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Effects of weaning onto a pelleted diet on docosahexaenoic acid (22:6*n*-3) levels in brain of developing turbot (*Scophthalmus maximus* L.)

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ABSTRACT

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The brain lipid and fatty acid compositions of turbot, *Scophthalmus maximus* (L.), were determined in unweaned fish and in fish from the same batch that had been weaned 1 week earlier. Fish were maintained on the same dietary regime until the time of weaning. Immediately prior to weaning fish were fed enriched *Artemia*. At weaning, one group of fish was fed a dry pelleted food whereas the other group remained on enriched *Artemia*. The dry pelleted diet contained 2-fold more eicosapentaenoic acid (EPA, 20:5*n*-3) and 13-fold more docosahexaenoic acid (DHA, 22:6*n*-3) per mg dry weight than the *Artemia*.

In weaned turbot, there were significant increases in brain dry weight (21.6% greater than in unweaned fish, $P<0.05$) and in the percentage of total polar classes in total lipid, due to a significant increase in the percentage of phosphatidylcholine (PC). There were no significant differences in the other lipid classes, with the exception of phosphatidylinositol (PI) which was significantly lower ($P<0.05$) in brain of weaned fish. The most striking effect of weaning on brain fatty acid composition was the rapid and specific incorporation of DHA into brain phosphoglycerides. The accumulation of DHA was highly significant in all phosphoglyceride classes, with the levels of DHA increased by factors of 52% in total lipid, 86% in PC, 62% in phosphatidylethanolamine (PE), 43% in phosphatidylserine (PS) and 31% in PI. The rapid incorporation of 22:6*n*-3 in turbot brain lipids was discussed with respect to the roles of this fatty acid in neutral tissues during development. The implication for the aquaculture of this species is that brain DHA levels may be directly related to larval performance, with the low levels of DHA in the brains of unweaned fish an important factor in the high mortality of larvae experienced during the stage when live feeds are being offered.

Abbreviations: DHA, docosahexaenoic acid; DMA, dimethyl acetal; EPA, eicosapentaenoic acid; FFA, free fatty acid; PA/CL, phosphatidic acid/cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SE, sterol ester; SM, sphingomyelin; TAG, triacylglycerol.

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INTRODUCTION

In recent years, increasing effort has been devoted to the study of lipid nutrition in fish during development (Sargent et al., 1989). In particular, considerable attention has been focused on the (*n*-3) polyunsaturated fatty acid (PUFA) requirements of marine fish larvae. Studies on species such as *Scophthalmus maximus* L. (Witt et al., 1984), *Sparus aurata* L. (Koven et al., 1989), and *Coryphaena hippurus* (Ostrowski and Divakaran, 1990) have shown that decosahexaenoic acid (DHA 22:6*n*-3) is strongly retained and is essential for these marine fish. Further nutritional studies have demonstrated that DHA was superior to eicosapentaenoic acid (EPA, 20:5*n*-3) as an essential fatty acid when each was provided to early larvae of red sea bream (*Pagrus major*) via rotifers (Watanabe et al., 1989a) or to later larval stages via *Artemia* (Izquierdo et al., 1989). Similar results were obtained for juvenile striped jack (*Pseudocaranx dentex*) or juvenile *P. major* by using inert artificial diets (Watanabe et al., 1989b; Takeuchi et al., 1990). In all cases, growth, survival and vitality were improved in fish fed higher levels of DHA. Therefore, in aquaculture, marine fish larvae are generally reared on (*n*-3)PUFA-enriched rotifers and *Artemia*. However, cultured turbot, *S. maximus*, displayed "better performance" based on several parameters when fed on calanoid copepods (Witt et al., 1984), which are richer in DHA than *Artemia* (Sargent et al., 1989), clearly implicating a role for DHA in turbot larval development.

Biochemical studies have shown that DHA is strongly retained in brain lipids of the sea bass, *Dicentrarchus labrax* L., even if deprived of (*n*-3)PUFA in the diet (Pagliarani et al., 1986). Fish, like mammals (Neuringer et al., 1988), have levels of DHA in brains and retinas well above those found in non-neural tissues, as shown in cod, *Gadus morhua* (Tocher and Harvie, 1988), and rainbow trout, *Oncorhynchus mykiss* (Tocher and Harvie, 1988; Bell and Tocher, 1989).

