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Incorporation and metabolism of ^{14}C-labelled polyunsaturated fatty acids in wild-caught juveniles of golden grey mullet, *Liza aurata, in vivo*

Gabriel Mourente[1] and Douglas R. Tocher[2]
[1]*Departemento de Biología, Facultad de Ciencias del Mar, Universidad de Cádiz, Polígono Río San Pedro, Apartado 40, 11510-Puerto Real, Cádiz, Spain;* [2]*NERC Unit of Aquatic Biochemistry, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland*

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Abbreviations: AA, 5,8,11,14-eicosatetraenoic acid (arachidonic acid, 20:4n – 6); CPL, diradyl (diacyl + alkenylacyl + alkylacyl) glycerophosphocholine; DHA, 4,7,10,13,16,19-docosahexaenoic acid (22:6n – 3); EPA, 5,8,11,14,17-eicosapentaenoic acid (20:5n – 3); EPL, diradyl (diacyl + alkenylacyl + alkylacyl) glycerophosphoethanolamine; HUFA, highly unsaturated fatty acids ($\geq C_{20}$ and with ≥ 3 double bonds); LA, 9,12-octadecadienoic acid (linoleic acid, 18:2n – 6); LNA, 9,12,15-octadecatrienoic acid (α-linolenic acid, 18:3n – 3) PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid(s).

Abstract

The incorporation, and the capacity for desaturation and elongation *in vivo*, of intraperitoneally-injected, ^{14}C-labelled n-3 and n-6 C_{18} and C_{20} polyunsaturated fatty acids (PUFA) were investigated in juvenile golden grey mullet, *Liza aurata*. The results indicate that juvenile mullet have only limited ability to convert C_{18} polyunsaturated fatty acids to C_{20} and C_{22} highly unsaturated fatty acids (HUFA) *in vivo*. This suggests that juvenile golden grey mullet require the provision of preformed $C_{20/22}$ HUFA, such as eicosapentaenoic and docosahexaenoic acids, in the diet. The impairment in the desaturase/elongase pathway was similar to that found in turbot, *Scophthalmus maximus*, and gilthead sea bream, *Sparus aurata*, being primarily at the level of Δ5-desaturase. The data from the largely herbivorous golden grey mullet juveniles are consistent with the hypothesis that marine fish in general, irrespective of dietary habits, have limited capacity for the desaturation and elongation of C_{18} PUFA. The defect in Δ5-desaturase activity combined with the consistent finding that arachidonic acid is selectively incorporated and retained in membrane phosphatidylinositol suggests that, like turbot and gilthead sea bream, golden grey mullet may also have a requirement for preformed arachidonic acid in the diet.

Introduction

The qualitative and quantitative requirements of fish for the dietary essential C_{18} fatty acids, linoleic acid (LA; 18:2n – 6) and linolenic acid (LNA; 18:3n – 3) are highly dependent upon the ability of the fish to convert the C_{18} polyunsaturated fatty acids (PUFA), *via* the desaturation/elongation pathways, to highly unsaturated C_{20} and C_{22} fatty acids (HUFA), $\geq C_{20}$ and with ≥ 3 double bonds). Based on data from nutritional studies and early isotope work on rainbow trout (*Oncorhynchus mykiss*) and turbot (*Scophthalmus maximus*) (Owen *et al.* 1975; Kanazawa *et al.* 1979), it has

been postulated that marine fish have only limited capacity to bioconvert C_{18} PUFA to $C_{20/22}$ HUFA (Sargent *et al.* 1989). Several more recent *in vivo* studies using intraperitoneally injected radioactivity labelled C_{18} and/or C_{20} PUFA have been carried out in order to determine the ability of different fish species to synthesize $C_{20/22}$ HUFA (Olsen *et al.* 1990; Linares and Henderson 1991; Olsen and Ringo 1992; Mourente and Tocher 1992a). The results of those experiments appear to confirm that there is a fundamental difference between freshwater fish and marine fish in their abilities to convert C_{18} PUFA to $C_{20/22}$ HUFA, with the freshwater species studied having a high capacity for desaturation and elongation of C_{18} PUFA, whereas the marine species studied had a low capacity for this conversion.

