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Chemical Composition and Size of Floating and Sunken Eggs of Olive Flounder *Paralichthys olivaceus*

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Olive flounder (*Paralichthys olivaceus*) is one of the most commercially important species of farmed marine fish in Korea. Although techniques for rearing the larva of this species are improving, production costs are also increasing due to microbial influences and genetic degradation caused by successive culturing. Since the demand for healthy seed, which requires eggs of good quality of, is high, we examined the effects of nutrient composition on the size of *P. olivaceus* eggs. We analyzed floating (live) and sunken (dead) eggs of *P. olivaceus* from five different hatcheries for their size and amino and fatty acid composition. The sizes of eggs and oil globules from floating vs. sunken eggs were significantly different at $p<0.05$. No significant relationships were observed, however, among larval length, hatching percentage, and egg and oil globule size. The dry weight and amino acid levels of floating eggs were greater than those of sunken eggs ($p< 0.05$), but no difference in fatty acid content was observed.

Key words: Amino acids, Egg quality, Fatty acids, Hatching, Olive flounder, *Paralichthys olivaceus*

Introduction

Cultured olive flounder *Paralichthys olivaceus* is one of the most commercially important fish in Korea, with production reaching 1,801 metric tons in 2003 (MOMAF, 2004). However, a major constraint on the seed supply of this fish is that good quality eggs and larva are required. Since the nutrients required for fish egg development are obtained from the parent broodstocks, determination of a suitable nutritive diet for brood stocks is essential. In addition, the amino and fatty acid composition of fish eggs may serve as a useful indicator for selection of appropriate live food for the fish larva after yolk depletion (Ringo et al., 1987; Sargent et al., 1989). Growth and survival of larva correlate with certain aspects of egg quality (Devauchelle et al., 1987), such as size (Hoar, 1969) and chemical composition, as well as the eggs' oil globule characteristics and hatching percentages (Watanabe et al., 1984a).

Optimal nutrition for successful larval production requires an understanding of the nutrient profiles of fed and starved larva. Larval nutritional requirements are expected to emerge from knowledge of patterns of conservation and loss of nutrients (Koven et al., 1989). The fatty and amino acid composition of fish larva is used as an indicator of their nutritional condition (Fraser et al., 1987).

Although Korean scientists have developed larval rearing techniques and feeding protocols for *P. olivaceus*, the cost of fry production is increasing owing to microbial influences and genetic degradation caused by successive culturing. Thus, the demand for healthy flounder larva, which requires good egg quality, is high. Egg quality is presumably improved by optimizing nutrients such as n-3 highly unsaturated fatty acids (HUFAs) (Furuita et al., 2002; Watanabe and Vassallo-Agius, 2003), arachidonic acid (AA) (Furuita et al., 2003a), and vitamin A (Furuita et al., 2001; 2003b). In addition, cultural environmental factors such as salinity (Hart and Purser, 1995), temperature (Avery et al., 2004), and light intensity (Watanabe et al., 1998) must be considered, and broodstock hormone doses are important (Watanabe and Carroll, 2001). Research progress in this area has been slow, however, owing to the myriad of interrelated factors that are thought to act as egg

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and larval quality determinants (Bromage, 1995). Therefore, we attempted to elucidate the major factors, such as size and amino and fatty acid composition, that affect the quality of flounder eggs.

Materials and Methods

P. olivaceus eggs from five different hatcheries were used. Eggs from the Nam-Jeju, Yeosu, and Taesong hatcheries were sampled from December 1991-March 1992, and those at the Geoje and Haeundae hatcheries were sampled from May-June 1992. A total of twelve trials were conducted during the spawning season. Eggs spawned naturally in rearing tanks were collected using a 200-μm plankton net during the night, and floating (live) and sunken (dead) eggs were separated using a 1,000-mL glass-mass cylinder. The salinity of the seawater at the hatcheries during the spawning season was about 33 psu. The morning following collection, eggs from the hatcheries (except Haeundae) were carried to the Institute of Fisheries Science at Haeundae in a plastic bag contained in a box.

Immediately upon arrival at Haeundae, floating eggs were put into an incubator tank to hatch out. Floating eggs were collected, put into 1,000 mL of filtered seawater (33 psu) in a flask, and incubated at 20°C. After hatching, the number of larvae and unhatched eggs were counted, and the hatching percentage was calculated. For each egg batch, three sets of 200 eggs each were incubated and analyzed in this manner. Random samples were also taken from the floating and sunken eggs (100 eggs each), and the diameters of the eggs and oil globules were measured using a stereomicroscope.

