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THE EFFECTS OF WEANING ON TO A DRY PELLET DIET ON BRAIN LIPID AND FATTY ACID COMPOSITIONS IN POST-LARVAL GILTHEAD SEA BREAM (*SPARUS AURATA* L.)

GABRIEL MOURENTE and DOUGLAS R. TOCHER*

Biología, Facultad de Ciencias del Mar, Universidad de Cádiz, Polígono del Río San Pedro, Apartado 40, E-11510 Puerto Real (Cádiz), Spain

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Abstract—1. Changes in brain lipid and fatty acid composition were investigated in gilthead sea bream (*Sparus aurata* L.) post-larvae during weaning from live food, enriched *Artemia*, to a dry pellet diet.

2. Although enriched *Artemia* supplied twice as much lipid and total (n-3) polyunsaturated fatty acids (PUFA) on a dry weight basis, the dry pellet diet supplied 6.5 times as much docosahexaenoic acid (22:6n-3) to the fish.

3. Brain weight was slightly increased by weaning on to the pellet diet for 7 days whereas brain protein and lipid contents and lipid class compositions were unaffected.

4. Weaning resulted in significant increases in 22:6n-3 and 18:2n-6 and significant decreases in 18:3n-3 and 20:5n-3 in brain total lipids without altering the total (n-6) or (n-3)PUFA contents.

5. The increased 22:6n-3 was mainly confined to choline- and ethanolamine-phospholipids.

6. The changes in other PUFA were found generally throughout the phospholipids with decreased 20:5n-3 and 20:4n-6 particularly prominent in phosphatidylinositol.

INTRODUCTION

Fish have high levels of docosahexaenoic acid (DHA; 22:6n-3) in brains and retinas, generally above those found in non-neural tissues, as shown in cod *Gadus morhua* (Tocher and Harvie, 1988), rainbow trout *Salmo gairdneri* (Tocher and Harvie, 1988; Bell and Tocher, 1989), hake *Merluccius hubbsi* and sea bass *Acanthusthius brasilianus* (Ayala *et al.*, 1991) and Atlantic herring *Clupea harengus* (Mourente and Tocher, 1992a). Nutritional studies in fish species such as turbot *Scophthalmus maximus* L. (Witt *et al.*, 1984), gilthead sea bream *Sparus aurata* L. (Koven *et al.*, 1989) and *Coryphaena hippurus* (Ostrowski and Divakaran, 1990) have shown that dietary DHA is essential for those marine fishes, especially during larval stages. Biochemical studies have also demonstrated that DHA is retained in brain lipids of adult sea bass *Dicentrarchus labrax* L. when fed diets deficient in (n-3) polyunsaturated fatty acids (PUFA) (Pagliarani *et al.*, 1986).

Recently we have shown that the level of DHA increases dramatically in brain lipids of turbot *S. maximus* during development of weaned fish (Mourente *et al.*, 1991). In addition, when post-larval turbot were weaned from (n-3)PUFA-enriched *Artemia*, containing high levels of eicosapentaenoic acid (EPA; 20:5n-3) but low levels of DHA, to a dry pellet food rich in DHA and EPA, a rapid and specific incorporation of DHA into brain phosphoglycerides was observed (Mourente and Tocher, 1992b). This was accompanied by a significant increase in brain weight of the weaned larvae in comparison with fish of the same batch that remained on the enriched *Artemia* (Mourente and Tocher, 1992b). Weaning of farmed marine fish is usually accompanied by a very significant decrease in larval mortality.

In the present paper, we investigated changes in brain lipid composition of gilthead sea bream (*Sparus aurata*. L.) post-larvae during weaning from enriched *Artemia* to a dry pellet food. Fish were maintained on the same dietary regime throughout early development, with all fish fed enriched *Artemia* immediately prior to the time of normal weaning. At this point, one group of fish was fed a dry pelleted food whereas the other group remained on enriched *Artemia*. Both populations were sampled 7 days later and the brains analysed for lipid content, and lipid class and fatty acid compositions.