Studies on turbot (Linares and Henderson 1991) and gilthead sea bream (*Sparus aurata*) (Mourente and Tocher 1993a), indicated that these species lack one or more of the desaturase enzymes required to synthesize HUFA. The impairment in the desaturase/elongase pathway was qualitatively and quantitatively similar in both species; primarily at the level of the Δ5-desaturase. Deficiency in the desaturase/elongase pathways enzymes, particularly Δ5-desaturase, has been postulated to be characteristic of marine fish in general (Henderson and Tocher 1987; Sargent *et al.* 1989). Therefore, the general inability of marine fish to desaturate and elongate C_{18} PUFA dictates the essential requirement of these fish for preformed C_{20} and C_{22} PUFA in their diet (Sargent *et al.* 1989). In addition, the fact that arachidonic acid (AA, 20:4n – 6) is selectively incorporated and retained in membrane phosphatidylinositol, suggested that, in addition to eicosapentaenoic (EPA, 20:5n – 3) and docosahexaenoic (DHA, 22:6n – 3) acids, these marine fish species may also have a requirement for preformed AA in the diet.

In terrestrial mammals, deficiencies in the desaturase/elongase pathways have been associated with dietary habits, with extreme carnivores, such as cats, lacking Δ6- and Δ5-desaturases (Rivers *et al.* 1975). The marine fish species studied, included turbot and gilthead sea bream, are highly carnivorous, as are most of the commercially important marine species. In order to elucidate the association between feeding habits and PUFA desaturase/elongase pathways, PUFA metabolism must be studied in marine species with different natural diets, particularly a strict or occasional marine herbivore and also a freshwater species that is an extreme carnivore.

Golden grey mullet, *Liza aurata*, is an important marine fish which is cultured or farmed in many countries, especially in the Mediterranean and South-East Asia. Mullet can be fed on a variety of food, including plankton, benthic algae, decaying organic matter, rice bran and groundnut meal; one of the reasons making it amenable to culture. In particular, it is well established that grey mullet fingerlings are herbivorous in the wild (Albertini-Berhaut 1973, 1974; Oren 1981). In the present study, the incorporation, and the capacity for desaturation and elongation, of C_{18} and C_{20} PUFA were investigated in wild-caught juvenile golden grey mullet *in vivo*.

Materials and methods

Fish

Fingerlings of the golden grey mullet, *Liza aurata* (Risso 1810) were captured with a hand scoop-net in shallow waters of the Bay of Cádiz (SW Spain) at the beginning of July 1992. Fish were transported to the laboratory in buckets containing well aerated sea-water and maintained in 50 l rectangular fibreglass tanks, supplied with underground sea water (salinity 32 g/l and temperature 20 ± 1°C) in an open circuit system with aeration. Prior to experimentation, no food was offered to the fish.

Injection of ^{14}C-labelled PUFA and experimental conditions

Six groups of three fish were starved from the time of capture until use in the experiment (> 1 week) to ensure no dietary input of $C_{20/22}$ HUFA and to maximize the rate of bioconversion of the injected ^{14}C-labelled PUFA. The fish in four of the groups

were injected with 3 μCi of either [1 – ^{14}C]LA, [1 – ^{14}C]LNA, [1 – ^{14}C]AA or [1 – ^{14}C]EPA suspended in 5 μl of ethanol (Linares and Henderson 1991; Olsen and Ringo 1992; Mourente and Tocher 1993a). The fish in a fifth group were injected with ethanol alone as an injection control. Fish were injected after anaesthetization by hypothermic shock (Summerfelt and Smith 1990), whereby the fish were placed in a beaker containing a 1:1 mixture of sea water and crushed ice for 1–2 min until immobilized. No mortalities occurred during starvation or during the experimental period after the fish were injected with radioactive PUFA. After injection, the groups of fish were maintained in cylindrical glass aquaria containing 2 l of sea water with aeration. Fish recovered from anesthesia and injection within 5 min with no apparent ill effects. After 48 h, fish were removed from the aquaria, carefully blotted with filter paper, killed by immersion in liquid nitrogen and wet weight determined. The sixth group of fish was used for dry weight determination; wet weight was determined as described above, then fish were finely chopped, desiccated at 110°C for 24 h and then cooled *in vacuo* before dry weight was determined.

