Dmrt1-siRNA Interference Led To Increased Female Number And Larger Size In Yellow Perch

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Introduction

- Yellow perch (*Perca flavescens*) is a species exhibiting male heterogametic sex determination (XX/XY) with a natural sex ratio of about 1:1.
- Females grow larger and faster than males, making monosex female production beneficial for aquaculture.
- Identifying key genes responsible for male sex determination and manipulating them via RNA interference (RNAi) could enable control over sex differentiation and facilitate the breeding of monosex populations.
- DMRT1 (Double Sex and Mab-3 Related Transcription Factor 1) is a crucial gene involved in sex determination across various organisms.
- This study aims to explore the role of the *dmrt1* gene in sex determination of yellow perch using RNAi technology to achieve male-to-female sex reversal.

Methods and Materials

- Yellow perch fry were raised and distributed into 32 tanks, eight experimental groups with three replicates each (Fig.1):
- PBS control, MT positive controls (MTL and MTH), *dmrt1* siRNA groups (dT1, dT2, and dT1+dT2), and siRNA negative controls (dNC1, dNC2, and dNC1+dNC2).

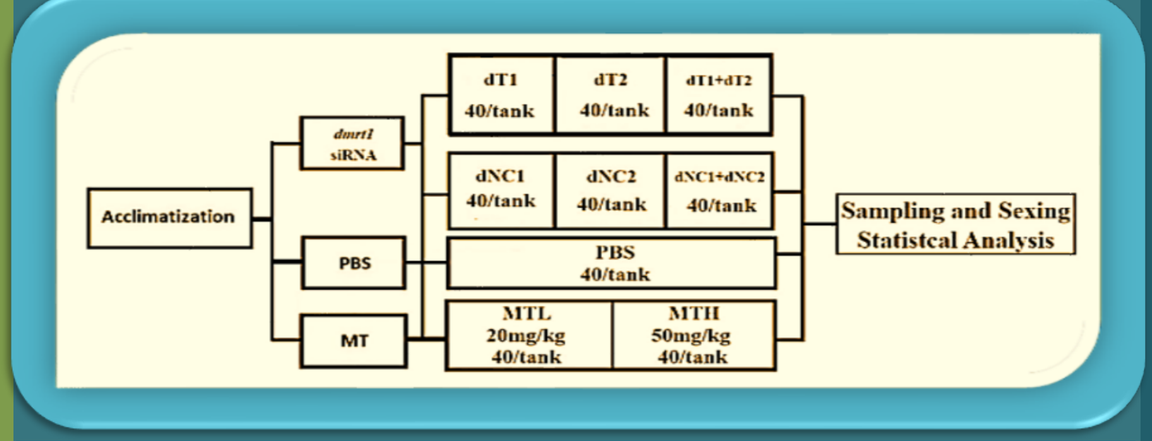

Fig. 1 Experimental design for examining the effects of siRNA silencing of *dmrt1* on sex differentiation and determination in yellow perch.

- RNAi treatment was administered through siRNA injections at 58-, 78-, 98-, and 121-days post-hatch (dph).
- Gene sequences of *dmrt1* and *cyp19a1* from homologous species were aligned with yellow perch sequences to design siRNA using Thermo Fisher BLOCK-iT RNAi Designer.
- Sequences were validated and synthesized in vivo (Table 1).

Gene	Group	siRNA sequence
dmrt1	Experimental group primer 1 Stealth-D1（dT1）	Sense: CCAGAUUUAUCUCCCUCCCAGGAUA
		Anti-Sense: UAUCCUGGGAGGGAGAUAAAUCUGG
	Positive control primer 1 Stealth-control-D1（dNC1）	Sense: CCAUUUCUACCUCUCACCGGGAAUA
		Anti-Sense: UAUUCCCGGUGAGAGGUAGAAAUGG
	Experimental group primer 2 Stealth-D2（dT2）	Sense: CCUGUCGACUCACAAUUAAUCUCUU
		Anti-Sense: AAGAGAUUAAUUGUGAGUCGACAGG
	Positive control primer 2 Stealth-control_D2（dNC2）	Sense: CCUCGACUCACAAUUAAUCUGUCUU
		Anti-Sense: AAGACAGAUUAAUUGUGAGUCGAGG