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 the present study, the influence of the weaning diet alone on brain lipid and fatty acid composition was investigated. The results were discussed with particular emphasis on the possible relationship between brain DHA levels and larval performance in commercial aquaculture.

MATERIALS AND METHODS

Fish

Turbot (*Scophthalmus maximus* L. 1758) were obtained from a commercial fish farm (Golden Sea Produce Ltd., Hunterston, Scotland, UK). The fish were maintained at the fish farm under normal operating conditions and

TABLE 1

Gross compositions (as percentage of dry wt.) of enriched *Artemia* and the pelleted diets. *Artemia* nauplii (Great Salt Lake, UT) were enriched as described in Materials and Methods. The pelleted diet was a dry compression pellet made from low temperature dried fillets, spray dried soluble fish protein, fish oil, vitamin and mineral mixes and binder. Three samples of *Artemia* and four samples of dry diet were analysed

Component	Composition	
	Enriched *Artemia*	Pelleted diet
Protein	60.1 ± 7.4	73.1 ± 6.0
Lipid	14.4 ± 4.6	15.9 ± 1.5
Carbohydrate	14.1 ± 3.3	0.9 ± 0.5
Ash	11.4 ± 2.8	10.1 ± 2.7

were fed the standard dietary regime. The time of weaning was determined by the size of the fish necessary to take the size of pellets of the weaning diet. The pellet size for the weaning diet was a balance between that optimal for the size of fish and practical considerations such as its stability in water. Too large a particle is poorly accepted by the fish whereas too small a particle causes operational difficulties due to breaking up in the water and clogging filters. Under the conditions prevailing at the time, and with this particular batch of fish, weaning occurred 54 days after hatching. At this point all fish were being fed enriched *Artemia*. One group of fish was then weaned on to the dry pelleted diet normally used at weaning, whereas the other group remained on enriched *Artemia*. Table 1 shows the gross compositions of the diets. Both populations were sampled 7 days later (61 days after hatching). Triplicate samples (containing 15 brains each) from the two groups of fish were dissected out on ice, immediately frozen in liquid nitrogen and stored at −80°C until analysis. Further samples (five brains each) were collected for dry weight determinations.

Diets

The newly hatched nauplii of *Artemia* (Great Salt Lake, UT; Sanders Brine Shrimp Company, Ogden, UT, USA) were enriched with (*n*-3)PUFA before they were supplied as live prey for turbot larvae. For enrichment, the *Artemia* were ongrown using a diet, prepared on-site, consisting of a soluble fish protein and fish oil mix base supplemented with minerals and vitamins. Particle size was small so that the diet was given essentially as a suspension. Fish oil levels in the diet were increased with growth of the *Artemia* culminating with a high-dose fish oil (enrichment) diet which was given during the 60 h prior to feeding to the fish. 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 pelleted diet during the 7 days. The fatty acid compositions of the diets are shown in Table 2.

Dry weight determination

Preweighed samples of five brains each were maintained at 110°C for 24 h. The dry weights were determined after cooling in vacuo for at least 1 h.

Total lipid extraction

Total lipid from the brain samples (~100 mg wet weight) was extracted, 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 class analysis

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 followed by calibrated densitometry using a Shimadzu CS-9000 dual-wavelength flying spot scanner and DR-2 recorder (Olsen and Henderson, 1989).

Fatty acid analysis

Individual phospholipid 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 phospholipid classes were prepared by acid-catalysed transmethylation for 16 h at 50°C, using nonadecanoic acid (19:0) as 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 Packard 436 gas chromatograph equipped with a chemically bonded CP Wax 52CB fused silica capillary column (50 m×0.34 mm i.d., Chrompack UK Ltd.), using on-column injection and hydrogen as carrier gas with a biphasic thermal gradient from 50°C to 235°C. After injection, the temperature was increased at 9°C/min until 180°C was reached and then increased at 1.5°C/min thereafter until 235°C was reached. Temperature was held at 235°C for a final phase of 20 min. Column head pressure was 50 kPa and carrier gas flow rate was 0.5 ml/min. Individual fatty acid methyl esters were identified by comparison with known standards and a well-characterised fish oil and by reference to published data as described previously (Tocher and Harvie, 1988), and quantified using a Shimadzu CR-3A recording integrator.