MATERIALS AND METHODS

Fish

Gilthead sea bream (*Sparus aurata*. L.) were obtained from a commercial fish farm (Cultivos Piscícolas Marinos S.A., Cupimar S.A.; Salina de San Juan Bautista, San Fernando, Cádiz, Spain). The fish were

*Present address: NERC Unit of Aquatic Biochemistry, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, U.K. (Tel 0786 67821; Fax 0786 64994).

maintained at the fish farm under normal culture conditions and were fed the dietary regime in routine operation at the farm. The time of weaning was determined by the size of the fish necessary to take the size of pellets of the weaning diet. With this particular batch of fish, weaning occurred 55 days after hatching. At this point, all fish were being fed enriched *Artemia*. One group of fish was then weaned on to the dry pellet diet normally used at weaning, whereas the other group remained on enriched *Artemia*. Both populations were sampled 7 days later (62 days after hatching). Triplicate samples (containing 25 brains each) from the groups of fish were dissected out on ice, immediately frozen in liquid nitrogen and stored at −80°C until analysis. Further samples (8 brains each) were collected for dry weight, ash and elemental composition determinations.

Diets

Artemia metanauplii were enriched with (n-3)PUFA by Selco enrichment emulsion (*Artemia* Systems), according to the manufacturer's recommendations, before they were supplied as live prey to gilthead sea bream post-larvae. The dry pelleted food was a commercial diet used for weaning marine fish (Ewos Start Marine, Ewos Aquaculture International). The stomach contents of weaned fish were observed (via transparency) in order to ensure that weaning had been successful and that the fish were consuming the pellet diet. The dry matter and gross composition (total protein, total lipid and total ash contents), and the fatty acid contents of both diets are shown in Table 1.

Table 1. Dry matter*, gross composition† and total lipid fatty acid contents‡ in diets used for rearing gilthead sea bream (*Sparus aurata*. L.)

	Artemia	Dry Food
Dry matter	9.3 ± 0.1	96.8 ± 0.1
Total protein content	51.8 ± 1.1	59.5 ± 1.3
Total lipid content	22.9 ± 1.4	10.6 ± 0.4
Total ash content	12.8 ± 0.5	15.4 ± 0.3
Fatty acid		
14:0	1.3 ± 0.2	2.9 ± 0.2
15:0	2.7 ± 0.2	1.2 ± 0.1
16:0	14.2 ± 0.6	11.7 ± 0.7
16:1n-7	5.7 ± 0.9	2.6 ± 0.1
16:2	1.2 ± 0.1	0.4 ± 0.1
16:3	1.1 ± 0.1	0.4 ± 0.1
18:0	7.3 ± 0.1	3.2 ± 0.2
18:1n-9	24.4 ± 1.5	8.7 ± 0.5
18:1n-7	7.8 ± 0.5	1.5 ± 0.1
18:2n-6	5.5 ± 0.2	9.1 ± 0.6
18:3n-3	20.5 ± 0.5	1.9 ± 0.1
18:4n-3	3.3 ± 0.2	1.8 ± 0.1
20:0	0.4 ± 0.1	0.2 ± 0.1
20:1n-9	0.9 ± 0.2	5.9 ± 0.5
20:4n-6	1.9 ± 0.1	0.4 ± 0.1
20:3n-3	0.5 ± 0.1	0.2 ± 0.1
20:4n-3	0.8 ± 0.1	0.5 ± 0.1
20:5n-3	9.2 ± 0.4	4.1 ± 0.3
22:1n-11	1.0 ± 0.3	8.5 ± 0.8
22:5n-6	0.5 ± 0.2	0.3 ± 0.1
22:5n-3	0.6 ± 0.1	0.8 ± 0.1
22:6n-3	1.4 ± 0.2	9.1 ± 0.9
Total Saturated	26.9 ± 1.1	19.7 ± 0.9
Total Monounsaturated	42.9 ± 2.9	27.8 ± 2.0
Total Polyunsaturated	50.2 ± 2.0	30.4 ± 2.2
Total (n-6)PUFA	10.5 ± 0.4	10.8 ± 0.7
Total (n-3)PUFA	39.3 ± 1.6	19.3 ± 1.5
Total (n-6)HUFA	3.2 ± 0.3	1.2 ± 0.1
Total (n-3)HUFA	13.2 ± 0.8	14.7 ± 1.4

Data are means ± SD of three samples. *percentage of wet weight; †percentage of dry weight; ‡μg fatty acid/mg dry weight, totals include some minor components (<0.1%) not shown. *Artemia* were enriched in (n-3)PUFA with Selco (Artemia System). Dry food was a commercial small size pellet diet (Ewos Start Marine) used for weaning marine fish. PUFA, polyunsaturated fatty acids: HUFA, highly unsaturated fatty acids (≥20:3)

