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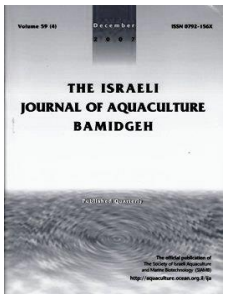

Effects of Different Dietary Lipid Sources on Spawning Performance, Egg and Larval Quality, and Egg Fatty Acid Composition in Tongue Sole *Cynoglossus semilaevis*

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Key words: *Cynoglossus semilaevis* broodstock, dietary lipid source, spawning performance, egg and larval quality

Abstract

A 60-day feeding experiment was conducted to investigate the effects of dietary lipid sources on reproduction of *Cynoglossus semilaevis*. Experimental diets were formulated with similar proximate compositions but different lipid sources (6.5%): fish oil (FO), soybean oil (SO) and olive oil (OO). The results showed that the relative fecundity in group FO and OO was significantly higher than that in group SO. Group OO showed a significantly higher buoyant egg rate than group FO and SO. The hatching rate and larval survival rate at 7 days post hatching were the highest in group FO, followed by group OO, and group SO recorded the lowest values. Group FO showed significantly higher egg diameter and larval survival activity index (SAI) and significantly lower larval deformity rate compared to group SO and OO. Fatty acid compositions of eggs reflected closely those of the diets. These results showed that the olive oil supplement in diets for tongue sole positively influenced the broodstock fecundity and buoyant egg rate though fish oil resulted in the highest hatching rate and best larval quality among the tested oils. The dietary soybean oil supplement reduced the spawning performance, and egg and larval quality.

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Introduction

Broodstock nutrition is recognized as a major factor influencing fish reproduction and larval quality. Lipid and fatty acids have been identified as major dietary factors that influence successful reproduction and survival of offspring (Izquierdo et al., 2001). In marine fish, fish oil, which is rich in long chain-polyunsaturated fatty acids (LC-PUFAs), i.e., docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6), was traditionally used as the lipid source in broodstock diets. It is widely known that LC-PUFAs which are essential to maintain the normal structure and function of biomembranes (Bell and Sargent, 2003), play critical roles in fish reproduction and larval development (Cerdá et al., 1995; Lavens et al., 1999; Mazorra et al., 2003; Nguyen et al., 2010; Zakeri et al., 2011; Reza et al., 2012). However, it has also been reported that excess LC-PUFAs could be detrimental to fish fecundity, egg quality and larval survival (Fernández-Palacios et al., 1995, 1997; Furuita et al., 2002, 2003; Li et al., 2005; Wilson, 2009). The inhibition of excess n-3 LC-PUFAs or ARA on fish reproduction has been observed in several marine species such as gilthead sea bream (Fernández-Palacios et al., 1995, 1997), Japanese flounder (Furuita et al., 2002, 2003), rabbitfish (Li et al., 2005), and Chilean flounder (Wilson, 2009). Thus, to obtain an appropriate fatty acid profile for marine broodstock diets, there is increasing interest in dietary supplementation of alternative oils, such as animal oils and vegetable oils which are LC-PUFAs deficient. The dietary supplementation of alternative oils can also help achieve an appropriate dietary ratio of n-3 fatty acids to n-6 fatty acids (n-3/n-6 ratio), which is very important for successful fish reproduction and larval survival. In addition, some fatty acids contained in alternative oils, such as C18:3n-3, C18:2n-6 and C18:1n-9, could have potential benefits for fish reproduction (Almansa et al., 1999). This makes the studies on supplementation of alternative lipid sources in broodstock diets more worthwhile.