Statistical analysis

The results shown are means ± s.d. ($n=3$) and the differences between means were studied by paired t-test analysis (small group of samples).

Materials

BHT and nonadecanoic acid (19:0) were from Sigma Chemical Co. (Poole, Dorset, UK). 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). Analar grade glacial acetic acid, sulphuric acid and iso-propanol were from BDH (Poole, Dorset, UK). All other solvents were of HPLC grade and were obtained from Rathburn Chemicals (Walkerburn, Peeblesshire, UK).

RESULTS

The enriched *Artemia* contained 7.6% dry matter compared with 93.3% for the pelleted diet (Table 2). Total lipid contents were identical in both diets at approximately 13% of the dry weight. However, the pelleted diet provided almost double the amount of total fatty acids per mg of dry weight compared with enriched *Artemia*, as pigments and cholesterol account for up to 45% of the total lipid extract in *Artemia* (J.C. Navarro, personal commun., 1991). More specifically, EPA and DHA were 2-fold and approximately 13-fold higher, respectively, on a dry weight basis in the pelleted diet. In contrast, 18:1n-7 and 18:3n-3 were approximately 2-fold and 5-fold higher, respectively, in the live prey.

The dry weights of the brains from turbot weaned on to the dry pelleted diet were significantly greater ($P<0.05$) than the brains of the fish that remained on enriched *Artemia* (Table 3). There was no significant difference between the total lipid contents of the brains expressed as a percentage of the dry weight, but total polar lipid contents were significantly higher ($P<0.05$) in brains of weaned fish. The major polar lipid classes were phosphatidylcholine (PC) and phosphatidylethanolamine (PE) followed by phosphatidylserine (PS). The major neutral lipid class was cholesterol which accounted for 31% of total lipids. There were virtually no significant differences between the lipid class compositions of the two groups other than the proportion of PC was slightly greater, and the proportion of phosphatidylinositol (PI) slightly lower, in the brains of weaned fish ($P<0.05$).

The total lipid fatty acid compositions of brains from weaned and unweaned 61-day-old turbot are presented in Table 4. There were no significant differences between the proportions of total saturates, total monoenes, total PUFA or total dimethylacetals (DMA) in the brains of turbot on the two diets. However, marked differences were found between the proportions of some individual fatty acids. The percentage of DHA was more than doubled

TABLE 2

Total lipids (as dry wt. percentage) and fatty acid contents (as μg fatty acid/mg dry wt.) of diets used for rearing turbot. *Artemia* were enriched with (*n*-3)PUFA for 60 h prior to being fed to fish. Data are means ± s.d. of three different samples. Totals include some minor components (<0.1%) not shown. PUFA = Polyunsaturated fatty acids; HUFA = highly unsaturated fatty acids

Diet	*Artemia*	Pelleted diet
Dry matter percentage	7.6 ± 0.2	93.3 ± 0.3
Total lipid content	12.9 ± 1.3	12.8 ± 1.0
Fatty acid		
14:0	0.9 ± 0.2	4.0 ± 0.3
15:0	0.2 ± 0.0	0.6 ± 0.1
16:0	8.1 ± 0.8	14.9 ± 0.5
16:1*n*-7	1.9 ± 0.2	5.9 ± 0.2
16:2	0.5 ± 0.3	0.8 ± 0.1
16:3	tr	0.6 ± 0.1
18:0	3.9 ± 0.3	3.7 ± 0.3
18:1*n*-9	13.7 ± 1.0	17.2 ± 0.4
18:1*n*-7	6.5 ± 1.1	3.7 ± 0.3
18:2*n*-6	4.0 ± 0.5	4.0 ± 0.2
18:3*n*-3	10.4 ± 1.0	2.2 ± 0.1
18:4*n*-3	1.5 ± 0.2	2.6 ± 0.1
20:0	0.3 ± 0.1	0.8 ± 0.4
20:1*n*-9	1.0 ± 0.2	5.9 ± 0.0
20:4*n*-6	0.6 ± 0.1	1.2 ± 0.1
20:3*n*-3	0.3 ± 0.0	0.2 ± 0.1
20:4*n*-3	0.3 ± 0.0	0.8 ± 0.0
20:5*n*-3	4.4 ± 0.3	9.2 ± 0.6
22:1*n*-11	0.4 ± 0.1	5.2 ± 0.3
22:5*n*-6	0.4 ± 0.2	0.2 ± 0.0
22:5*n*-3	0.2 ± 0.0	1.4 ± 0.1
22:6*n*-3	1.0 ± 0.1	12.8 ± 2.9
Total saturated	14.1 ± 1.6	24.2 ± 1.1
Total monounsaturated	24.1 ± 2.5	40.7 ± 0.8
Total polyunsaturated	23.8 ± 2.3	38.5 ± 3.0
Total (*n*-6)PUFA	5.6 ± 0.7	7.2 ± 0.2
Total (*n*-3)PUFA	18.2 ± 1.6	31.2 ± 2.8
Total (*n*-6)HUFA	1.1 ± 0.1	2.0 ± 0.2
Total (*n*-3)HUFA	6.2 ± 0.4	24.8 ± 2.4