Lipid extraction

Lipids were extracted from weighed fish by homogenization in chloroform/methanol (2:1, v/v), containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant, according to Folch *et al.* (1957). Solvent was evaporated under a stream of nitrogen and lipid extracts desiccated overnight *in vacuo* before weighing. Lipid extracts were redissolved in chloroform/methanol (2:1, v/v) at a concentration of 50 mg/ml and stored under an atmosphere of nitrogen at –20°C until analysis. An aliquot of the total lipid was taken for determination of radioactivity as described below.

Lipid class quantification

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

Fatty acid analysis

Individual glycerophospholipid classes were separated by thin-layer chromatography (TLC) using methyl acetate/isopropanol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.) as developing solvent (Vitiello and Zanetta 1978). For the separation of neutral lipid classes hexane/diethyl ether/acetic acid (80:20:2, v/v/v) was used (Christie 1989). Fatty acid methyl esters were prepared from total lipids and individual glycerophospholipid and neutral lipid classes by acid-catalyzed 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 analyzed 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 Inc., Bellefonte, USA), using hydrogen as carrier gas with a thermal gradient from 185°C to 235°C. Individual fatty acid methyl esters were identified and quantified by reference to authentic standards and to a well characterized fish oil, using a Hewlett-Packard 3394 recording integrator (Tocher and Harvie 1988). All solvents contained 0.01% BHT as an antioxidant).

Incorporation of radioactivity into total lipids and individual lipid classes

Samples of total lipids (3 mg) from ^{14}C-PUFA-injected mullet were applied in 4 cm streaks to TLC plates and polar lipid and neutral lipid classes separated as described above. Lipid classes were visualized by brief exposure to iodine vapour, bands

marked and the iodine removed under vacuum (Tocher and Harvie 1988). Individual classes were scraped into scintillation mini vials, 4 ml of liquid scintillation cocktail (Ready Safe, Beckman) added and radioactivity determined in a Beckman LS 5000 CE liquid scintillation spectrophotometer. Results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions.

Incorporation of radioactivity into fatty acids from total lipids

Fatty acid methyl esters from total lipids were prepared as described above. Methyl esters were separated by argentation-TLC, using 2% silver nitrate-impregnated TLC plates with toluene/acetonitrile (95:5, v/v) as developing solvent (Wilson and Sargent 1992). This system resolves the methyl esters into discrete bands based on both degree of unsaturation and chain length (Christie 1989; Wilson and Sargent 1992). Developed TLC plates were subjected to autoradiography for 14 days using Kodak X-OMAT AR-5 X-ray film, and the labelled bands scraped into scintillation vials and radioactivity determined as above. Identification of labelled bands was confirmed by using authentic unlabelled standards run on parallel plates, with visualization by charring.

Materials

[1-^{14}C] PUFA (all 50–53 mCi/mmol and 99% pure) were obtained from NEN Dupont, Investigación Técnica Inductrial S. A. (ITISA), Madrid, Spain. BHT, silver nitrate and potassium chloride were from Sigma Chemical Co. Ltd. (Poole, Dorset, U.K.). TLC (20 × 20 cm × 0.25 mm) and HPTLC (10 × 10 × 0.15 mm) glass plates, precoated with silica gel 60 (without fluorescent indicator), were purchased from Merck (Darmstadt, Germany). All solvents were from HPLC grade and were obtained from Fluka Chemical Co. (Glossop, Derbyshire, U.K.).

