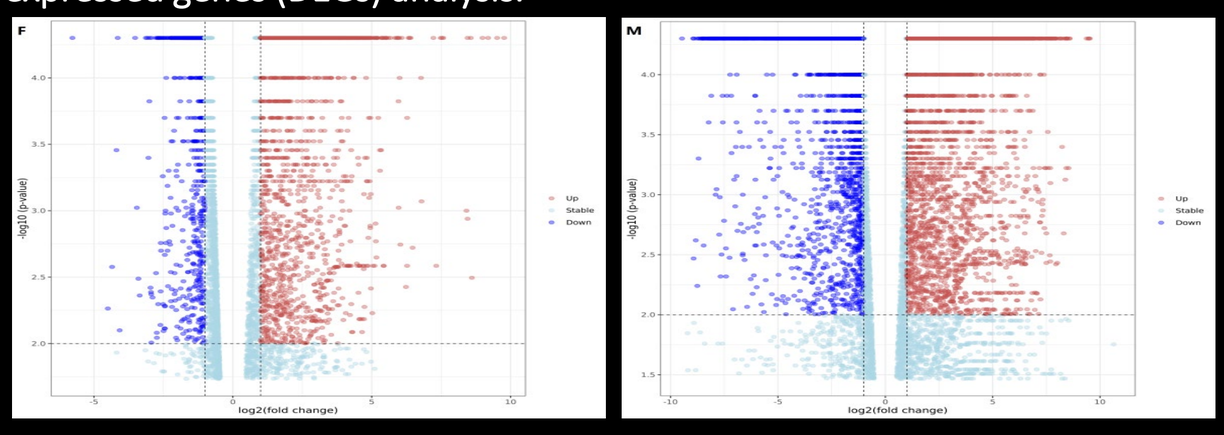


Fig. 7 Volcanic map of differential expression of genes of male and female gonads. F, ♀ gonad; M, ♂ gonad. Red, upregulation; Blue, downregulation

- 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. 8A).
- 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. 8B). The expression of *cyp19a1 and cyp19a2* in liver could not be detected in all the samples.

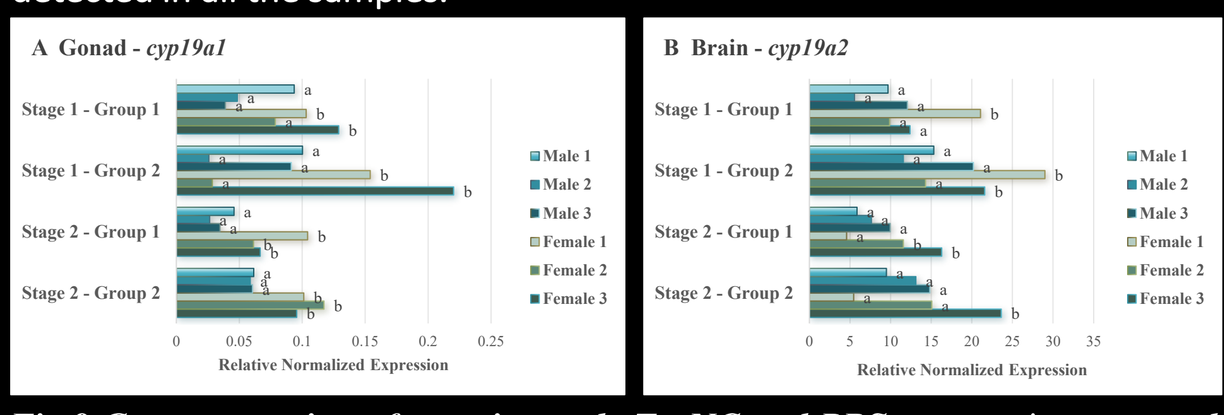


*Fig.8 Gene expression of experimental cT, cNC and PBS groups. *represented significant; ** represented highly significant (P < 0.01)*

- 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. 9)
- 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. 9)

*Fig.9 The relative expression of experimental cT, cNC, and PBS groups. The data represent the mean ± SE. *represented significant; ** represented highly significant (P < 0.01)*

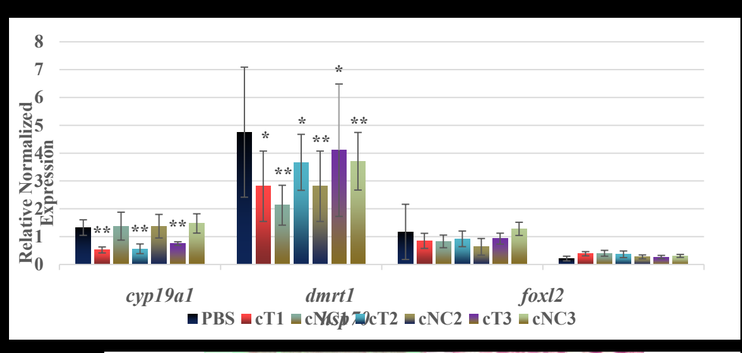
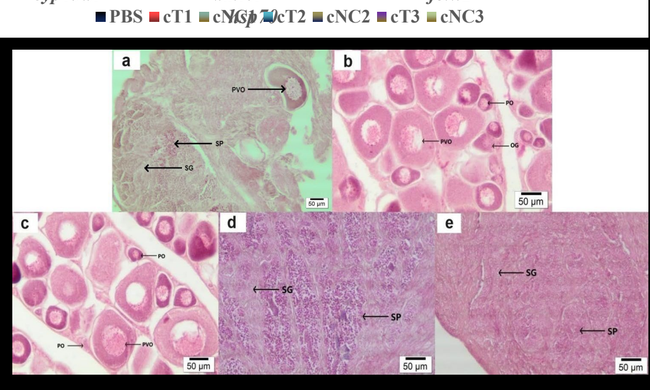


Fig. 10 Histological observation of gonads of cT group (a), cNC group (b), PBS group (c), MTH group (d), MTL group (e). OG, oogonium; PO, perinucleolar oocyte; PVO, previtellogenic oocyte; SG, spermatogonia; SP, sperm



Gonadal development and structure

- Some male germ cells were identified in the *cyp19a1* treatment group although it contained various stages of oocytes (Fig. 10a).
- The male germ cells identified were similar to those observed in the MTL group (Fig. 10d) and MTH group (Fig. 10e).
- 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. 10b) and PBS groups (Fig. 10c)