Dry weight, ash and elemental composition determinations

Four replicates of preweighed samples were maintained at 110°C for 24 hr. The dry weights were determined after cooling *in vacuo* for at least 1 hr. Ash content was determined by combusting preweighed samples of dry matter at 550°C in a furnace and reweighing the residues. The elemental composition (C, N, H) analyses were performed in a Carlo Erba 1106 Elemental Analyser, using cyclohexanone 2,4-dinitrophenylhydrazone as standard. Samples were washed with distilled water, desiccated to constant weight and replicates of 1 mg dry weight analysed. Carbon, nitrogen and hydrogen contents were obtained by multiplying their respective percentage values by the dry weight. Protein content was obtained by multiplying the nitrogen content by 6.25.

Total lipid extraction, lipid class and fatty acid analysis

Total lipid was extracted from samples after homogenization in a teflon pestle glass homogenizer, in 10 ml of chloroform/methanol (2:1, v/v) containing 0.01% (w/v) butylated hydroxytoluene (BHT) basically according to Folch *et al.*, 1957, as detailed previously (Tocher and Harvie, 1988).

Lipid classes were separated by high-performance thin-layer chromatography (HPTLC) using a single-dimension double-development method described previously (Tocher and Harvie, 1988; Olsen and Henderson, 1989). The classes were quantified by charring (Fewster *et al.*, 1969) followed by calibrated densitometry using a Shimadzu CS-9000 dual-wavelength flying pot scanner and DR-13 recorder (Olsen and Henderson, 1989).

Individual phosphoglyceride classes were separated by thin layer chromatography (TLC) according to the method of Vitiello and Zanetta (1978). Fatty acid methyl esters from total lipids and individual phosphoglyceride classes were prepared by acid-catalysed transmethylation for 16 hr at 50°C, using nonadecanoic acid (19:0) as an internal standard (Christie, 1989). Methyl esters were extracted and purified as described previously (Tocher and Harvie, 1988). The fatty acid methyl esters were analysed in a Hewlett-Packard 5890 A Series II gas chromatograph equipped with a chemically bonded (PEG) Omegawax 320 fused silica wall-coated capillary column (30 m × 0.32 mm i.d., Supelco, Bellefonte, U.S.A.) using hydrogen as carrier gas with a thermal gradient from 185 to 235°C. Individual fatty acid

methyl esters were identified as described previously (Tocher and Harvie, 1988) and quantified using a Hewlett-Packard 3394 recording integrator.

Statistical analysis

Results are presented as means ± SD ($N = 3$). The significance of differences between means were determined by paired t-test analysis (small group of samples) (Zar, 1984).

Materials

Potassium bicarbonate, potassium chloride, BHT and nonadecanoic acid (>99% pure) were from Sigma Chemical Co. (Poole, Dorset, U.K.). TLC (20 × 20 cm × 0.25 mm) and HPTLC (10 × 10 × 0.15 mm) plates precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). Glacial acetic acid, sulphuric acid (analar grade) and all solvents (HPLC grade) were purchased from Fluka Chemicals Co. (Glossop, Derbyshire, U.K.).

RESULTS

The enriched *Artemia* contained 9.3% dry matter compared with 96.8% for the dry pellet diet (Table 1). Total protein and ash contents were slightly higher in the dry food, whereas total lipid content was 2-fold greater in enriched *Artemia*. Enriched *Artemia* provided 1.5-fold more total fatty acids per mg dry weight, with, in particular, 18:1 isomers, 18:3n-3, 20:4n-6 and EPA all in greater amounts compared with the dry pellet food. In contrast, the dry pellet food provided more DHA (6.5-fold greater), 18:2n-6, 20:1n-9 and 22:1n-11 on a μg fatty acid per mg dry weight of diet basis (Table 1).

The dry weights of brains from gilthead sea bream weaned on to the dry pellet diet were slightly greater than those of unweaned fish, although the difference was not significant (Table 2). Weaning had no significant effect on brain total protein or total lipid contents. The major polar lipid classes were diradyl glycerophosphocholines (CPL) and diradyl glycerophosphoethanolamines (EPL), followed by phosphatidylserine (PS). The major neutral lipid was cholesterol, which accounted for approximately 20% of total lipids, whereas triacylglycerol and steryl esters amounted to about 12 and 15%, respectively. Weaning on to the pelleted diet had no significant effect on the brain lipid class composition (Table 2).