Several studies have shown that the combined use of fish oil and alternative oils such as beef tallow (Fernández-Palacios et al., 1995), lard (Li et al., 2005), and corn oil (Furuita et al., 2007; Wilson, 2009) in marine fish diets resulted in improved spawning performance and larval quality compared with using fish oil as the sole lipid source. Japanese flounder fed palm olein as the sole lipid source produced more eggs than fish fed pollock visceral oil as the sole lipid source (Furuita et al., 2000). However, the study on yellowfin sea bream showed that the supplementation of sunflower oil in broodstock diet reduced the spawning performance (Zakeri et al., 2011), while another study on cobia (Nguyen et al., 2010), demonstrated that inclusion of up to 10% canola oil in the broodstock diet did not affect the spawning quality. It seems that the influence of dietary lipid sources on fish reproduction is highly related to fish species and the dietary fatty acid profile. The present study was designed to evaluate the effects of different lipid sources, i.e., the lipid source with fish oil alone or those with supplemented vegetable oils, on reproductive performance in flatfish tongue sole *Cynoglossus semilaevis*.

Tongue sole *Cynoglossus semilaevis* is economically valuable for aquaculture and is extensively exploited in northern China. However, reproduction remains a bottleneck for commercial culture. Although considerable efforts have been made in breeding this fish, little is known about the nutritional requirements of the broodstock, especially the lipid and fatty acid requirements. The present study was conducted to evaluate the effects of different dietary lipid sources on *Cynoglossus semilaevis* reproduction in terms of spawning performance, and egg and larval quality.

Materials and Methods

Experimental diets. Three isonitrogenous and isoenergetic experimental diets were formulated (Table 1). The ingredient compositions of all diets were similar, with the exception of the added oils. Experimental fish meal based diets were supplemented with fish oil (Diet FO), soybean oil (Diet SO) or olive oil (Diet OO) at a ratio of 6.5% (of dry weight). The corresponding fatty acid profiles of the diets, analyzed by high-performance gas chromatography (GS, Agilent 7890A, USA), are presented in Table 2.

Table 1. Diet formulation and proximate composition of the experimental diets (g/kg diet, of dry matter).

Ingredients (g 100/ g dry diet)	*Diets*		
	FO	*SO*	*OO*
Fish meal[1]	65.00	65.00	65.00
Casein	2.00	2.00	2.00
Krill meal	5.00	5.00	5.00
Wheat gluten meal	15.50	15.50	15.50
Soy lecithin	2.00	2.00	2.00
Choline chloride	1.00	1.00	1.00
Monocalcium phosphate	1.50	1.50	1.50
Vitamin mix[2]	0.50	0.50	0.50
Mineral mix[3]	0.50	0.50	0.50
Ascorbyl polyphosphate	0.50	0.50	0.50
Fish oil[1]	6.50		
Soybean oil		6.50	
Olive oil			6.50
Proximate composition (%)			
Crude protein	52.70	51.90	52.90
Crude lipid	11.50	11.90	11.70
Ash	20.80	20.30	20.90
Gross energy (MJ/kg)	18.30	18.90	18.50

[1] Fish meal: White fish meal (Chilean). Fish oil: Menhaden oil.
[2] Vitamin premix (mg or g/kg diet): thiamin 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12} (1%), 10 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin, 200 mg; folic acid, 20 mg; biotin (2%), 60 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; vitamin E 120 mg; wheat middling 3.76 g.
[3] Mineral premix (mg or g/kg diet): $MgSO_4 \cdot 7H_2O$, 1200 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MnSO_4 \cdot H_2O$, 45 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $NaSeSO_3 \cdot 5H_2O$ (1%), 20 mg; KI, 0.8 mg; zoelite, 3.537 g.

Table 2. Fatty acid compositions of the experimental diets (% of total fatty acids).

Fatty acid	*Diets*		
	FO	*SO*	*OO*
Total saturates	38.37	29.96	28.76
C18:1n-9	12.28	19.08	47.60
Total monoenes	24.17	20.43	47.74
C18:2n-6 (LA)	5.55	30.02	7.16
C20:4n-6 (ARA)	1.86	1.06	1.15
Total n-6 PUFA*	7.95	31.14	8.67
C18:3n-3 (LNA)	1.30	3.98	1.02
C20:5n-3 (EPA)	12.83	2.37	2.60
C22:6n-3 (DHA)	10.96	5.78	6.23
Total n-3 PUFA*	28.48	12.70	10.58
n-3/n-6 ratio	3.58	0.40	1.22
EPA/DHA ratio	1.18	0.41	0.42
EPA/ARA ratio	6.90	2.24	2.28
C18:1n-9/n-3	0.43	1.50	4.50

* PUFA: Polyunsaturated fatty acid.