and 15:0 and 16:1*n*-7 were also significantly higher in the brains of weaned fish. In contrast, the proportions of 18:2*n*-6, 18:3*n*-3 and 20:3*n*-3 were significantly lower in total lipid from brains of weaned fish.

The fatty acid compositions of brain PC and PE from weaned and un-

TABLE 3

Total lipid contents (as dry wt. percentage) and lipid class compositions (as total lipid percentage) of total lipids from brains of turbot fed on (A) enriched *Artemia*, (B) dry pelleted food for 1 week after weaning. Data are means ± s.d. of three different samples. Differences between the two groups of means were analysed by a paired *t*-test and were significant ($P<0.05$) where indicated (*)

Diet	A	B
Brain dry wt. (mg/brain)	0.76 ± 0.05	0.97 ± 0.05*
Total lipid content	33.8 ± 1.1	30.5 ± 0.4
Total polar lipid	62.8 ± 0.6	64.7 ± 0.7*
Total neutral lipid	37.2 ± 0.6	35.3 ± 0.7*
Polar lipid		
Phosphatidylcholine	22.9 ± 0.7	25.7 ± 0.2*
Phosphatidylethanolamine	21.0 ± 0.7	20.9 ± 0.3
Phosphatidylserine	9.0 ± 0.2	9.3 ± 0.1
Phosphatidylinositol	2.3 ± 0.1	2.0 ± 0.1*
Phosphatidic acid/cardiolipin	2.7 ± 0.3	2.8 ± 0.1
Sulphatide	0.7 ± 0.2	0.8 ± 0.1
Cerebroside	2.3 ± 0.7	2.5 ± 0.1
Sphingomyelin	0.6 ± 0.1	0.6 ± 0.1
Neutral lipid		
Cholesterol	31.2 ± 0.6	31.0 ± 0.5
Free fatty acid	0.9 ± 0.4	0.3 ± 0.2
Triacylglycerol	2.1 ± 0.7	1.3 ± 0.2
Sterol ester	3.0 ± 2.2	2.8 ± 0.6

weaned turbot are shown in Table 5. In PC, total PUFA and total (*n*-3)PUFA were significantly higher in brains of weaned fish, whereas total (*n*-6)PUFA (primarily 18:2*n*-6) were more abundant in *Artemia*-fed fish. Highly unsaturated fatty acids (HUFA; carbon chain length ≥ 20 and with three or more double bonds) such as 22:5*n*-3 and DHA were almost 2-fold and 7-fold higher, respectively, in brains of weaned turbot. In PE, the proportion of total saturates was significantly higher in weaned fish whereas the proportions of total monoenes, total DMA, and total (*n*-6)PUFA were significantly lower. The proportions of total PUFA and total (*n*-3)PUFA in PE were not significantly different between the two groups of turbot. However, the proportion of DHA was ~2.5-fold greater in PE from weaned fish, whereas the proportion of EPA was ~2.5-fold greater in PE from unweaned fish. The percentage of 22:5*n*-3 was also significantly higher in PE in unweaned turbot.