Table 1. Wet and dry weight, total length, lipid content and lipid class composition of juvenile golden grey mullet after starvation for 1 week

Fish total length (cm)	6.4 ± 1.5
Fish wet weight (g)	2.4 ± 0.6
Fish dry weight (g)	0.6 ± 0.1
Lipid content (percentage of dry weight)	5.7 ± 1.5
Lipid class composition (percentage of total lipid)	
Total polar lipids	65.8 ± 6.6
Diradyl glycerophosphocholines	27.3 ± 2.0
Diradyl glycerophosphoethanolamines	15.1 ± 1.1
Phosphatidylserine	5.2 ± 1.1
Phosphatidylinositol	3.3 ± 0.2
Phosphatidic acid/cardiolipin	4.0 ± 0.2
Sphingomyelin	4.8 ± 1.0
Cerebrosides	2.7 ± 0.2
Sulfatides	0.6 ± 0.1
Lyso-phosphatidylcholine	2.9 ± 1.3
Total neutral lipids	34.2 ± 6.6
Cholesterol	16.6 ± 2.0
Free fatty acid	0.4 ± 0.1
Triacylglycerol	10.7 ± 7.9
Steryl esters	6.4 ± 0.6

Data are means ± SD (n = 15 for total length, wet and dry weights and lipid content, and n = 3 for lipid class composition).

Statistical analysis

Results are presented as means ± SD of triplicate experiments. The data were checked for homogeneity of the variances by the Bartlett test and, where necessary, the data were arc-sin transformed before further statistical analyses. Differences between mean values for incorporation of radioactivity into total lipids and individual lipid classes were analyzed by one-way ANOVA followed (where appropriate) by a multiple comparison test (Tukey) (Zar 1984).

Results

Biometric data of fish (total length and dry weight) are shown in Table 1. Total lipid accounted for 5.7% of the dry body weight with total polar lipids

Table 2. Fatty acid compositions of lipid classes from golden grey mullet after starvation for 1 week