Ingredients were ground into fine powder through 200 μm mesh. All ingredients were thoroughly mixed with the respective oils, and water was added to produce stiff dough. The dough was then pelleted (4×5 mm) with an experimental feed mill and dried for about 12 h in a ventilated oven at 50°C. After drying, the diets were stored at -20°C until use.

Feeding procedure. Three-year-old tongue sole broodstocks, that had been fed a commercial diet (Qihao Biotech Co. Ltd., Qingdao, China), were used in the present experiment. The mean initial weight of females and males was 1.58±0.18 kg and 0.20±0.05 kg, respectively. Prior to the start of the feeding trial, the experimental fish were reared in concrete ponds (75 m^3) and fed a commercial low-lipid diet (50% protein, 15% lipid) at 1% body weight per day for 15 days to acclimate to the experimental conditions. At the onset of the experiment, the experimental fish were randomly distributed into 6 concrete ponds (2.0 m×2.0 m×1.8 m) with flowing filtered seawater at a rate of 100 l/min. Each pond was stocked with 20 fish at a male/female ratio of 1:3 and the fish were implanted with a passive integrated transponder tag containing a code specific to each individual. Fish were hand-fed to apparent satiation twice daily (09:00 and 19:00) for 60 days before and during the spawning season. The ponds were cleaned daily by siphoning out the residual feed and feces. During the experimental period, the water temperature was maintained between 22~24°C, salinity 30~33 ‰, pH 7.6~8.2, and dissolved oxygen approximately 6 mg/l.

Evaluation of fecundity, egg and larval quality. Eggs produced by the broodstock were gathered in a collecting net fixed to the drainpipe outlet. The eggs were harvested twice daily. The number of eggs produced was estimated by weight, with the number of eggs in 1 g counted. The relative fecundity was calculated by dividing the total number of eggs by the total weight of the spawning females. The buoyant egg rate was measured in beakers, as normal eggs float while abnormal eggs or eggs of poor quality sink. Buoyant eggs samples (about 100 randomized samples) were incubated in petri dishes at 22 °C to determine the hatching rate, as well as the larval normality and survival rate of larvae at 7 days post hatching (DPH). The hatching rate was determined by dividing the total number of larvae by the total number of fertilized eggs. Deformity was defined as the proportion of larvae with abnormal body curvature. The egg diameter of 35 buoyant eggs and the total length of 35 larvae at 1 DPH were measured with a micrometer under the microscope. A total of 3~7 batches of eggs and larvae were evaluated for each group.

The survival activity index (SAI) was determined by the starvation tolerance test as described in several studies (Furuita et al., 2000; Almansa et al., 2001; Emata et al., 2003). One hundred newly hatched larvae were placed in a 5 l plastic tank containing filtered seawater (30~33 ‰, 22~24 °C) and dead larvae were counted and removed every day until total larval mortality SAI was calculated (Zakeri et al. 2011):

$$\text{SAI} = \sum_{i=1}^{k}(N - h_i)\times i/N$$

where N is the total number of supplied larvae; h_i is the cumulative mortality by the i-th day, and k is the number of days elapsed until all larvae died due to starvation.

Chemical analysis. The proximate composition of experimental diets was analyzed using standard methods (Association of Official Analytical Chemists). Protein was determined by measuring nitrogen using the Kjeldahl method (N×6.25); lipid by petroleum ether extraction using Soxhlet; ash by combustion at 550 °C. The fatty acid content of diets and eggs was determined as described by Mourente et al. (1999) with minor modifications using gas chromatography (with Agilent 7890A, USA; methyl ester of fatty acids were used). Results are expressed as the percentage of each fatty acid with respect to total fatty acids.