The total lipid fatty acid compositions of brains from weaned and unweaned 62-day-old gilthead sea bream post-larvae are presented in Table 3. The proportion of total saturated fatty acids was significantly greater in brains of weaned fish, whereas there were no differences in total monoenes or total PUFA. However, the proportions of some individual fatty acids were significantly affected by weaning. In particular, the percentage of DHA was significantly increased by 1.4-fold in brain total lipid from weaned fish. Similarly, 16:0, 18:0 dimethyl acetal, 18:2n-6 and 20:1n-9 were significantly higher in brains of weaned fish. In contrast, the proportions of 18:3n-3, EPA and 18:1n-7 were significantly lower in total lipid from brains of weaned fish.

Table 2. Brain dry weights*, total protein and lipid contents† and lipid class compositions‡ of total lipids from brains of 62-day-old gilthead sea bream (*Sparus aurata*. L.) postlarvae fed: (A) only enriched-*Artemia*, (B) enriched-*Artemia* until day 55 and then dry pellet food for 1 week after weaning

	(A)	(B)
Brain dry weight	0.51 ± 0.08	0.56 ± 0.14
Protein	58.6 ± 2.4	57.4 ± 0.4
Lipid	44.8 ± 3.9	43.9 ± 2.0
Total polar lipids	49.5 ± 0.3	49.1 ± 1.1
Diradyl glycerophosphocholines	16.7 ± 0.8	16.2 ± 0.9
Diradyl glycerophosphoethanolamines	15.8 ± 0.7	15.6 ± 0.5
Phosphatidylserine	6.2 ± 0.2	6.2 ± 0.2
Phosphatidylinositol	1.6 ± 0.2	1.4 ± 0.1
Phosphatidic acid/cardiolipin	2.2 ± 0.3	1.9 ± 0.2
Glycosylglyceride	5.3 ± 0.5	6.1 ± 0.8
Sulphatide	0.3 ± 0.1	0.3 ± 0.1
Cerebroside	0.9 ± 0.2	0.8 ± 0.2
Sphingomyelin	0.4 ± 0.1	0.5 ± 0.2
Total neutral lipids	50.5 ± 0.3	50.9 ± 1.3
Cholesterol	19.2 ± 0.4	20.4 ± 0.8
Free fatty acid	3.6 ± 0.4	3.6 ± 0.6
Triacylglycerol	12.1 ± 0.1	11.2 ± 0.8
Steryl ester	15.5 ± 0.8	15.1 ± 0.3

Data are means ± SD of three samples. *mg/brain; †percentage of dry weight; ‡percentage of total lipid. Differences between the two groups of means were analysed by a paired t-test and were significant ($P < 0.05$) where indicated (§).

The fatty acid compositions of brain CPL and EPL from weaned and unweaned gilthead sea bream post-larvae are shown in Table 4. DHA and 18:2n-6 were significantly greater, and 18:3n-3 and 18:1n-7 were significantly lower, in both brain CPL and EPL in weaned fish. In addition, the percentages of EPA and 20:4n-6 were significantly reduced in brain EPL in weaned larvae.

The fatty acid compositions of brain PS and phosphatidylinositol (PI) from weaned and unweaned gilthead sea bream post-larvae are presented in Table 5. In PS, the proportion of total saturates, mainly 16:0, were significantly higher and the proportions of total (n-3)PUFA, mainly EPA, were significantly lower in weaned fish. The main effects of weaning on brain PI were significantly reduced EPA, 20:4n-6 and 18:1 isomers and significantly increased saturated fatty acids, especially 18:0, 16:0 and 14:0 (Table 5). The percentages of DHA were greater, but not significantly so, in brain PS and PI of weaned fish.

DISCUSSION

Values of 8.5 mg (n-3)HUFA/g dry wt of rotifers (Koven *et al.*, 1990) and 3.0 mg (n-3)HUFA/g dry wt of *Artemia* (Kissil, 1991) provide good growth in gilthead sea bream larvae, although they are possibly not optimum values (Kissil, 1991). However, no (n-3)HUFA requirements for 1 g or larger gilthead sea bream have been reported (Kissil, 1991). The

Table 3. Fatty acid compositions of brain total lipids from 62-day-old gilthead sea bream (*Sparus aurata*. L.) postlarvae fed: (A) only enriched-*Artemia* and (B) enriched-*Artemia* until day 55 and then dry pellet food for 1 week after weaning