Fatty acid	TL	TPL	TAG	SE	CPL	EPL	PS	PI
14:0	1.2±0.2	0.7±0.1	4.3±0.2	7.1±0.3	1.1±0.0	0.4±0.1	1.1±0.0	1.4±0.1
15:0	1.9±0.4	0.5±0.1	3.0±0.7	1.8±0.8	1.5±0.4	1.5±0.4	4.8±0.2	6.1±1.5
16:0DMA	0.6±0.0	0.9±0.0	nd	nd	nd	2.8±0.1	nd	nd
16:0	15.6±0.9	17.9±0.7	17.6±1.5	38.2±2.5	31.3±1.0	7.0±0.3	7.1±0.9	8.5±0.3
16:1(n−7)	2.9±0.7	2.5±0.2	9.2±1.6	3.3±0.1	2.8±0.5	1.3±0.0	0.9±0.1	1.5±0.5
16:2	0.8±0.0	0.5±0.1	1.4±0.2	2.1±0.4	0.4±0.0	0.4±0.2	0.9±0.1	1.1±0.2
16:3	0.5±0.1	0.8±0.3	1.7±0.1	1.3±0.1	0.7±0.1	0.9±0.0	1.1±0.1	0.9±0.0
16:4	0.9±0.2	0.3±0.1	0.5±0.1	1.9±0.4	0.7±0.2	0.8±0.1	0.4±0.1	0.5±0.1
18:0DMA	1.0±0.2	1.4±0.1	nd	nd	nd	6.3±0.4	nd	nd
18:1(n−9)DMA	0.2±0.0	0.3±0.0	nd	nd	nd	1.2±0.1	nd	nd
18:1(n−7)DMA	0.1±0.0	0.2±0.0	nd	nd	nd	1.0±0.1	nd	nd
18:0	11.6±1.1	13.1±0.6	5.3±0.1	2.9±0.2	7.7±0.8	16.6±0.4	37.3±3.5	29.8±1.7
18:1(n−9)	11.1±1.1	11.7±0.9	10.5±2.0	3.5±0.5	12.2±0.4	7.6±0.6	5.8±0.6	4.9±0.8
18:1(n−7)	3.5±0.2	3.3±0.1	3.9±0.0	1.1±0.1	2.8±0.2	3.2±0.2	1.6±0.0	3.2±1.0
18:2(n−6)	2.1±0.2	1.8±0.2	3.9±0.4	1.0±0.1	1.7±0.1	1.3±0.1	0.7±0.0	2.0±0.4
18:3(n−3)	0.3±0.0	0.2±0.0	0.6±0.1	1.5±0.4	0.1±0.0	0.2±0.1	0.9±0.2	0.4±0.1
18:4(n−3)	0.3±0.1	0.2±0.0	1.0±0.0	0.5±0.1	0.1±0.0	0.2±0.0	0.2±0.1	0.5±0.1
20.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.1	0.2±0.0	0.4±0.0	0.9±0.0	0.3±0.1
20:1(n−9)	1.7±0.7	0.9±0.1	5.5±1.2	1.3±0.2	0.8±0.1	1.0±0.2	0.8±0.1	0.7±0.2
20:1(n−7)	0.7±0.2	0.6±0.1	0.8±0.1	0.5±0.2	0.5±0.2	0.7±0.2	0.7±0.1	0.4±0.1
20:2(n−6)	0.4±0.1	0.2±0.1	0.4±0.0	nd	0.2±0.0	0.3±0.0	0.9±0.1	1.3±0.3
20:3(n−6)	0.2±0.1	0.1±0.1	0.7±0.4	0.8±0.4	0.2±0.0	0.1±0.0	0.1±0.0	0.2±0.1
20:4(n−6)	4.0±0.7	4.3±0.4	1.5±0.3	0.4±0.2	2.7±0.2	4.2±0.2	2.0±0.1	7.2±1.4
20:3(n−3)	0.3±0.1	0.1±0.0	0.4±0.1	0.4±0.3	0.7±0.1	0.1±0.0	0.3±0.1	1.4±0.4
20:4(n−3)	0.6±0.2	0.2±0.0	0.2±0.1	0.4±0.1	0.2±0.0	0.3±0.0	0.4±0.0	0.2±0.0
20:5(n−3)	8.5±0.5	8.6±0.6	3.8±0.2	2.0±0.6	7.9±1.0	6.5±1.0	2.2±0.3	3.0±0.5
22:1(n−11)	0.6±0.3	0.1±0.0	3.1±0.9	nd	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
22:4(n−6)	0.3±0.1	0.1±0.0	0.1±0.0	nd	0.1±0.0	tr	0.1±0.0	0.2±0.1
22:3(n−3)	0.2±0.0	0.3±0.0	0.3±0.0	nd	0.1±0.0	0.4±0.1	0.5±0.0	0.2±0.1
22:5(n−6)	0.6±0.0	0.8±0.1	0.6±0.2	0.6±0.3	0.3±0.1	1.4±0.4	0.1±0.0	0.9±0.3
22:5(n−3)	2.6±0.2	2.6±0.2	1.9±0.2	1.4±0.1	1.5±0.1	2.7±0.2	3.1±0.2	1.5±0.8
22:6(n−3)	16.4±1.2	17.8±1.3	5.6±0.8	4.8±1.1	10.7±0.8	20.4±2.1	12.7±0.7	4.6±1.6
24:1(n−9)	0.6±0.1	0.3±0.2	0.1±0.0	nd	0.7±0.2	nd	0.1±0.0	0.6±0.1
Total SFA	31.8±1.0	33.6±0.3	30.9±2.6	50.4±3.1	43.9±0.9	25.9±0.7	51.6±2.4	47.2±3.1
Total MUFA	21.7±2.1	20.1±0.7	34.0±2.8	9.8±0.3	21.1±0.5	14.1±1.0	10.4±1.4	11.6±2.5
Total PUFA	40.3±0.9	39.9±0.9	25.5±0.2	20.8±2.4	29.3±1.3	41.9±2.9	27.4±0.5	29.4±2.9
Total DMA	2.0±0.2	2.8±0.1	nd	nd	nd	11.4±0.3	nd	nd
(n−6)PUFA	9.0±0.8	8.3±0.4	8.9±1.0	5.7±0.7	5.9±0.4	8.3±0.2	5.3±0.2	13.6±0.7
(n−3)PUFA	30.9±1.2	31.2±1.1	16.1±1.3	14.4±1.8	23.1±1.8	32.9±2.9	21.9±0.4	13.9±2.4