Statistics. All data were subjected to one-way analysis of variance in SPSS 16.0 for Windows. All percentage data were arcsine transformed before analysis. Differences between the means were tested by Tukey's multiple range test. The level of significance was chosen at $P<0.05$ and the results are presented as means±S.E.M. (standard error of the mean).

Results

Spawning performance and egg and larval quality. The relative fecundity in group SO was significantly ($P<0.05$) lower compared to group FO and OO but no significant ($P>0.05$) difference in relative fecundity was observed between group FO and OO (Fig. 1).

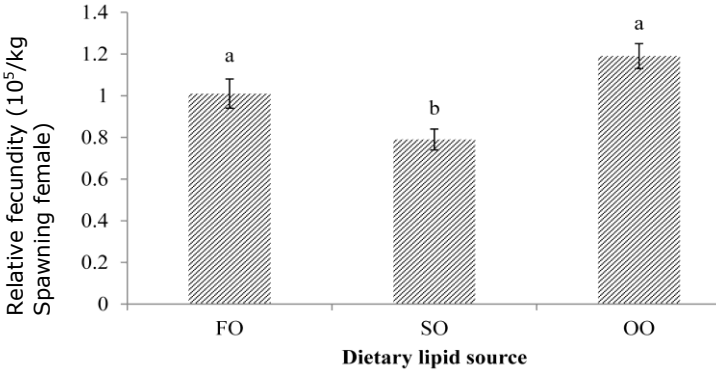

Fig. 1. Relative fecundity of tounge sole broodstock (*Cynoglossus semilaevis*) fed experimental diets with different lipid sources. Bars denoted by the different letters are significantly different (P<0.05) (n=6).

The buoyant egg rate in group OO was significantly higher ($P<0.05$) compared to other groups and group SO showed the lowest value ($P<0.05$) (Table 3).

Table 3. Quality of egg and larvae of tongue sole fed experimental diets with different lipid sources[1].

Quality parameters	*Diets*		
	FO	*SO*	*OO*
Egg quality			
Buoyant egg rate (%)	39.34±0.93^{b}	35.15±0.97^{c}	54.60±0.61^{a}
Egg diameter (mm)	1.28±0.03^{a}	1.23±0.01^{b}	1.25±0.05^{b}
Larval quality			
Larval survival rate at 7 DPH[2] (%)	58.48±1.11^{a}	34.85±0.56^{c}	45.57±0.86^{b}
Larval deformity rate (%)	3.12±0.29^{c}	17.35±0.25^{a}	8.85±0.36^{b}
Larval length at 1 DPH[2] (mm)	2.64±0.16	2.56±0.15	2.50±0.10
Survival activity index (SAI)	24.28±2.14^{a}	14.99±0.58^{b}	16.56±1.55^{b}

[1] Data were means±S.E.M (n=6). Values with different superscript letters in the same row are significantly different (P<0.05).
[2] DPH: Day post hatching.

The hatching rate and larval survival rate at 7 DPH significantly (*P<0.05*) ranked as follows: FO>OO>SO while the larval deformity rate recorded an opposite significant (*P<0.05*) rank: SO>OO>FO (Fig. 2, Table 3). The egg diameter in group FO was significantly (*P<0.05*) higher compared with group SO and OO but no significant difference was observed between group SO and OO. There was no significant difference in larval length at 1 DPH among dietary treatments (*P>0.05*). The SAI showed a similar pattern with the egg diameter.

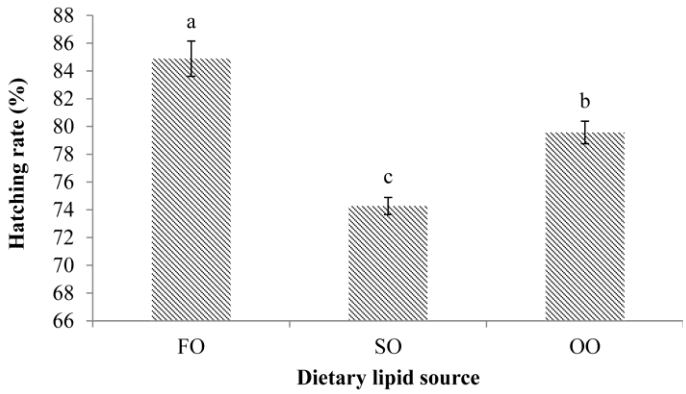

Fig. 2. Hatching rate of eggs form experimental tounge sole broodstock (*Cynoglossus semilaevis*) fed diets with different lipid sources. Bars denoted by the different letters are significantly different (P<0.05) (n=6).

