Optimization of neo-female induction technology and breeding of supermale in large yellow croaker (*Larimichthys crocea*)

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Abstract

Male large yellow croaker, favored for their slender build, can improve breeding economic benefits with all-male cultivation. Sex reversal to produce neo-female and supermale fish is method for all-male populations within the XY sex system. The study induced neo-female fish by feeding diets with E_2 concentrations (0, 0.2, 1, 5 mg/kg) to large yellow croaker. E_2 was found to inhibit growth, lower survival, and boost antioxidant enzyme activity, showing a dose-dependent effect. Sex reversal rates were 67%, 95%, and 100% in respective treatment groups, making 1 mg/kg E_2 the best induction dose. At six months, neo-female fish ovaries were histologically similar to controls, but development lagged after nine months. These fish, like females, expressed only the female gene cyp19a1a, not the male gene dmrt1. Molecular and histological confirmation showed neo-female fish could produce supermales when mated with males. The study established effective methods for inducing neo-female and cultivating supermale large yellow croaker, supporting all-male production with theory and tech know-how.

Project designes

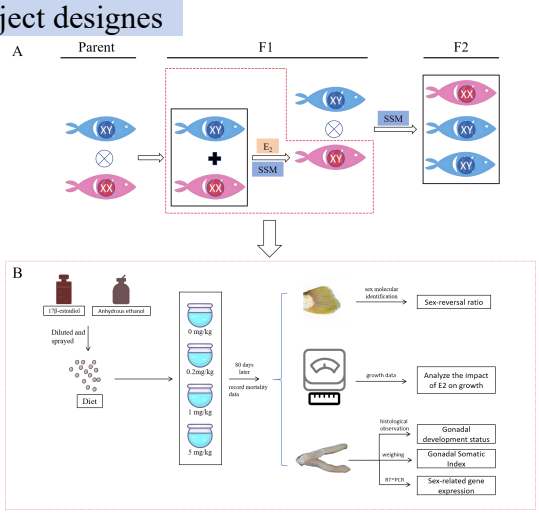

Fig. 1. Schematic diagram of approach for supermale production (A). The technical route for optimizing the induction technique of neo-female large yellow croaker (B).

Results

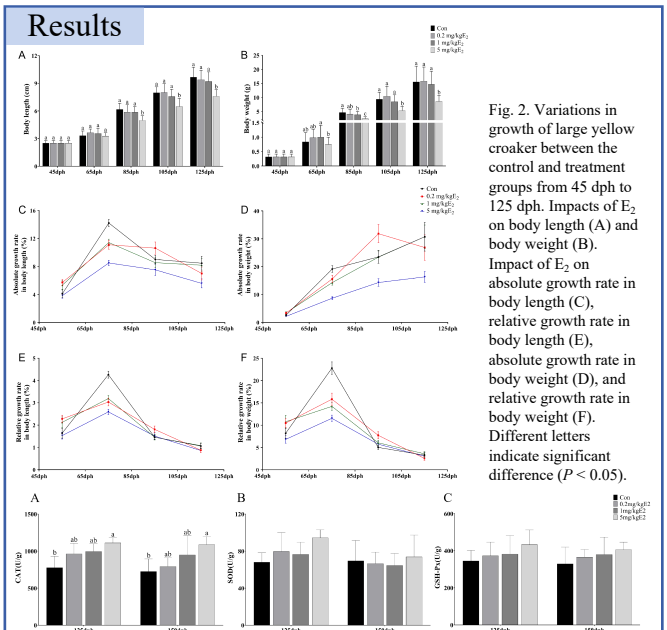

Fig. 2. Variations in growth of large yellow croaker between the control and treatment groups from 45 dph to 125 dph. Impacts of E_2 on body length (A) and body weight (B). Impact of E_2 on absolute growth rate in body length (C), relative growth rate in body length (E), absolute growth rate in body weight (D), and relative growth rate in body weight (F). Different letters indicate significant difference ($P < 0.05$).

Fig. 3. Effect of E_2 treatment on serum biochemical indices in large yellow croaker. (A) catalase, CAT; (B) superoxide dismutase, SOD; (C) glutathione peroxidase, GSH-PX. Different letters indicate significant difference ($P < 0.05$).

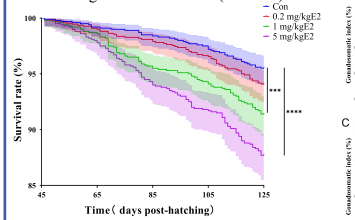

Fig. 4. The impact of E_2 on the survival of large yellow croaker during the treatment period (45dph-125dph). Values are considered to be significantly different among groups at $P <$ 0.001 (***) and $P < 0.0001$(****).

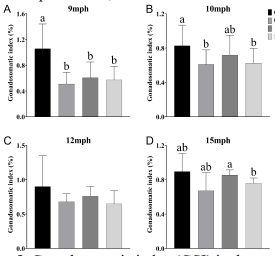

Fig. 5. Gonadosomatic index (GSI) in the control and E_2-treated groups at 9 mph (A), 10 mph (B), 12 mph (C), and 15 mph (D). Different letters indicate significant differdence ($P < 0.05$).