Data are expressed as percentage of weight and represent the mean ± SD (n = 3). SD = 0.0 implies an SD < 0.05. Totals include some other components not shown. nd, not detected; tr, trace < 0.1%; CPL, total diradyl glycerophosphocholine; DMA, dimethyl acetal; EPL, total diradyl glycerophosphoethanolamine; MUFA, monounsaturated fatty acid; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SE, steryl esters; SFA, saturated fatty acid; TAG, triacylglycerol; TL, total lipids; TPL, total polar lipids.

(65.8% of the total lipids) predominating over total neutral lipids (34.2%). The predominant polar lipid classes were total diradyl (diacyl + alkenylacyl + alkylacyl) glycerophosphocholines (CPL, 27.3% of total lipid) and total diradyl glycerophosphoethanolamines (EPL, 15.1%), followed by phosphatidylserine (PS, 5.2%), sphingomyelin (4.8%), phosphatidic acid/cardiolipin (4.0%) and phosphatidylinositol (PI, 3.3%) (Table 1). Cholesterol (16.6%) was the predominant neutral lipid, fol-

Table 3. Incorporation of radioactivity from injected ^{14}C-polyunsaturated fatty acids into lipid classes in juvenile golden grey mullet

	^{14}C-polyunsaturated fatty acid			
	18:2(n−6)	18:3(n−3)	20:4(n−6)	20:5(n−3)
Incorporation into total lipid (dpm × 10^6/fish)	1.3±0.2^{ab}	0.9± 0.1^{a}	2.2±0.0^{c}	1.4±0.4^{b}
Recovery in total lipid	12.0±1.7^{a}	8.6± 0.7^{a}	20.2±0.4^{b}	12.3±4.1^{ab}
Recovery in lipid classes				
Total polar lipids	79.6±8.9^{a}	39.1± 1.0^{b}	70.8±11.8^{a}	80.7±8.4^{a}
Glycerophosphocholines	53.1±6.9^{a}	25.4± 3.4^{b}	35.7±5.2^{b}	54.4±1.4^{a}
Glycerophosphoethanolamines	10.4±0.5^{ab}	6.0± 0.8^{a}	13.4±1.7^{bc}	15.6±1.5^{c}
Phosphatidylserine	2.2±0.4^{ab}	1.3± 0.2^{a}	4.6±0.5^{c}	3.6±0.7^{bc}
Phosphatidylinositol	2.0±0.1^{a}	1.2± 0.1^{a}	13.6±2.8^{b}	3.1±0.1^{c}
Phosphatidic acid/cardiolipin	8.7±2.8^{a}	3.2± 0.4^{b}	1.1±0.3^{b}	1.0±0.1^{b}
Cerebrosides/sulfatides	1.0±0.1^{a}	0.8± 0.1^{a}	1.7±0.1^{b}	1.6±0.0^{c}
Sphingomyelin	2.2±0.3^{a}	1.2± 0.3^{b}	0.7±0.0^{b}	1.4±0.2^{ab}
Total neutral lipids	20.4±6.9^{a}	60.9± 4.2^{b}	29.2±4.9^{a}	19.3±4.8^{a}
Monoacylglycerol	2.9±0.8	1.8± 1.4	1.7±0.4	1.3±0.5
Diacylglycerol	2.7±0.5	6.0± 3.4	4.9±0.9	2.9±1.3
Free fatty acid	0.2±0.0	0.2± 0.0	0.2±0.0	0.3±0.1
Triacylglycerol	12.4±7.7	47.8±15.6	19.3±6.2	11.9±9.1
Steryl esters	2.7±0.9	5.1± 4.6	3.1±0.6	2.9±1.4