Fatty acid composition of diets and eggs. Diet FO showed high levels of long chain-polyunsaturated fatty acids (LC-PUFAs) i.e., DHA (C22:6n-3), EPA (C20:5n-3), and ARA (C20:4n-6) but it also showed a higher level of saturated fatty acids compared to Diet SO and OO (Table 2). Diet SO showed a high level of C18:2n-6, which is a characteristic fatty acid of soybean oil, and subsequently a higher level of n-6 fatty acids and a lower n-3/n-6 ratio. Diet OO showed a high level of monounsaturated fatty acids, mainly C18:1n-9, which is a characteristic fatty acid of olive oil. The egg fatty acid profiles reflected those of the diets (Table 4).

Table 4. Fatty acid compositions of eggs in tongue sole fed diets with different lipid sources (% of total fatty acids)[1].

Fatty acid	*Diets*		
	FO	*SO*	*OO*
Total saturates	31.06±1.23[a]	26.87±1.27[b]	26.60±1.32[b]
C18:1n-9	20.58±0.76[b]	20.44±0.82[b]	32.68±0.85[a]
Total monoenes	32.57±2.12[b]	30.11±2.31[c]	42.22±2.62[a]
C18:2n-6 (LA)	7.87±0.34[c]	22.15±0.32[a]	9.13±0.35[b]
C20:4n-6 (ARA)	1.61±0.03[a]	1.22±0.00[c]	1.39±0.02[b]
Total n-6 PUFA[2]	9.94±0.94[b]	23.87±1.24[a]	10.99±1.05[b]
C18:3n-3 (LNA)	0.91±0.02[b]	2.27±0.13[a]	1.02±0.06[b]
C20:5n-3 (EPA)	4.78±0.45[a]	1.57±0.22[c]	2.61±0.27[b]
C22:6n-3 (DHA)	18.98±0.15[a]	14.30±0.12[c]	15.30±0.14[b]
Total n-3 PUFA[2]	25.74±0.22[a]	18.59±0.16[c]	19.65±0.17[b]
n-3/n-6 ratio	2.59±0.22[a]	0.78±0.06[c]	1.79±0.15[b]
EPA/DHA ratio	0.25±0.02[a]	0.11±0.01[b]	0.17±0.01[b]
EPA/ARA ratio	2.97±0.27[a]	1.29±0.14[b]	1.88±0.19[b]
C18:1n-9/n-3	0.80±0.07[b]	1.10±0.12[b]	1.66±0.15[a]

[1] Data were means±S.E.M (n=6). Values with different superscript letters in the same row are significantly different ($P<0.05$).
[2] PUFA: Polyunsaturated fatty acid.

The contents of LC-PUFAs (C22:6n-3, C20:5n-3 and C20:4n-6) and saturated fatty acids in eggs of group FO were significantly (*P<0.05*) higher compared to group SO and OO. Group SO showed a significantly (*P<0.05*) higher C18:2n-6 content and group OO showed a significantly (*P<0.05*) higher C18:1n-9 content compared to group FO. The ratios of EPA/DHA and EPA/ARA were the highest in group FO and the lowest in group SO, with group OO showing moderate levels. The n-3/n-6 ratio in eggs from group SO was low (0.78) while the C18:1n-9/n-3 ratio in eggs from group OO was relatively high (1.66).