The fatty acid compositions of brain PS and PI from weaned and unweaned turbot are presented in Table 6. In PS, the proportions of total PUFA and total (*n*-3)PUFA were significantly greater, and the proportions of total

TABLE 4

Fatty acid compositions (wt. percentage) of brain total lipids from turbot fed with (A) enriched *Artemia* and (B) dry pelleted food for 1 week after weaning. Data are means ± s.d. of three different samples. Statistical analysis as described in Table 3. PUFA = Polyunsaturated fatty acids; HUFA = highly unsaturated fatty acids; DMA = dimethyl acetal; tr = trace amount < 0.05%

Fatty acid	A	B
14:0	0.7±0.1	1.9±0.9
15:0	0.3±0.1	0.4±0.0*
16:0DMA	0.5±0.1	0.5±0.2
16:0	13.3±1.6	14.9±1.0
16:1*n*-7	1.4±0.1	3.6±1.4*
16:2	0.4±0.1	0.7±0.3
16:3	1.3±0.2	1.1±0.5
18:0DMA	1.1±0.2	1.1±0.4
18:1*n*-9DMA	1.1±0.2	0.6±0.2
18:1*n*-7DMA	0.3±0.4	tr
18:0	7.8±0.7	6.6±0.8
18:1*n*-9	15.7±1.8	13.5±3.3
18:1*n*-7	4.1±0.5	2.9±0.7
18:2*n*-6	6.7±2.4	1.7±0.2*
18:3*n*-3	2.1±0.3	0.8±0.1*
18:4*n*-3	0.7±0.2	1.2±0.7
20:0	0.6±0.3	0.5±0.3
20:1*n*-9	1.4±0.7	2.8±0.7
20:1*n*-7	0.4±0.2	0.4±0.1
20:2*n*-6	0.4±0.2	0.5±0.2
20:3*n*-6	0.3±0.1	0.2±0.0*
20:4*n*-6	1.4±0.3	1.4±0.3
20:3*n*-3	1.1±0.2	0.4±0.1*
20:4*n*-3	0.5±0.1	0.5±0.1
20:5*n*-3	6.3±0.8	6.0±2.6
22:1	1.0±0.7	2.5±1.6
22:4*n*-6	tr	tr
22:3*n*-3	0.1±0.1	0.1±0.1
22:5*n*-6	0.1±0.1	0.2±00
22:5*n*-3	2.1±0.5	1.7±0.4
22:6*n*-3	5.2±0.8	10.9±1.9*
24:1*n*-9	0.9±0.1	1.0±0.0
Total saturated	23.4±1.8	24.7±1.0
Total monounsaturated	26.6±2.0	27.3±5.9
Total PUFA	29.4±2.8	28.1±4.8
Total DMA	2.9±0.9	2.2±0.6
Total (*n*-6)PUFA	9.7±2.2	5.2±0.3*
Total (*n*-3)PUFA	19.7±2.6	22.9±4.7
Total (*n*-6)HUFA	2.1±0.4	2.2±0.1
Total (*n*-3)HUFA	15.3±2.4	19.8±4.1

TABLE 5

Fatty acid compositions (wt. percentage) of brain phosphatidylcholine and phosphatidylethanolamine from turbot fed with (A) enriched *Artemia* and (B) dry pelleted food for 1 week after weaning. Data are means ± s.d. of three different samples. Statistical analysis as in Table 3. PUFA = Polyunsaturated fatty acids; HUFA = highly unsaturated fatty acids; DMA = dimethyl acetals; tr = trace amount < 0.05%; n.d. = not detected