Table 1 The designed siRNA sequences of gene *dmrt1* for the experiment

- Total RNA was extracted from gonads of 180 dph yellow perch.
- cDNA was synthesized using a high-capacity cDNA reverse transcription kit.
- qPCR was employed with the internal reference primers β-actin to detect the expression *of dmrt1, cyp19a1, foxl2*, and *hsp70*.
- Gonads were examined macroscopically and microscopically to determine sex and assess development.
- Histological analysis involved paraffin sectioning and staining to evaluate cellular structures.
- Sex ratios and gene expression levels were analyzed using Chi-square tests and ANOVA.
- Gonadosomatic, hepatosomatic, and viscerosomatic indices were calculated and compared across groups.

Conclusion

- This study successfully demonstrated that RNAi targeting *dmrt1* can manipulate sex differentiation in yellow perch.
- The significant down-regulation of *dmrt1* and up-regulation of *cyp19a1* resulted in phenotypic sex reversal.
- The findings contribute to advancing aquaculture practices by enabling the production of monosex female populations, optimizing growth, and improving yield.
- Future research should explore the long-term effects and ecological impacts of genetically manipulated populations in aquaculture systems.

Acknowledgments

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Results

Gene Expression

PCR verification confirmed the presence of *dmrt1* in YP. qPCR analysis revealed significant downregulation of *dmrt1* and upregulation of *cyp19a1* in *dmrt1* siRNA groups compared to controls, indicating successful gene interference.

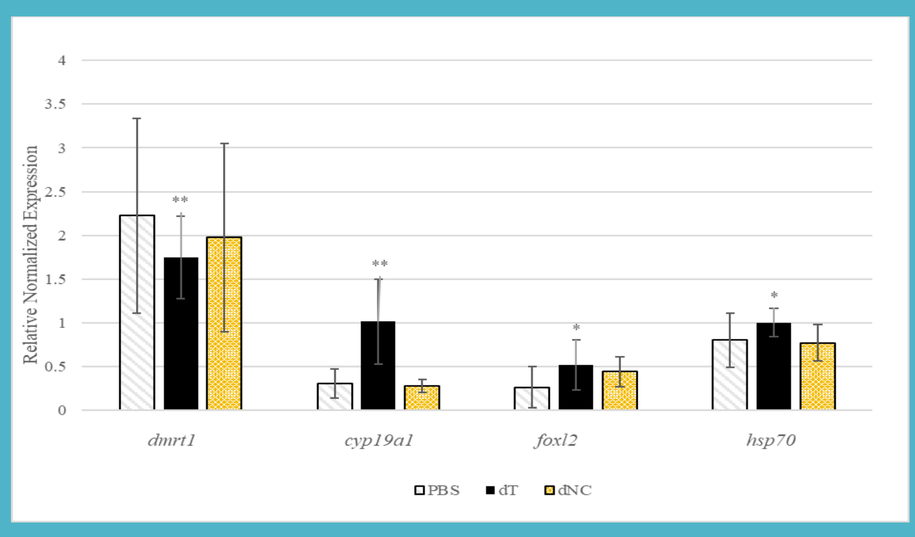

Figure 2 The relative expression of experimental dT, dNC, and PBS groups in double gonad tissues. The data represent the mean±SE. *represented significant; ** represented highly significant ($P < 0.01$).

Gonadal Development

siRNA treatment resulted in altered gonadal morphology, with the dT group exhibiting enlarged gonads compared to controls (Fig. 3, 4). Histological analysis showed distinct male and female gonadal structures in controls (Fig. 5, 6).