Discussion

Broodstock diets of marine species traditionally depend on trash fish for the supply of lipid and other nutrients. With the expansion of aquaculture, the use of commercially formulated feed allows greater control over the components of dietary lipids and fatty acids according to the requirements of cultured fish (Bruce et al., 1999; Mazorra et al., 2003). Previous studies have demonstrated that formulated diets enriched in LC-PUFAs, i.e., DHA, EPA, and ARA, resulted in good reproductive performance of marine fish, and fish oil, squid oil, and tuna orbital oil, which are rich in LC-PUFAs, were all proved to be efficient lipid sources for marine broodstock diets (Fernández-Palacios et al., 1997; Lavens et al., 1999; Mazorra et al., 2003; Nguyen et al., 2010; Zakeri et al., 2011; Reza et al., 2013).

In the present study, higher hatching rate and better larval quality was observed in tongue sole broodstock fed fish oil as the lipid source compared to fish fed diets with 6.5% supplemented vegetable oils, indicating the beneficial effects of fish oil on reproduction of tongue sole. This was in agreement with studies on other marine species such as gilthead seabream (Almansa et al., 1999) and yellowfin sea bream (Zakeri et al., 2011), which demonstrated that fish oil resulted in better reproductive performance than vegetable oils. However, other studies reported that excess LC-PUFAs in fish oils could negatively affect the spawning performance and larval quality of marine fish. 2.18% and 3.15% (of dry weight) n-3 LC-PUFA in diets of gilthead sea bream broodstock resulted in significantly lower spawn production compared to 1.60% dietary n-3 LC-PUFA (Fernández-Palacios et al., 1995). In Japanese flounder (Furuita et al., 2002, 2003), rabbitfish (Li et al., 2005), Chilean flounder (Wilson, 2009), and gilthead sea bream (Fernández-Palacios et al., 1997), evidence has shown that excess dietary LC-PUFA contents lead to reduced reproductive performance. Excess DHA, EPA or ARA can impair fish reproduction through varied mechanisms such as increasing oxidation (Lavens et al., 1999), altering the profile of eicosanoids which are probably involved in embryogenesis, egg hatching and larval development (Sargent, 1995; Bell et al., 1997), and decreasing the concentrations of saturated and monounsaturated fatty acids which provide metabolic energy for egg and larval development (Sargent, 1995; Bell et al., 2003). Taking into consideration the inhibition of excess LC-HUFAs on fish reproduction, more attention is being paid to the partial replacement of fish oil with alternative lipid sources for manipulating the fatty acid profile of marine broodstock diets.

In the present study, broodstock fed diets with supplementation of 6.5% olive oil showed significantly higher buoyant egg rate, an important parameter for evaluating egg quality in pelagic egg spawner (Furuita et al. 2000, 2002), compared to fish given fish oil.

Olive oil is rich in oleic acid (C18:1n-9), which is a major energy source during egg and larval development (Van der Meeren et al., 1991). Positive significant correlations between the n-9 fatty acids (mainly C18:1n-9) content of fish eggs, and egg viability or hatching percentages have been observed in gilthead seabream broodstock (Fernández-Palacios et al., 1997). A more balanced C18:1n-9/n-3 ratio could contribute to the beneficial effects of C18:1n-9 rich lipid sources on marine fish reproduction (Almansa et al., 1999). The beneficial effects of C18:1n-9 rich lipid sources on fish reproduction was also indicated in another study on Japanese flounder, which showed that the broodstock diet with 10% palm olein produced higher total egg production than the diet with 10% pollock visceral oil (Furuita et al., 2000). However, inclusion of up to 10% canola oil, which is also rich in C18:1n-9, in diets of cobia broodstock did not affect the spawning quality (Nguyen et al., 2010). The other possibility explanation for the improvement of egg production by diets with C18:1n-9 rich lipid sources is that the improvement is due to the avoidance of excess dietary LC-PUFAs. The reduction of buoyant egg rate by excess dietary LC-PUFAs has been reported in several studies (Fernández-Palacios et al., 1995; Li et al., 2005; Wilson, 2009). Further studies are needed to elucidate the mechanism involved in the effects of C18:1n-9 rich lipid sources on marine fish reproduction.