Fatty acid	Phosphatidylcholine		Phosphatidylethanolamine	
	A	B	A	B
14:0	0.7±0.1	1.2±0.1*	0.3±0.0	0.2±0.0
15:0	0.4±0.0	0.4±0.0	0.3±0.2	0.1±0.0
16:0DMA	n.d.	n.d.	1.2±0.0	1.1±0.0
16:0	26.0±4.2	30.2±2.3	6.1±0.3	8.8±0.2*
16:1*n*-7	3.4±0.5	3.1±0.2	2.0±0.3	1.5±0.1
16:2	0.4±0.2	0.5±0.1	0.6±0.3	0.6±0.0
16:3	1.5±0.2	1.0±0.2	1.4±0.2	0.7±0.0*
18:0DMA	n.d.	n.d.	3.3±0.2	3.1±0.1
18:1*n*-9DMA	n.d.	n.d.	3.3±0.2	2.9±0.0*
18:1*n*-7DMA	n.d.	n.d.	2.9±1.1	1.7±0.1
18:0	5.4±0.6	5.1±0.1	10.6±0.7	10.9±0.2
18:1*n*-9	23.4±3.6	21.6±1.2	8.4±0.4	6.6±0.1
18:1*n*-7	3.6±0.5	3.3±0.2	4.9±0.3	3.0±0.0*
18:2*n*-6	4.6±1.7	2.1±0.0	2.8±0.2	1.3±0.0*
18:3*n*-3	2.6±0.0	0.9±0.1*	2.2±0.2	0.7±0.0*
18:4*n*-3	0.3±0.3	0.2±0.2	0.5±0.1	0.3±0.1
20:0	0.8±0.9	0.7±0.6	0.3±0.1	0.3±0.0
20:1*n*-9	1.2±0.8	1.1±0.4	0.7±0.2	1.0±0.1
20:1*n*-7	0.3±0.4	0.3±0.2	0.2±0.2	0.5±0.0
20:2*n*-6	0.6±0.4	0.2±0.2	0.3±0.1	0.3±0.0
20:3*n*-6	0.7±0.2	0.2±0.1	0.3±0.0	0.5±0.0*
20:4*n*-6	0.7±0.2	0.7±0.0	2.9±0.1	1.7±0.1*
20:3*n*-3	0.8±0.1	0.5±0.1*	2.5±0.1	0.9±0.0*
20:4*n*-3	0.5±0.2	0.3±0.1	1.0±0.0	0.7±0.1*
20:5*n*-3	3.5±0.3	3.5±0.2	14.2±0.8	5.6±0.1*
22:1	0.1±0.1	0.3±0.0*	tr	0.1±0.0
22:4*n*-6	tr	tr	tr	0.2±0.1
22:3*n*-3	tr	0.1±0.1	tr	tr
22:5*n*-6	tr	0.1±0.1	0.1±0.1	0.2±0.0
22:5*n*-3	0.7±0.1	1.3±0.1*	4.5±0.2	3.6±0.1*
22:6*n*-3	1.2±0.1	8.3±0.7*	10.2±0.5	26.5±0.7*
24:1*n*-9	0.9±0.6	1.7±0.1*	tr	tr
Total saturated	34.2±4.5	38.4±1.9	17.6±0.9	20.5±0.3*
Total monounsaturated	33.3±2.9	32.2±1.2	16.3±0.3	12.9±0.1*
Total PUFA	18.2±0.7	20.3±0.2*	43.8±1.6	44.3±0.7
Total DMA	n.d.	n.d.	10.8±0.8	8.9±0.2*
Total (*n*-6)PUFA	6.9±1.2	4.1±0.2*	7.2±0.3	5.2±0.2*
Total (*n*-3)PUFA	11.3±0.6	16.3±0.3*	36.5±1.7	39.1±0.6
Total (*n*-6)HUFA	1.3±0.7	1.2±0.2	3.5±0.4	2.9±0.2
Total (*n*-3)HUFA	6.8±0.3	14.1±0.8*	32.4±1.5	37.3±0.7*

TABLE 6

Fatty acid compositions (wt. percentage) of brain phosphatidylserine and phosphatidylinositol from turbot fed with (A) enriched *Artemia* and (B) dry pelleted food for 1 week after weaning. Data are means ± s.d. of three different samples. Statistical analysis as in Table 3. PUFA = Polyunsaturated fatty acids; HUFA = highly unsaturated fatty acids; tr = trace amount < 0.05%