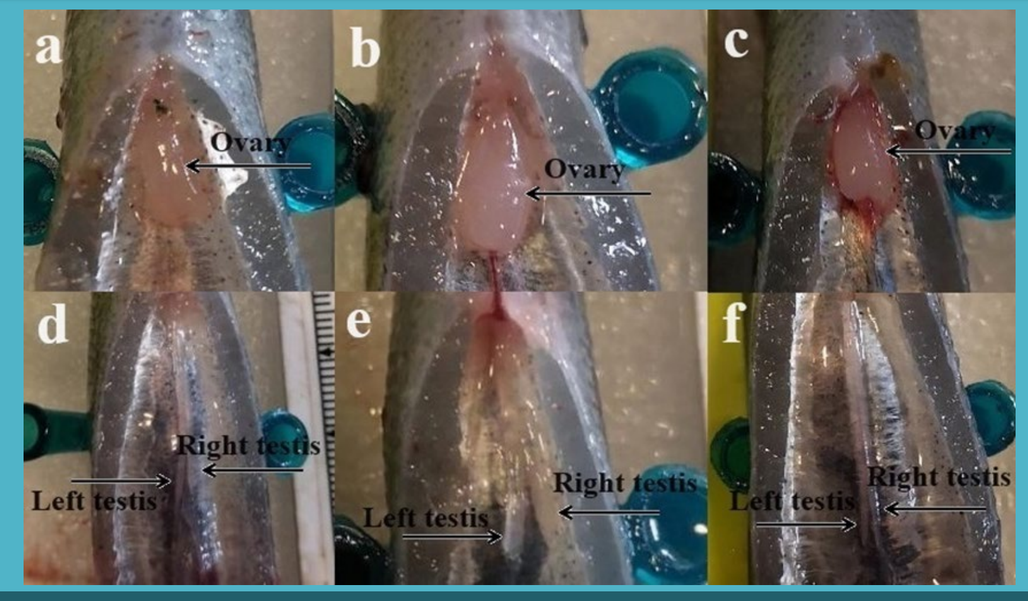

Fig. 3 Morphological observation of female gonads: PBS group (a), dT group (b), and dNCgroup (c), and male gonads: PBS group (d), dT group (e), and dNCgroup (f).

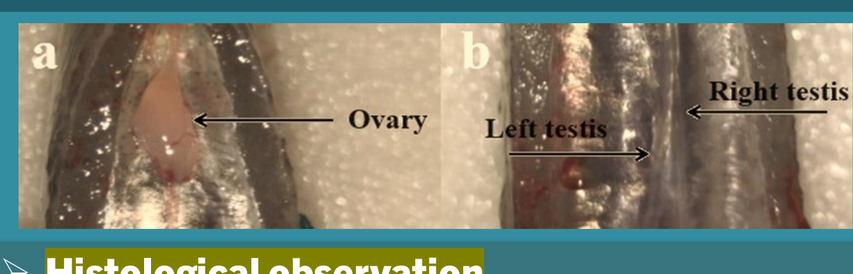

Fig. 4 Visual observation of single gonad (a) of a female and double gonad (b) of a male in control group.

Histological observation

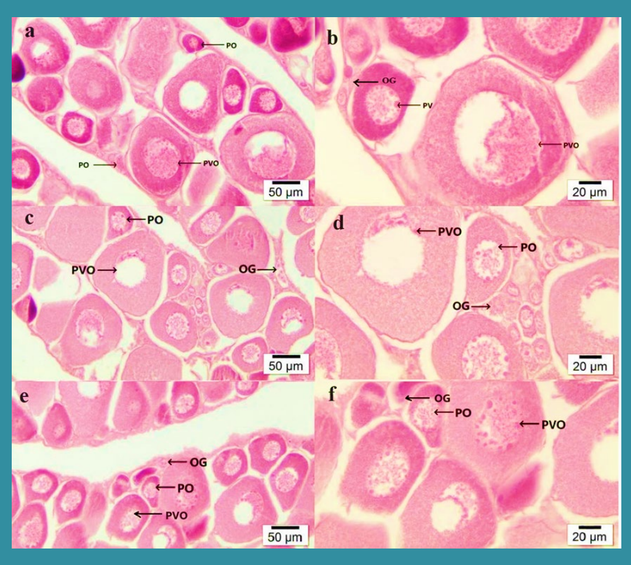

Fig. 5 Histological observation of single gonads (♂) of PBS (a,b), dT group (c,d), dNC group (e,f). OG, oogonium; PO, perinucleolar oocyte; PVO, previtellogenic oocyte.