Among the dietary treatments, the diet with supplementation of 6.5% soybean oil produced the worst reproductive performance of tongue sole, decreasing all the parameters tested compared to the other two treatments. A study on tongue sole suggests that the high C18:2n-6 content and the subsequent high level of n-6/n-3 ratio in soybean oil probably contributed to the inhibition of soybean oil supplements on fish reproduction (Liang et al., 2014). Another C18:2n-6 rich oil, sunflower oil, has also been reported to reduce the spawning performance and larval quality in diets of yellowfin sea bream broodstock (Zakeri et al., 2011). A higher dietary n-6/n-3 ratio caused by the corn oil supplement negatively affected the embryogenesis in Japanese eel (Furuita et al., 2007). However, the buoyant egg rate and fertilization rate in Japanese eel fed diets with corn oil alone were higher than those in fish fed diets with pollack oil alone or a combination of pollack oil and corn oil. These studies suggest that a high dietary n-6/n-3 ratio may reduce fish reproduction but this effect possibly depends on fish species and reproduction stage.

In the present study, the fatty acid composition of eggs reflected those of the diets, which were largely influenced by the dietary lipid sources. It has been widely demonstrated that the egg and larval quality is highly related to the egg fatty acid compositions (Izquierdo et al., 2001), and the regulation of fish reproduction by dietary lipids was commonly parallel with the alterations in fatty acid profiles of broodstock and gametes (Almansa et al., 1999; Furuita et al., 2002, 2007; Li et al., 2005; Wilson, 2009; Nguyen et al., 2010; Zakeri et al., 2011). In the present study, the altered fatty acids content of eggs by dietary lipids, mainly the contents of LC-PUFAs, n-6 fatty acids, and monounsaturated fatty acids, probably directly and indirectly affected the egg and larval quality of tongue sole. Moreover, with the supplementation of vegetable oils, the content of EPA and ARA in experimental eggs decreased by approximately 56% and 50% respectively, whereas the DHA content decreased only by approximately 22%. This indicates that DHA was selectively accumulated in eggs and could play an important role in embryogenesis and larval development. Similar results have also been observed in cobia (Nguyen et al., 2010), Atlantic herring (Tocher et al., 1985), Atlantic halibut (Bruce et al., 1999; Mazorra et al., 2003), and Japanese flounder (Furuita et al., 2000, 2002). The present study has also shown that the C18:1n-9 content in eggs (e.g. 20.58% of TFA in group FO) was much higher than that in the diet (e.g. 12.28% of TFA in Diet FO). This indicates that C18:1n-9 is another important fatty acid sequestered in the eggs, indicting its importance for the formation of new tissue in growing embryo and early larva. This phenomenon has been observed in turbot (Peleteiro et al., 1995; Lavens et al., 1999) and gilthead seabream too (Almansa et al., 2001) and it could contribute to the improvement of egg quality with the dietary supplementation of C18:1n-9 rich olive oil in the present study. In other marine species high incorporations of EPA or ARA into eggs and their importance in fish reproduction was also observed (Fernández-Palacios et al.,

1997; Almansa et al., 2001; Emata et al., 2003; Mazorra et al., 2003). The fatty acid profiles of broodstock or eggs generally vary according to species (Emata et al., 2003; Støttrup et al., 2013) and reproduction stage (Tocher et al., 1985) which could explain that the effects of certain dietary fatty acids on fish reproduction. In addition, since an alteration of lipid source always leads to alterations in a series of dietary fatty acids other than in a single one, studies with purified fatty acids to obtain dietary grades of a single fatty acid are needed to elucidate the mechanisms involved in the reproductive regulation by dietary fatty acids.

In conclusion, results of the present study show that the supplementation of 6.5% olive oil in the fish meal based diets for tongue sole broodstock positively influenced the fecundity and the rate of buoyant eggs however among the tested oils, dietary fish oil resulted in the highest hatching rate and best larval quality in terms of survival, morphology, and starvation tolerance of larvae. The dietary supplementation of soybean oil (6.5%) reduced the spawning performance, and egg and larval quality of tongue sole compared to fish oil and olive oil.

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