Fatty acid	Phosphatidylserine		Phosphatidylinositol	
	A	B	A	B
14:0	0.3±0.0	0.2±0.0*	0.7±0.2	0.5±0.1
15:0	0.3±0.1	0.2±0.0	0.5±0.1	0.6±0.6
16:0	3.3±0.3	2.8±0.1	5.9±0.8	6.8±0.9
16:1*n*-7	1.5±0.0	1.1±0.2*	1.2±0.2	1.3±0.9
16:2	0.4±0.1	0.4±0.0	0.2±0.1	0.1±0.0
16:3	0.7±0.3	0.5±0.0	0.1±0.1	0.3±0.2
18:0	20.6±0.7	22.7±0.4*	22.7±0.9	24.6±3.6
18:1*n*-9	7.9±0.4	6.2±0.1*	10.8±0.7	7.5±1.0*
18:1*n*-7	3.0±0.1	1.8±0.1*	3.7±0.2	2.5±0.1*
18:2*n*-6	1.9±0.2	0.9±0.0*	1.1±0.1	0.5±0.3*
18:3*n*-3	1.4±0.1	0.4±0.0*	0.4±0.3	0.2±0.1
18:4*n*-3	0.2±0.1	tr	tr	tr
20:0	0.4±0.2	0.3±0.0	0.1±0.1	0.3±0.2
20:1*n*-9	0.9±0.0	0.9± 0.0*	0.1±0.1	0.3±0.2
20:1*n*-7	0.3±0.1	0.2±0.0*	tr	tr
20:2*n*-6	0.4±0.1	0.2±0.0*	0.1±0.1	0.3±0.1
20:3*n*-6	0.9±0.1	0.5±0.1*	0.2±0.2	0.3±0.2
20:4*n*-6	1.7±0.2	0.6±0.0*	11.8±0.5	10.9±1.4
20:3*n*-3	tr	tr	0.2±0.2	0.1±0.1
20:4*n*-3	1.1±0.1	0.6±0.0*	0.1±0.1	0.2±0.1
20:5*n*-3	5.0±0.0	1.9±0.1*	17.7±0.8	13.1±1.8*
22:1	0.3±0.1	0.2±0.0	tr	tr
22:4*n*-6	0.6±0.1	0.2±0.0*	tr	tr
22:3*n*-3	0.6±0.1	0.6±0.0	tr	tr
22:5*n*-6	0.7±0.1	0.4±0.0*	0.1±0.1	tr
22:5*n*-3	7.9±0.5	7.7±0.2	2.8±0.2	1.5±1.1
22:6*n*-3	18.4±1.6	32.5±0.1*	10.8±2.5	15.7±2.7
24:1*n*-9	0.9±0.1	1.0±0.1	tr	tr
Total saturated	25.3±1.0	26.7±0.2	30.0±1.6	32.8±3.9
Total monounsaturated	15.1±0.5	11.6±0.4*	15.9±0.6	11.7±0.4*
Total PUFA	42.9±1.8	48.7±0.4*	46.1±1.8	43.6±3.3
Total (*n*-6)PUFA	7.0±0.1	3.8±0.2*	13.7±0.5	12.2±0.5*
Total (*n*-3)PUFA	35.9±1.9	44.9±0.3*	32.4±2.3	31.4±2.7
Total (*n*-6)HUFA	4.3±0.1	2.3±0.1*	12.2±0.1	11.3±1.1
Total (*n*-3)HUFA	33.2±2.6	43.8±0.4*	31.8±2.8	30.7±3.5

monoenes and total (*n*-6)PUFA significantly lower, in weaned fish. The proportions of total saturated fatty acids were not significantly different in the two groups. The percentage of DHA in PS was almost doubled in weaned turbot, whereas EPA in PS was 2.6-fold greater in unweaned fish. The percentage of DHA also increased in PI from weaned turbot, although the difference was not statistically significant. As with the other phosphoglycerides, other (*n*-3)PUFA, such as EPA and 22:5*n*-3, and total monoenes and total (*n*-6)PUFA were found in higher percentages in the group fed only with *Artemia*. The higher percentage of (*n*-6)PUFA in PI was due mainly to significantly higher 18:2*n*-6, and no significant difference was found between the percentages of 20:4*n*-6, which accounted for ~11% of total fatty acids in PI from both groups of fish.