For dry weight and chemical composition analysis, floating and sunken eggs were collected using a 200-μm plankton net, washed, and stored at -86°C until analyzed. For dry weight analysis, about 0.5-1.0 g of each sample was maintained at 110°C until a constant dry weight was achieved. Protein and lipid contents were determined by semimicro Kjedahl analysis (Joo et al., 1992) and the method of Folch et al. (1957), respectively. Amino and fatty acids were analyzed using an LKB 4150 Alpha amino acid analyzer (Pharmacia Instruments, England) and a Model 8700 gas chromatograph (Perkin Elmer Ltd., USA) according to the method of Cabrera and Hur (2001).

Student's t-test was used to compare the average diameters of the eggs and oil globules and the dry weights of the floating and sunken eggs using the SPSS/PC statistical computer package (version 10.1.3, SPSS Inc., Chicago, Illinois). Correlations between egg or oil globule diameter and hatching percentage or total length of the hatched larva were considered to be significant if $p<0.05$.

Results

P. olivaceus eggs were small and contained a single oil globule. Floating eggs were generally larger than sunken eggs, and their oil globules were generally larger. The average diameters of floating and sunken eggs varied from 0.902-0.968 mm and from 0.888-0.950 mm, respectively. The average diameters of oil globules from floating and sunken eggs ranged from 0.154-0.193 mm and from 0.151-0.188 mm, respectively (Table 1). A t-test of the mean values of egg and oil globule diameters showed a significant difference between floating and sunken eggs ($p<0.05$). However, the sunken eggs from Geoje were larger than the floating eggs from Geoje, although the floating eggs had larger oil globules. Furthermore, in the 7th trial, egg and oil globule diameters were the same for floating vs. sunken eggs.

Eggs at 20°C hatched out within 48-72 hr of spawning, and the hatching percentage ranged from 37.5-81.4%. No significant correlations between hatching percentage and egg or oil globule diameter were observed. In addition, no significant correlations were observed among larval length, hatching percentage, and egg or oil globule diameter.

Floating eggs were always heavier than sunken eggs. Dry weights of floating eggs ranged from 40.8-50.9 μg, whereas those of the sunken eggs ranged from 32.5-50.2 μg. For eggs from Nam-Jeju and Geoje, the mean weights of the floating and sunken eggs were significantly different at $p<0.05$ (Table 2).

The protein and total amino acid contents of floating eggs were higher than those of sunken eggs (Table 3). The levels of each individual amino acid were also higher in the floating eggs.

Glutamic acid in the floating eggs was the most abundant amino acid, at 1.13 μg. Methionine was present at a much lower amount in sunken eggs than in floating eggs (0.02 vs. 0.18 μg, respectively), whereas tyrosine levels were similar (0.07 vs. 0.08 μg, respectively). Essential amino acid (EAA) levels were higher in the floating eggs than in the sunken eggs, as was the EAA/non-EAA ratio.

The total fatty acid contents of the floating and sunken eggs were not significantly different; the total fatty acid content of the floating and sunken eggs ranged from 2.30-11.92 μg and from 3.07-12.07 μg, respectively, per 100 μg dry weight (Table 4). Floating and sunken eggs from all batches exhibited a high

Table 1. Diameter of egg and oil globule of floating and sunken egg, hatching percentage, and total length of newly hatched larva of *Paralichthys olivaceus*

Trial No.	Hatchery	Spawning day	Egg diameter (mm)		Oil globule diameter (mm)		Hatching (%)	Total length (mm)
			Floating egg	Sunken egg	Floating egg	Sunken egg		
1	Nam-Jeju	Dec. 27	0.965±0.020	0.938±0.030	0.193±0.011	0.188±0.018	56.0	2.73±0.21
2	Nam-Jeju	Jan. 7	0.924±0.016	0.915±0.017	0.154±0.009	0.151±0.010	75.0	2.56±0.14
3	Nam-Jeju	Jan. 27	0.924±0.044	0.880±0.029	0.169±0.009	0.158±0.008	79.0	2.88±0.25
4	Nam-Jeju	Feb. 16	0.914±0.018	0.902±0.028	0.169±0.021	0.164±0.010	52.0	2.87±0.17
5	Nam-Jeju	Mar. 5	0.936±0.036	0.914±0.048	0.168±0.011	0.161±0.020	79.5	NA
6	Geoje	May 25	0.919±0.012	0.927±0.015	0.193±0.000	0.183±0.012	78.8	2.44±0.24
7	Geoje	May 31	0.937±0.018	0.937±0.020	0.169±0.003	0.169±0.002	73.0	2.61±0.13
8	Geoje	June 3	0.921±0.020	0.926±0.022	0.189±0.009	0.176±0.012	65.0	2.41±0.11
9	Haeundae	May 4	0.946±0.021	0.941±0.020	0.173±0.004	0.169±0.006	81.4	NA
10	Haeundae	June 4	0.968±0.036	0.950±0.030	0.185±0.010	0.180±0.017	75.0	2.69±0.16
11	Taesong	Mar. 10	0.912±0.020	0.892±0.030	0.167±0.010	0.169±0.012	37.5	NA
12	Yeosu	Mar. 10	0.902±0.013	0.898±0.020	0.190±0.009	0.168±0.010	56.4	2.58±0.10
	Mean±S.D.		0.931±0.148^{a}	0.918±0.004^{b}	0.177±0.083^{a}	0.170±0.001^{b}	67.4±14.0	2.64±1.68