Results

Table 1. Impact of different concentrations of E_2 on the sex reversal ratio of large yellow croaker.

Mph	Groups	Sample size	Phenotypic female	Phenotypic male	Phenotype-uncertain	Genotypic female	Genotypic male	Sex-reversal ratio
4	Con	20	65%	35%	0	65%	35%	—
	0.2mg/kg	20	65%	5%	30%	45%	55%	37%
	1mg/kg	20	90%	0	10%	40%	60%	83%
	5mg/kg	20	100%	0	0	70%	30%	100%
6	Con	20	40%	60%	0	40%	60%	—
	0.2mg/kg	20	75%	20%	5%	50%	50%	50%
	1mg/kg	20	100%	0	0	40%	60%	100%
	5mg/kg	20	100%	0	0	55%	45%	100%
9	Con	20	50%	50%	0	50%	50%	—
	0.2mg/kg	20	80%	20%	0	10%	90%	78%
	1mg/kg	20	100%	0	0	50%	50%	100%
	5mg/kg	20	100%	0	0	45%	55%	100%
15	Con	20	55%	45%	0	55%	45%	—
	0.2mg/kg	20	70%	30%	0	10%	90%	67%
	1mg/kg	20	100%	0	0	50%	50%	100%
	5mg/kg	20	100%	0	0	40%	60%	100%

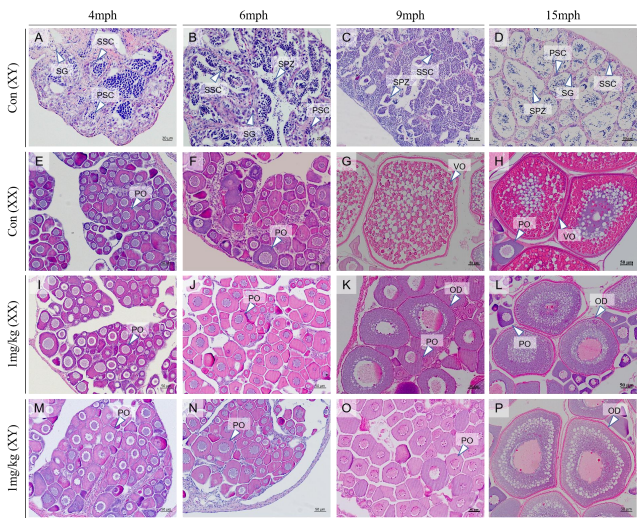

Fig. 6. Histological examination of groups with control (A-H) and the 1mg/kg E_2 (Q-X) large yellow croaker gonads at 4, 6, 9 and 15 month post-hatch(mph). A-D, ovaries of control fish with XX genotype; E-H, testes of control fish with XY genotype, Q-T, ovaries of 1mg/kg E_2 fish with XX genotype; U-X, ovaries of 1mg/kg E_2 fish with XY genotype; PO, peri-nucleolus oocyte; OD, oil-droplet stage oocytes; VO, vitellogenesis oocytes; SG, spermatogonia; PSC, primary spermatocyte; SSC, second spermatocyte; SPZ, spermatozoa.

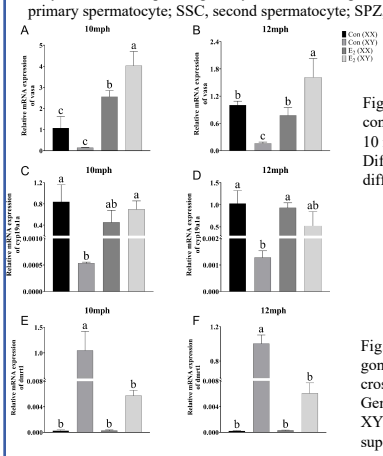

Fig. 7. Gonadosomatic index (GSI) in the control and E_2-treated groups at 9 mph (A), 10 mph (B), 12 mph (C), and 15 mph (D). Different letters indicate significant differdence ($P < 0.05$).

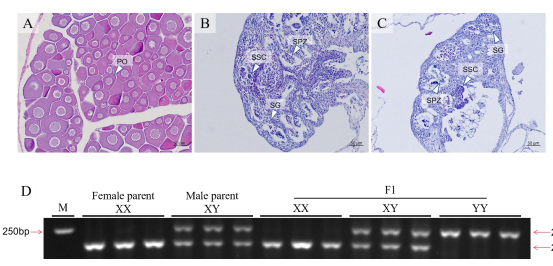

Fig. 8. Histological observation of the gonads in the offspring at 140dph from the crossbreeding of neo-female and male fish. Genotype XX female fish (A), genotype XY male fish (B), and genotype YY supermale fish (C). D shows the genetic sex identification. PO, peri-nucleolus oocyte; SG, spermatogonia; SSC, second spermatocyte; SPZ, spermatozoa.

Conclusion

(1) This study demonstrates that oral administration of E_2 can effectively induce feminization in large yellow croaker.
(2) Considering all the effects of E_2 on growth (during E_2 treatment), survival, sex ratio, and gonadal development, we concluded that a dietary dose of 1 mg/kg E_2 is the optimal dosage for the feminization of large yellow croaker.
(3) We successfully bred supermale large yellow croaker, providing a theoretical basis and technical support for the production of all-male large yellow croaker.