DISCUSSION

It has been shown that turbot require DHA for healthy development (Bell et al., 1985). The minimum levels of dietary (*n*-3)HUFA, including DHA, required by turbot for optimum growth and development have been reported as 1.3%–1.7% of the diet dry weight for larvae (Le Milinaire et al., 1983; Robin et al., 1987) and 0.57% of diet dry weight for adult fish (Gatesoupe et al, 1977; Leger et al., 1979). In marine-type *Artemia*, which contain high levels of EPA (Watanabe et al., 1978), the amount of (*n*-3)HUFA in newly hatched nauplii is inadequate for turbot larvae (Robin et al., 1987), and even the best quality (with respect to (*n*-3)HUFA content) *Artemia* strain contains only trace amounts of DHA (Leger et al., 1986). Therefore, for marine fish culture, DHA must be added to *Artemia* via enrichment techniques (Leger et al., 1987). In most cases, it is difficult to get an adequate (with respect to (*n*-3)HUFA levels) and clean enrichment of *Artemia*. Therefore, in the present study, although a significant level of DHA was achieved (1% of total fatty acids), the total (*n*-3)HUFA content was still only 0.6% of the dry weight. This is below the amount reported by Le Milinaire et al. (1983) and Robin et al. (1987) to satisfy turbot larvae requirements, but it should be sufficient for adult fish. The fish in the present study were juveniles and, although the exact (*n*-3)HUFA requirement is not known it is likely to be closer to the value for larvae. The pelleted diet used to wean the turbot contained twice as much EPA and almost 13-fold more DHA per mg of dry weight than the enriched *Artemia*. Total (*n*-3)HUFA in the dry food was 2.9% of diet dry weight, well above the established minimum requirement for turbot. Therefore, in the present study, turbot were weaned from what may be an (*n*-3)HUFA-deficient diet (enriched *Artemia*) to a sufficient diet (dry pellet).

Weaned turbot had significantly larger brains and higher levels of DHA in brain total lipid than unweaned fish. Brain dry weight was 20% greater and DHA was more than doubled after only 7 days consuming the pelleted diet.

In the brain phosphoglycerides, DHA levels increased almost 7-fold in PC, 2.6-fold in PE, 1.8-fold in PS and 1.4-fold in PI. In comparison, diet has been shown to alter fatty acid composition in rat brain membranes within 24 days (Foot et al., 1982). In the present study, DHA was incorporated by turbot brain in preference to other fatty acids, and this has also been demonstrated in rats (Anderson and Connor, 1988). The incorporation of DHA into the phosphoglycerides can occur via both de novo biosynthesis of phospholipid and turnover via the processes of deacylation/reacylation. The relative importance of each pathway is not clear, but obviously de novo synthesis will account for significant incorporation as the fish were growing and, in particular, brain weights were increased significantly. However, considering the relatively short period of time (7 days), combined with the large increases in DHA, turnover may also account for a significant amount of the incorporation. It is thought that the processes of deacylation/reacylation could have an important role in maintaining optimal membrane composition without the high energy cost associated with de novo synthesis (Connor et al., 1990). What is clear is the avidity with which brain phosphoglycerides retain DHA. The resultant membrane remodelling will probably affect and, perhaps, enhance the physiological functions of the central nervous system in the weaned turbot.

DHA may have physiological roles in the nervous system related to the biophysical properties of membranes (Neuringer et al., 1988; Bazan, 1990), as a modulator of lipid–protein interactions and neural enzyme activity (Neuringer et al., 1988), and as a precursor of lipoxygenase products (Bazan, 1990). 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 is 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. In particular, in a predator like the turbot in which the visual system is highly developed, with the eyes set upward on the left side of the body and capable of precise binocular vision, accurate development of all aspects of the visual processes are paramount.

The natural food of turbot larvae includes a wide variety of planktonic crustaceans, primarily cladocerans and calanoid copepods (Last, 1979). However, natural zooplankton is rarely available in fish hatcheries for rearing marine fish larvae, and the utilization of (*n*-3)HUFA-enriched rotifers and *Artemia* is generally obligatory. Cultured turbot larvae show prey selection (e.g. preferring calanoid copepods to *Artemia*) (Kuhlmann et al., 1981; Van der Meeren, 1991) and displayed "better performance" based on several parameters when fed on calanoid copepods (Witt et al., 1984), which are richer in DHA than *Artemia* (Sargent et al., 1989). The fish in the present study did

not show any signs of gross essential fatty acid deficiency, but the results obtained in this biochemical study suggest that improving the (*n*-3)-PUFA and DHA content of the live foods may improve the condition of the fish. This could lead to lower mortalities before weaning and may reduce the time for weaning to be reached by improved growth parameters. Therefore, not only should adequate amounts of (*n*-3)HUFA be provided to broodstocks before spawning (Watanabe, 1985; Mourente and Odriozola, 1990), but also the enrichment techniques for the live prey (rotifers and *Artemia*) commonly used to feed marine fish larvae should be improved to ensure an adequate supply of DHA during these critical early periods of development.

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