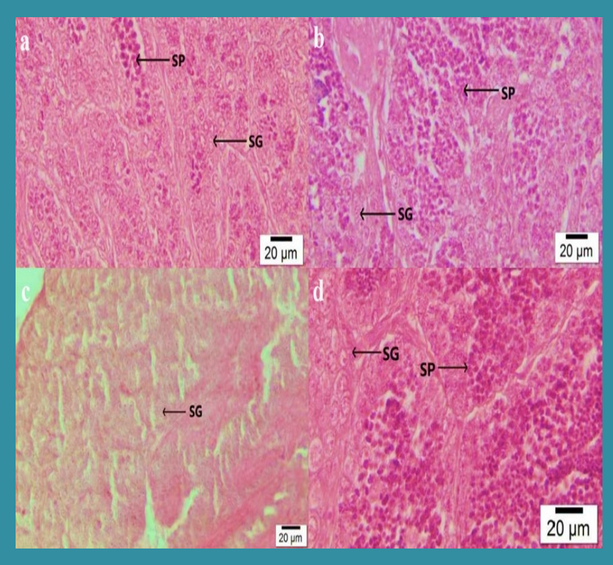

Fig. 6 Histological observation of double gonads (♂) of MTL group (a), MTH group (b), dT group (c), dNC group (d). SG, spermatogonia; SP, sperm.

Sex Ratios

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).

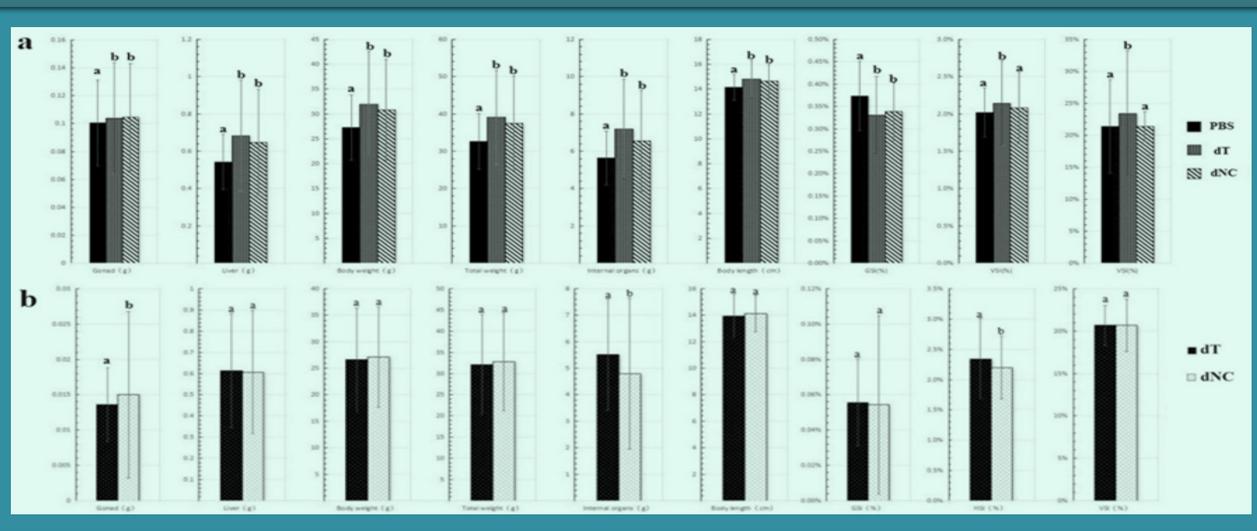

Fig. 7 Column chart of *dmrt1*-siRNA groups statistical comparison in single (a) and double (b) gonad anatomy. The data represent the mean±SD. The groups not sharing common letters are significantly different ($P<0.05$)