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Effects of fish oil on ovarian development in spotted scat (*Scatophagus argus*)

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ABSTRACT

The effects of different concentrations of dietary fish oil (0, 2%, or 6%) on ovarian development in 2-year-old female *Scatophagus argus* were investigated. The levels of serum sex steroid hormones (estradiol-17β, E_2; testosterone, T), protein phosphorus (SPP), and protein calcium (SPC), as well as vitellogenin (*vtg*) mRNA expression in livers and ovaries were measured. Over the eight week experimental period, oocytes did not develop further and remained at phase III in fish fed with the control diet with no supplement of fish oil. Fish fed with 2% fish oil supplement had oocytes at transition phase from III to IV. Fish fed with 6% fish oil supplement had oocytes at late phase IV. Higher gonadosmatic index, serum E_2, SPP, SPC, and liver *vtg* expression were found in 6% fish oil group compared to that in the 2% fish oil group (except E_2) and the control group ($P < 0.05$). In addition, *vtg* expression in livers was 600–1000 times higher than that in the ovaries. Gonadosmatic index, E_2, and SPP, as well as liver *vtg* expression increased during the experiment and peaked at the end of experiment. However, hepatosomatic index, serum T, and ovarian *vtg* expression peaked at 4 weeks, and then decreased at 8 weeks, with no significant difference among the 3 groups. In summary, we showed that 6% fish oil supplementation in *S. argus* could effectively promote ovarian development, with associated increases in E_2 secretion and increased liver *vtg* mRNA expression.

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1. Introduction

Hormonal, environmental, social and nutritional factors contribute to the regulation of reproduction in fish. Dietary lipids, as nutritional factors, can modulate reproductive function. Marine fish can synthesize neither shorter chain precursors such as 18:3*n*-3 nor *n*-3 long-chain (≥C20) polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) *de novo*, therefore, DHA and EPA supplement are required in diet (Cejas et al., 2003; Rennie et al., 2005). Fish oil contains high concentrations of DHA and EPA, and is heavily exploited globally in aquatic feeds (Jackson, 2006; Tacon and Metian, 2008). Although fish oil in aquatic feeds has been replaced with alternative oils, several studies have shown that decreasing dietary *n*-3 PUFA significantly altered the fatty acid composition of tissues (Bransden et al., 2003; Codabaccus et al., 2011; Brown et al., 2010; Trushenski and Boesenberg, 2009). Therefore, an appropriate amount of fish oil was still required during the finishing period to restore the fatty acid composition of fish fed with alternative oils. Previous nutrition studies have focused on the effects of dietary composition on body composition, growth performance, egg quality or larval survival (Bransden et al., 2003; Rennie et al., 2005; Zakeri

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et al., 2011). Few studies have investigated physiological and biochemical responses of ovarian development. In *Dicentrarchus labrax*, dietary PUFA significantly facilitates the annual changes of sex steroids and liver vitellogenin (*vtg*) expression (Prat et al., 1990; Mananos et al., 1994; Navas et al., 1998). Fremont et al. (1984) also showed that the synthesis of the yolk precursor, *vtg*, was facilitated by *n*-3 PUFA. Furthermore, long-term dietary deficiencies in *n*-3 PUFA may affect plasma lipids patterns and induce early gonadal atresia. Cerdá et al. (1995) showed that dietary *n*-3 PUFA is positively correlated with liver *vtg* expression.

Spotted scat, *Scatophagus argus*, an euryhaline subtropical fish, is widely distributed in India-Pacific waters including Guangdong, Taiwan and Guangxi coast in China (Barry et al., 1993). At present, juvenile spotted scat must be harvested from the wild and the quantity available is limited. There is increasing demand of spotted scat seedlings. In *S. argus*, female sexual maturation is later than the males, and their ovaries cannot fully mature for breeding in artificial environments (Lan et al., 2005). Some studies investigated the reproductive biology of *S. argus* (Phuang et al., 2004; Cai et al., 2010). Research on breeding was mainly concentrated on artificial induction of sperm and egg maturation in some parts of southeast Asia (Barry et al., 1991, 1993; Fast, 1988; Biona et al., 1988), but female maturation could not be induced artificially. Studies have confirmed that spotted scat are omnivorous, eating algae, detritus, diatoms, and crustaceans, but showing a preference for algae (Sivan and Radhakrishnan, 2011), therefore, the composition, especially fish oil content, are important for the gonadal development in spotted scat fed with formulated diets. However, information concerning the effect of dietary fish oil content on ovarian development, serum sex steroid and *vtg* mRNA expression in livers and ovaries is limited in spotted scat.