Different superscripts in the mean value of egg and oil globule diameter mean significant difference between floating and sunken egg at $p<0.05$. NA, not available. Nam-Jeju, Geoje and Yeosu are national hatcheries belong to The National Fisheries Research and Development Institute. Haeundae is the Institute of Marine Science belong to the Pykyong National University. Taesong is a commercial flounder farming company in Busan.

Table 2. Dry weight (μg) of floating and sunken egg of *Paralichthys olivaceus*

Hatchery	Spawning day	Floating egg	Sunken egg
Nam-Jeju	Jan. 7	40.80	32.50
Nam-Jeju	Jan. 27	42.10	36.50
Geoje	May 25	43.18	35.84
Geoje	June 3	50.91	50.18
Mean S.D.		44.25 4.55^{a}	38.76 7.82^{b}

Different superscripts in the mean value of dry weight mean significant difference at $p<0.05$.

proportion of saturated and monounsaturated fatty acids and a low proportion of polyunsaturated fatty acids. In all samples, 16:0, 18:0, 16:1, and 18:1 were the major fatty acids. The fatty acid 22:1 was present at high levels, representing 23% of the total fatty acid content of the floating eggs from the Haeundae and Nam-Jeju hatcheries.

The major HUFAs were 18:2n-6, 20:3n-6, and 20:4n-6. Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are considered essential n-3 HUFAs for fish larva, were not assessed in this study. However, AA (20:4n-6) was found in all eggs except floating eggs from the Haeundae hatchery. AA was present at 1.46-3.59% and was present at higher levels in floating eggs than in sunken eggs.

Table 3. Amino acid compositions (μg/dry egg) of floating and sunken egg of *Paralichthys olivaceus* from Nam-Jeju hatchery

Amino acids	Floating egg	Sunken egg
Aspartic acid	0.59	0.42
Threonine	0.50	0.36
Serine	0.55	0.34
Glutamic acid	1.13	0.74
Proline	0.66	0.49
Glycine	0.38	0.28
Alanine	0.47	0.33
Cystine	0.13	0.07
Valine	0.84	0.55
Methionine	0.18	0.02
Isoleucine	0.55	0.38
Leucine	0.81	0.55
Tyrosine	0.07	0.08
Phenilalanine	0.59	0.43
Histidine	0.55	0.38
Lysine	0.59	0.40
Arginine	0.22	0.16
Total	8.81	5.98
EAA	4.82	3.24
NEAA	3.99	2.74
EAA/NEAA	1.21	1.18
Protein	9.33	6.38
Protein (%)	22.87	19.90

EAA, essential amino acids; NEAA, non-essential amino acids.

Discussion

The size of a newly hatched larvae depends on the size of its progenitor egg (Hoar, 1969). Egg size in turn depends on various characteristics of the brood-stock, including genetic factors (Scott, 1962), number of eggs spawned (Niklosky, 1963; Kuo et al., 1972), age (Schoenberr, 1977), food availability (Banegal, 1971), and environmental conditions, such as temperature, salinity, and light intensity (Bromley et al.,

Table 4. Fatty acid compositions (% area) of floating and sunken eggs of *Paralichthys olivaceus*