Fatty acid	(A)	(B)
14:0	0.4 ± 0.1	0.5 ± 0.1
15:0	3.4 ± 0.6	2.8 ± 1.2
16:0DMA	0.3 ± 0.0	0.3 ± 0.0
16:0	13.3 ± 1.0	16.7 ± 0.4*
16:1n-9	0.9 ± 0.1	1.0 ± 0.1
16:1n-7	1.4 ± 0.1	1.4 ± 0.0
16:2	0.5 ± 0.1	0.4 ± 0.1
16:3	0.6 ± 0.2	0.8 ± 0.2
18:0DMA	1.1 ± 0.3	1.7 ± 0.0*
18:1n-9DMA	0.4 ± 0.1	0.6 ± 0.0
18:1n-7DMA	0.1 ± 0.0	0.1 ± 0.0
18:0	11.3 ± 0.7	11.7 ± 0.8
18:1n-9	13.8 ± 0.9	13.5 ± 0.4
18:1n-7	4.9 ± 0.3	3.9 ± 0.1*
18:2n-6	2.5 ± 0.2	3.1 ± 0.2*
18:3n-3	4.0 ± 0.3	2.2 ± 0.1*
18:4n-3	0.5 ± 0.0	0.3 ± 0.0
20:0	0.2 ± 0.0	0.2 ± 0.0
20:1n-9	0.4 ± 0.0	0.8 ± 0.0*
20:1n-7	0.2 ± 0.0	0.2 ± 0.1
20:2n-6	0.3 ± 0.1	0.3 ± 0.0
20:3n-6	0.3 ± 0.0	0.2 ± 0.0
20:4n-6	2.6 ± 0.1	2.6 ± 0.1
20:3n-3	0.7 ± 0.0	0.5 ± 0.0
20:4n-3	0.7 ± 0.0	0.6 ± 0.0
20:5n-3	9.8 ± 0.6	8.5 ± 0.4*
22:1	0.3 ± 0.1	0.6 ± 0.2
22:4n-6	0.2 ± 0.0	0.3 ± 0.1
22:3n-3	0.2 ± 0.0	0.5 ± 0.0
22:5n-6	0.4 ± 0.1	0.5 ± 0.1
22:5n-3	3.1 ± 0.2	3.2 ± 0.4
22:6n-3	9.0 ± 0.6	13.1 ± 1.0*
24:0	0.3 ± 0.0	0.4 ± 0.2
24:1n-9	0.5 ± 0.1	0.6 ± 0.1
24:1n-7	0.2 ± 0.1	0.2 ± 0.0
Total Saturated	29.6 ± 1.8	33.1 ± 0.7*
Total Monounsaturated	21.9 ± 1.5	22.4 ± 0.5
Total Polyunsaturated	37.2 ± 2.8	38.4 ± 1.8
Total (n-6)PUFA	7.3 ± 0.8	7.2 ± 0.1
Total (n-3)PUFA	29.3 ± 2.1	30.6 ± 1.8

Data are percentages of weight and are means ± SD of three samples. Totals include some minor components (<0.1%) not shown. SD = 0.0 implies an SD < 0.05. DMA, dimethyl acetal; PUFA, polyunsaturated fatty acids. Differences between the two groups of means were analysed by a paired *t*-test and were significant ($P < 0.05$) where indicated (*).

enriched *Artemia* and the weaning diet used in the present study provided levels of (n-3)HUFA well over those mentioned above; 13.2 mg/g dry wt for enriched *Artemia* and 14.7 mg/g for the dry pellet food. However, the enriched *Artemia* only provided 1.4 mg DHA/g dry wt, whereas the pellet diet contained 9.1 mg/g. Standard enrichment techniques are generally unable to routinely raise levels of DHA in *Artemia* higher than 1–1.5 mg/g dry wt. The absolute requirement for DHA in most larval marine fish, including gilthead sea bream, is unknown but it is probable that *Artemia*, enriched by standard techniques, represent a deficient diet with respect to DHA. In consequence, marine fish larvae are subjected to an abrupt increase in dietary DHA levels during weaning from enriched *Artemia* to a dry pellet food.