In the present study, spotted scat were reared with formulated diets which contained different levels of fish oil (0, 2%, and 6%, respectively). The aim was therefore to evaluate serum sex steroid hormone (estradiol-17β, E_2; testosterone, T), protein phosphorus (SPP), and protein calcium (SPC) levels, as well as *vtg* expression in livers and ovaries in 2-year-old female spotted scat. This study will provide information on ovarian development and benefit artificial breeding of spotted scat.

2. Materials and methods

2.1. Experimental fish

Two-year-old female fish (body weight, 316.3 ± 70.3 g, body length, 21.4 ± 2.0 cm) were obtained from Zhuhai Longsheng Fry Cultivation Base (Zhuhai, Guangdong, China), and feeding experiment lasted 8 weeks. Before the experiment, the fishes were domesticated for one week. All of the experimental fishes were reared at indoor conditions. The water salinity was 10 ± 2, and the water temperature was 20 ± 2 °C. Fish were fed with Guangdong Tongwei marine fish feed once a day.

Table 1
Ingredients, formulation and proximate composition of the experimental diets containing different levels of fish oil.

Ingredient composition (g/kg as fed basis)	Diets		
	Control	2% fish oil	6% fish oil
White fishmeal	300	300	300
Casein	250	250	250
Peeled soybean meal	50	50	50
Vital wheat gluten	50	50	50
Corn starch	193.2	193.2	193.2
Sodium alga acid	30	30	30
Fish oil	0	20	60
Corn oil	80	60	20
Soybean lecithin	20	20	20
L-Ascorbicacid 2-phosphate-Mg	0.5	0.5	0.5
Choline chloride	5	5	5
Vitamin premix	3	3	3
Mineral premix	7	7	7
Attractant	1	1	1
Ethoxyquin	0.3	0.3	0.3
Calcium monophosphate	10	10	10
Proximate composition (%)			
Crude protein	48.11	47.89	47.65
Crude lipid	13.84	13.69	13.49

2.2. Experimental design and sampling procedures

Two-year-old female fish were divided into 3 groups (20 per group), and fed with diets containing different levels of fish oil (0, 2%, or 6%) (Table 1) in 1 m × 1 m × 2 m net cages. Table 2 shows fatty acid composition of the experimental diets. At 0, 4, and 8 weeks, 6 fish were collected randomly from each group, and then were bled with sterile syringe from the caudal vasculature (about 1–2 ml) after anaesthesia with clove oil (Linda Biological Technology Co., Zhanjiang, Guangdong). Sera were collected by centrifugation (at 6000 r/min for 5 min at 4 °C) and kept frozen (−20 °C) until assay of sex steroid hormone, SPP and SPC. Livers and ovaries were quickly dissected and frozen in liquid nitrogen, and then stored at −70 °C for assay of *vtg* expression. A sample from the central part of the ovary was fixed in Bouin's solution, dehydrated with gradient alcohol, cleared with xylene and

Table 2
Proximate analysis of fatty acids in the experimental diets.

Fatty acids	Control	2% fish oil	6% fish oil
16:00	19.30	19.83	18.34
16:1*n* − 9	0.29	0.13	0.29
17:00	1.25	0.64	0.81
18:00	10.40	10.24	10.71
18:1*n* − 9	18.49	18.41	16.46
18:2*n*-6	15.90	14.98	10.95
20:00	0.54	0.72	0.62
18:3*n*-3	0.68	1.41	1.51
22:00	2.62	1.21	1.69
20:4*n*-6	2.42	2.17	1.70
EPA	11.67	12.11	13.38
DHA	16.43	18.15	23.54
$\sum$*n*-3 PUFA	28.78	31.67	38.43
$\sum$*n*-6 PUFA	18.32	17.15	12.65
n-3/*n*-6 PUFA	1.57	1.85	3.04
DHA/EPA	1.41	1.50	1.76

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