Recovery in total lipid is presented as the percentage of injected radioactivity. Recoveries in lipid classes are percentages of the total radioactivity recovered in lipid. All data are means ± SD (n = 3). SD = 0.0 implies an SD < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $p < 0.05$. If no superscript appears, values are not different.

lowed by triacylglycerol (10.7%) and steryl esters (6.4%).

Saturated fatty acids were predominant in CPL, PS, PI and steryl esters, whereas monoenes predominated in triacylglycerol and total PUFA predominated in total lipid, total polar lipids and EPL (Table 2). PUFA comprised 25.5% and 20.8% of the total fatty acids in triacylglycerol and steryl esters, respectively, whereas the glycerophospholipid classes contained higher proportions of PUFA than the neutral lipid classes. The proportions of n−3 PUFA were greater than those of n−6 PUFA and the levels of DHA were higher than those of EPA in all lipid classes studied (Table 2). Indeed, DHA was the predominant PUFA in total lipid, total polar lipids and all individual lipid classes other than PI in which AA was the predominant PUFA. The percentage of LA was greatest in triacylglycerol and exceeded that of LNA in all lipid classes with the exception of PS and steryl esters. In contrast, the percentages of EPA greatly exceeded those of AA in all lipid classes with the notable exception of PI (Table 2).

The percentage of radioactivity recovered in body total lipid from injected ^{14}C-labelled AA, at over 20%, was significantly greater compared with the percentages of radioactivity recovered from injected ^{14}C-LA, LNA and EPA (Table 3). In all cases, less than 0.4% of the total radioactivity recovered in the body lipids was present as free fatty acids, the chemical form in which the isotopes were injected. The recovery of radioactivity in total polar lipids from injected ^{14}C-labelled LNA was significantly lower than the recoveries of ^{14}C-LA, AA and EPA, with no significant difference between the recoveries of radioactivity in polar lipids from the latter three labelled fatty acids. In polar lipids, the radioactivity from all four isotopes was predominantly incorporated into CPL (25.4% to 54.4% of total radioactivity incorporated), generally followed by the incorporation into EPL (6.0% to 15.6%) (Table 3). However, with ^{14}C-AA, PI (13.6%) and EPL (13.4%) were equally labelled. The incorporation of radioactivity into CPL was

Table 4. Metabolism of injected ^{14}C-polyunsaturated fatty acids *via* the desaturase/elongase pathway in juvenile golden grey mullet

	^{14}C-polyunsaturated fatty acid injected			
Fatty acid fraction	18:2(n−6)	18:3(n−3)	20:4(n−6)	20:5(n−3)
18:2(n−6)	83.3±1.1	–	–	–
18:3(n−6)	3.3±0.0	–	–	–
18:3(n−3)	–	73.3±0.5	–	–
18:4(n−3)	–	2.1±0.0	–	–
20:2(n−6)	7.4±0.9	–	–	–
20:3(n−6)	2.9±0.0	–	–	–
20:3(n−3)	–	9.7±1.9	–	–
20:4(n−6)	0.4±0.0	–	83.8±0.2	–
20:4(n−3)	–	4.7±1.5	–	–
20:5(n−3)	–	3.8±0.1	–	61.0±1.2
22:2(n−6)	1.9±0.1	–	–	–
22:3(n−3)	–	2.9±0.0	–	–
22:4(n−6)	0.4±0.0	–	10.1±0.1	–
22:5(n−6)	0.4±0.0	–	6.1±0.1	–
22:5(n−3)	–	1.7±0.1	–	26.3±1.0
22:6(n−3)	–	1.8±0.1	–	8.2±0.3
24:5(n−3)	–	tr	–	2.2±0.1
24:6(n−3)	–	tr	–	2.3±0.0