The brains of the gilthead sea bream post-larvae in the present study contained lower proportions of total polar lipids and the major phosphoglyceride classes (Table 2) than brains of turbot post-larvae of the same age (Mourente and Tocher, 1992b). Particularly noteworthy was the presence of significant levels of glycosylglycerides in brain lipids of gilthead sea bream. Glycosylglycerides are also present in small but significant amounts in lipids from brain and retina of rainbow trout (*Oncorhynchus mykiss*) and cod (*Gadus morhua*) (Tocher and Harvie, 1988; Tocher and Sargent, 1990), and seem to be involved in myelination processes (Sastry, 1985). In contrast, brain lipids of gilthead sea bream post-larvae contained larger proportions of neutral lipids, primarily triacylglycerol and steryl esters than the brains of turbot post-larvae (Tocher and Mourente, 1992b). The neutral glycerides may supply precursor diacylglycerol moieties for phospholipid biosynthesis and serve as sources of cholesterol and fatty acid in the course of biomembrane formation in the rapidly developing neural tissues (Sastry, 1985).

In a previous study with turbot *Scophthalmus maximus* L. we observed that DHA was specifically accumulated in brain phosphoglycerides after weaning from enriched *Artemia* to a pellet diet (Mourente and Tocher, 1992b). In the present study, the increased DHA levels in brain phospholipids in weaned gilthead sea bream compared to unweaned fish were lower than the increase in phospholipid DHA observed in the earlier turbot study (Mourente and Tocher, 1992b). This may be a consequence of differences between the diets used in the two studies. In the present study, the enrichment procedure produced *Artemia* that were more lipid rich and actually supplied more fatty acid (mg/g of dry wt) than the dry pellet food. As a result, the differences in brain lipid class and fatty acid compositions between weaned and unweaned fish were generally less striking. Furthermore, the enriched *Artemia* in the present study contained more than 1.4-fold more DHA than the enriched *Artemia* used to feed the turbot post-larvae in the earlier study. In consequence, the unweaned gilthead sea bream contained higher proportions of DHA in brain total lipids, CPL, EPL and PS than brains from the unweaned turbot (Mourente and Tocher, 1992b). Finally, the dry pellet diet used to wean the gilthead sea bream in the present study supplied less DHA than the weaning diet used in the earlier turbot study (Mourente and Tocher, 1992b). Therefore, although quantitatively less, the results of the present study are consistent with the earlier data showing a specific accumulation of DHA in brain phospholipids after weaning on to a DHA-rich diet.

It has become increasingly accepted recently that (n-3)PUFA, specifically DHA, are essential for neural development and photoreceptor function (Neuringer *et al.*, 1988; Bazan, 1990). Accumulation may occur either via *de novo* biosynthesis of DHA from shorter chain precursors in the brain or may result from uptake of dietary DHA (*via* the liver) (Crawford, 1987; Neuringer *et al.*, 1988; Scott and Bazan, 1989) with incorporation into phospholipids *via* turnover

Table 4. Fatty acid compositions of brain diradyl glycerophosphocholines and diradyl glycerophosphoethanolamines from 62-day-old gilthead sea bream (*Sparus aurata*. L.) postlarvae fed: (A) only enriched-*Artemia* and (B) enriched-*Artemia* until day 55 and then dry food for 1 week after weaning