Fatty acids	Hatchery / Spawning day							
	Nam-Jeju/Jan. 7		Nam-Jeju/ Jan. 27		Geoje/ May 26		Haeundae/May 31	
	F	S	F	S	F	S	F	S
14:0	6.62	8.26	4.97	5.34	3.00	3.44	4.67	4.68
16:0	29.11	35.31	28.96	28.44	27.96	28.88	33.49	36.72
18:0	4.61	5.95	5.96	5.34	8.88	7.17	6.92	9.87
20:0	-	0.25	-	0.39	-	-	-	-
Total saturated (%)	40.34	49.77	39.90	41.18	39.83	38.49	45.07	51.27
14:1 n-5	-	0.23	-	-	-	-	-	0.54
16:1 n-7	7.56	10.63	6.59	7.25	4.85	6.65	7.43	8.47
18:1 n-7 + n-9	18.68	26.37	16.23	17.82	11.65	16.20	16.90	23.54
20:1 n-9 + n-11	2.57	2.20	1.36	1.52	-	0,64	1.41	2.16
22:1 n-9+ n-11	23.69	-	-	-	-	-	23.91	-
Total monounsaturated (%)	52.20	39.43	52.59	51.60	51.79	49.59	49.65	34.71
18: 2 n-6	3.42	5.67	4.29	4.15	0.97	0.75	1.32	1.89
18:3 n-3+ n-6	1.04	1.27	1.10	0.92	0.75	0.58	0.82	1.07
20:2	-	0.47	-	0.68	-	2.33	-	-
20:3 n-6	-	0.62	0.39	-	3.06	4.82	3.14	7.77
20:4 n-6	2.71	1.71	1.73	1.46	3.59	3.48	-	3.28
20:5 n-3	-	1.06	-	-	-	-	-	-
Total polyunsaturated (%)	7.17	10.80	7.52	7.21	8.38	11.93	5.27	14.01
Total fatty acids (μg /100 μg dry weight)	3.68	3.14	2.30	3.07	6.54	12.07	11.92	8.39

F, floating eggs; S, sunken eggs; -, not detected.

1986; Hart and Purser, 1995; Watanabe et al., 1998).

The average size of *P. olivaceus* eggs observed in this study was slightly less than that previously observed by Fukuhara (1986). The reason for this size discrepancy cannot be determined, however, since neither the broodstock nor the environmental conditions were explicitly examined in either study. In the current study, no significant correlation between hatching percentage and egg diameter or oil globule diameter was observed. However, the egg and oil globule diameters were larger for floating eggs than for sunken eggs.

In *Solea solea*, egg size and protein and carbohydrate content are inversely related (Devauchelle et al., 1987). A similar result has been reported for *Pseudopluronectes americanus* and for red sea bream (*Pagrus major*) (Cetta and Cappuzo, 1982). In the present study, we found that the amino acid content of floating eggs was higher than that of sunken eggs, suggesting that the observed differences in hatching percentages may be related more to differences in chemical composition than to differences in size.

The hatching percentages observed in this experiment were lower than those reported by Min (1987) for the same species, and they varied from trial to trial and with hatchery. Differences in the broodstock's nutrition may affect hatchability (Watanabe et al., 1984a; Furuita et al., 2003a). Intrinsic differences in egg viability (Min, 1987), stress induced by transportation of the eggs, and hatchery-specific variables contributing to low fertilization of spawned eggs (Lavens and Sorgeloos, 1991) may also affect hatchability.

Average dry weight, total protein content, and amino acid content were higher for floating eggs than for sunken eggs. These factors appeared to be directly related to hatching success. The lipid content observed in this research was in the range previously described for marine fish (Sargent et al., 1989), although it varied among the egg batches, as was also observed for *Scophthalmus maximus* (Lavens and Sorgeloos, 1991). On the other hand, the fatty acid composition (Watanabe et al., 1984a; Devauchelle et al., 1987; Furuita et al., 2003a) and vitamin content (Furuita et al., 2003b) of the broodstock's diet have also been reported to affect egg quality. Eggs spawned by a single female cod in the same batch vary in fatty acid composition (Ulvend and Grahl-Nielsen, 1988). In the present experiment, the most common fatty acids found in *P. olivaceus* eggs were 16:0, 16:1, and 18:1. These fatty acids were also found in *P. major* (Watanabe et al., 1984a) and *S. maximus* eggs (Witt et al., 1984).

We could not determine the relevance of HUFA content to hatching percentage in this study, since HUFAs such as EPA and DHA were not detected by the methods used. In previous reports, no clear relationship between fatty acids and hatching percentage was observed for either *P. major* (Watanabe et al., 1984b) or *S. maximus* (Planas et al., 1991). However, Furuita et al. (2002) reported that a high level of n-3 HUFA in the broodstock's diet was associated with high egg production but negatively affected egg quality in *P. olivaceus*, leading to decreased percentages of floating eggs and of hatching. Subsequently, a high level of AA in the diet (1.2% w/w) was shown to negatively affect *P. olivaceus* egg quality, possibly due to an inhibitory effect on EPA (Furuita et al., 2003b).

The biochemical composition of fish eggs can be used as an indicator of the nutritional requirements of their larva (Falk-Petersen et al., 1986; Komis et al., 1991). However, a clear picture of the determinants of fish egg quality has not yet emerged, owing to the myriad of possible causative agents. In this study, we found that the amino acid content of *P. olivaceus* eggs is an indicator for floating (live) eggs of large size. However, further studies of the relationship between egg quality and the amounts of HUFA and amino acids in the broodstock diet will be essential in determining the optimal diet for production of high-quality eggs.

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