	Diradyl glycerophosphocholines		Diradyl glycerophosphoethanolamines	
Fatty acid	(A)	(B)	(A)	(B)
14:0	0.6 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
15:0	1.2 ± 0.1	1.2 ± 0.2	1.3 ± 0.1	1.6 ± 0.0*
16:0DMA	nd	nd	0.6 ± 0.0	0.7 ± 0.0
16:0	32.4 ± 0.8	32.7 ± 2.0	6.1 ± 0.3	7.5 ± 0.3*
16:1n-9	1.8 ± 0.1	1.6 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
16:1n-7	1.9 ± 0.0	1.7 ± 0.2	0.6 ± 0.0	0.7 ± 0.1
16:2	0.7 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.4 ± 0.1
16:3	1.1 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.1
18:0DMA	nd	nd	2.4 ± 0.0	2.3 ± 0.1
18:1n-9DMA	nd	nd	0.6 ± 0.0	0.6 ± 0.0
18:1n-7DMA	nd	nd	0.4 ± 0.0	0.4 ± 0.0
18:0	7.9 ± 0.1	7.7 ± 0.6	17.9 ± 0.3	18.3 ± 0.4
18:1n-9	18.4 ± 0.4	18.5 ± 1.6	6.7 ± 0.2	6.6 ± 0.3
18:1n-7	3.5 ± 0.1	2.9 ± 0.3*	5.5 ± 0.1	4.4 ± 0.0*
18:2n-6	2.8 ± 0.1	3.6 ± 0.4*	1.3 ± 0.0	1.6 ± 0.0*
18:3n-3	2.7 ± 0.1	1.7 ± 0.3*	1.7 ± 0.0	1.0 ± 0.0*
18:4n-3	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
20:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:1n-9	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.7 ± 0.0*
20:1n-7	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:2n-6	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
20:3n-6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0*
20:4n-6	1.0 ± 0.0	1.2 ± 0.2	4.5 ± 0.2	3.9 ± 0.1*
20:3n-3	0.3 ± 0.0	0.3 ± 0.1	0.9 ± 0.0	0.7 ± 0.0*
20:4n-3	0.3 ± 0.0	0.3 ± 0.1	0.9 ± 0.0	0.7 ± 0.0*
20:5n-3	5.0 ± 0.2	5.3 ± 0.9	17.6 ± 0.6	13.3 ± 0.7*
22:1	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:4n-6	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.3	0.7 ± 0.1
22:3n-3	0.5 ± 0.2	0.3 ± 0.2	0.8 ± 0.1	0.5 ± 0.4
22:5n-6	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.2
22:5n-3	0.5 ± 0.1	0.8 ± 0.1*	4.9 ± 0.1	4.5 ± 0.3
22:6n-3	1.6 ± 0.1	4.4 ± 0.7*	14.6 ± 0.1	18.6 ± 0.6*
24:1n-9	0.5 ± 0.1	0.8 ± 0.1*	0.5 ± 0.2	0.6 ± 0.2
Total saturated	44.2 ± 0.7	44.3 ± 2.9	26.6 ± 0.1	28.8 ± 0.4*
Total monounsaturated	27.1 ± 0.4	26.8 ± 2.2	14.4 ± 0.1	13.7 ± 0.4
Total PUFA	20.4 ± 0.4	23.2 ± 2.2	51.7 ± 0.9	49.2 ± 1.1
Total (n-6)PUFA	5.9 ± 0.3	7.0 ± 0.7	8.3 ± 0.2	8.4 ± 0.1
Total (n-3)PUFA	13.5 ± 0.2	15.2 ± 1.8	43.1 ± 0.7	40.8 ± 0.9

Data are percentages of weight and are means ± SD of three samples. Totals include some minor components (<0.1%) not shown. SD = 0.0 implies an SD < 0.05. Statistical analysis as in Table 3. DMA, dimethyl acetal; PUFA, polyunsaturated fatty acids; nd = not detected.

(deacylation/reacylation) and/or direct transacylation (MacDonald and Sprecher, 1991). There are no data concerning these pathways in fish. However, in radiotracer studies performed in isolated brain cells from newly-weaned and 4-month-old turbot, the rank order of incorporation of [1-^{14}C]DHA into phosphoglycerides was CPL > EPL > PS > PI and this was independent of the age of the fish and the time of incubation (Tocher *et al.*, 1992). There was little specificity between DHA and 18:3n-3 in the uptake processes and there was only very limited capacity to convert 18:3n-3 to DHA although there was significant conversion of EPA to DHA in the isolated turbot brain cells (Tocher *et al.*, 1992). Similar studies are required in gilthead sea bream to determine the capacity of fatty acid transformation by brain and liver cells in order to predict how much DHA can be synthesized in either brain or liver and how much DHA must be supplied by the diet.

The possible physiological role(s) of DHA in neural tissues are under intense investigation (Neuringer *et al.*, 1988; Bazan, 1990) and the evidence indicates that, in mammals, DHA is most highly concentrated in synaptic membranes and in the disk membranes of photoreceptor cells of the retina (Bazan, 1990). Therefore, during development and differentiation of the central nervous system, DHA may be required especially for synaptogenesis, biogenesis of photoreceptor membranes and vision in general. This could be one of the most important and essential roles that this fatty acid plays during the early developmental stages of marine fish, particularly predatory species, in which prey capture is so essential at, and beyond, first feeding.

The natural diet of gilthead sea bream larvae and post-larvae includes primarily copepods, polychaetes, amphipods, mysidacea and ostracods (Arias and Drake, 1990). These are generally richer in DHA than the enriched rotifers or *Artemia* used in hatcheries for rearing marine fish larvae and post-larvae (Sargent *et al.*, 1990). Therefore, wild populations of gilthead sea bream will be able to obtain a sufficient and consistent supply of DHA in their natural diet.

Table 5. Fatty acid compositions of brain phosphatidylserine and phosphatidylinositol from 62-day-old gilthead sea bream (*Sparus aurata*. L.) postlarvae fed: (A) only enriched-*Artemia* and (B) enriched-*Artemia* until day 55 and then dry pellet diet for 1 week after weaning

	Phosphatidylserine		Phosphatidylinositol	
Fatty acid	(A)	(B)	(A)	(B)
14:0	0.6 ± 0.3	0.2 ± 0.0*	0.8 ± 0.2	1.7 ± 0.3*
15:0	2.2 ± 0.2	3.1 ± 0.1*	6.5 ± 1.4	8.7 ± 0.5
16:0	2.5 ± 0.1	3.2 ± 0.4*	7.5 ± 0.4	10.2 ± 0.2*
16:1n-7	0.6 ± 0.1	0.4 ± 0.2	0.8 ± 0.4	0.4 ± 0.1
16:2	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.3
17:0	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
16:3	0.4 ± 0.2	0.7 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
18:0	29.9 ± 0.8	31.2 ± 0.4	25.6 ± 1.6	32.3 ± 0.6*
18:1n-9	4.4 ± 0.1	3.9 ± 0.4	7.4 ± 0.7	4.7 ± 0.2*
18:1n-7	2.8 ± 0.1	2.1 ± 0.1*	3.2 ± 0.2	1.5 ± 0.1*
18:2n-6	0.8 ± 0.0	0.8 ± 0.1	1.2 ± 0.1	1.2 ± 0.0
18:3n-3	1.0 ± 0.1	0.5 ± 0.0*	1.1 ± 0.2	0.7 ± 0.3
18:4n-3	0.3 ± 0.0	0.4 ± 0.1	1.2 ± 0.1	1.0 ± 0.5*
20:0	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.1
20:1n-9	0.4 ± 0.0	0.4 ± 0.1	0.7 ± 0.4	0.6 ± 0.4
20:1n-7	0.3 ± 0.0	0.2 ± 0.0	tr	0.1 ± 0.0
20:2n-6	0.2 ± 0.1	0.3 ± 0.1	0.8 ± 0.5	0.8 ± 0.5
20:3n-6	0.5 ± 0.0	0.4 ± 0.1	0.6 ± 0.1	0.3 ± 0.2
20:4n-6	1.1 ± 0.0	0.9 ± 0.1	8.1 ± 0.7	5.7 ± 0.1*
20:3n-3	1.0 ± 0.0	0.6 ± 0.2*	0.4 ± 0.2	0.6 ± 0.1
20:4n-3	1.1 ± 0.1	0.7 ± 0.0*	0.9 ± 0.5	0.5 ± 0.3
20:5n-3	5.8 ± 0.2	4.0 ± 0.2*	8.9 ± 1.0	5.1 ± 0.3*
22:1	0.2 ± 0.1	0.3 ± 0.0	tr	tr
22:3n-3	0.3 ± 0.1	0.4 ± 0.2	tr	tr
22:5n-6	1.0 ± 0.1	0.7 ± 0.4	1.2 ± 0.4	0.8 ± 0.1
22:5n-3	8.9 ± 0.3	8.7 ± 0.1	2.1 ± 0.5	1.8 ± 0.2
22:6n-3	28.1 ± 1.4	28.3 ± 0.8	4.8 ± 0.8	5.7 ± 0.6
Total saturated	35.6 ± 1.0	39.1 ± 0.7*	41.3 ± 3.0	54.1 ± 1.3*
Total monounsaturated	8.8 ± 0.3	8.0 ± 0.5	12.1 ± 1.0	7.4 ± 0.5*
Total PUFA	51.2 ± 1.8	49.6 ± 0.5	36.1 ± 2.8	27.6 ± 1.9*
Total (n-6)PUFA	3.7 ± 0.2	4.4 ± 0.5	13.7 ± 1.8	9.7 ± 1.4
Total (n-3)PUFA	47.1 ± 1.9	41.9 ± 0.9*	21.7 ± 1.3	15.9 ± 0.6*

Data are percentages of weight and are means ± SD of three samples. Totals include some minor components (<0.1%) not shown. SD = 0.0 implies an SD < 0.05. Statistical analysis as in Table 3. PUFA, polyunsaturated fatty acids; tr = trace amount <0.1%.

Studies on the evolution of brain fatty acid compositions during the development of wild populations of marine fish are required to provide additional comparative data (Mourente and Tocher, 1992a).

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