Values represent the radioactivity found in each fatty acid fraction expressed as a percentage of total radioactivity recovered and are means ± SD (n = 3). SD = 0.0 implies an SD < 0.05

significantly greater in fish injected either with ^{14}C-LA or EPA (53.1% and 54.4% of total radioactivity incorporated, respectively) than in fish which had been injected with ^{14}C-LNA or AA (25.4% and 35.7%). However, the incorporation of radioactivity from both ^{14}C-labelled C_{20} PUFA into EPL and PS was greater than the incorporation of radioactivity from either ^{14}C-labelled C_{18} PUFA into these glycerophospholipids (Table 3). The incorporation of radioactivity into sphingomyelin and cerebrosides/sulfatides was probably due to the combined effects of oxidation of the injected labelled PUFA and *de novo* fatty acid synthesis utilizing the resultant labelled acetyl-CoA.

Table 4 shows the percentage distribution of radioactivity from injected ^{14}C-PUFA in specific fatty acid fractions from total body lipids from mullet 48 h after injection. With ^{14}C-LA, 83.3% of the radioactivity present in total lipid fatty acids was recovered as the LA moiety itself. Only 16.7% was recovered in all other fatty acid fractions and just 7.4% as more unsaturated fatty acids. In comparison, significantly less of the injected ^{14}C-LNA was recovered unmetabolized (73.3%), although the percentage metabolized by desaturase activities was still relatively small (14.1%), with 3.8% recovered in the EPA fraction and only 1.8% recovered as DHA (Table 4). With ^{14}C-AA, 83.8% of the recovered radioactivity was located in the AA fraction, with 10.1% in 22:4n−6 and 6.1% in 22:5n−6 (Table 4). As with the (n−3) and (n−6) C_{18} PUFA, significantly less of the radioactivity from the injected ^{14}C-labelled EPA was recovered unmetabolized (61.0%) compared to ^{14}C-AA, with 26.3% recovered in 22:5n−3, 8.2% recovered in DHA and a total of 4.5% of radioactivity recovered in C_{24} PUFA (Table 4).

Discussion

The wild-caught juvenile golden grey mullet used in the present study contained less neutral lipid, after starvation for one week, than cultured juvenile turbot (Linares and Henderson 1991) or cultured juvenile gilthead sea bream (Mourente and Tocher

1993a) under similar experimental conditions. The triacylglycerol/steryl ester ratio was also much lower in the wild caught mullet than in the cultured turbot or sea bream, both indices perhaps indicating a poorer nutritional condition for the wild mullet (Fraser *et al.* 1987).

The polar lipid class composition of mullet was similar to compositions previously reported for other fish species with CPL and EPL accounting for approximately 45% and 25% of total polar lipids, respectively, with between 8% and 5% each of PS, PI, cardiolipin/phosphatidic acid and sphingomyelin (Henderson and Tocher 1987; Linares and Henderson 1991; Mourente and Tocher 1993a). The distribution of the individual fatty acids between the separate glycerophospholipid classes in mullet, with high 16:0 and relatively low PUFA in CPL, high PUFA in EPL, high 18:0 and PUFA, predominantly C_{22} PUFA, in PS and high 18:0 and C_{20} PUFA, particularly AA, in PI, was similar to that noted previously for whole turbot (Linares and Henderson 1991) and gilthead sea bream (Mourente *et al.* 1992; Mourente and Tocher 1993a), turbot (Mourente *et al.* 1991; Mourente and Tocher 1992a) and sea bream brain lipids (Mourente *et al.* 1992; Mourente and Tocher 1993b) and in several previous studies using a variety of fish tissues or cells (Bell *et al.* 1983; Tocher and Sargent 1984; Tocher and Harvie 1988; Linares and Henderson 1991; Mourente *et al.* 1991; Mourente and Tocher 1992b). However, the proportions of PUFA in the different glycerophospholipid classes were lower than those found in cultured juveniles of turbot and gilthead sea bream (Linares and Henderson 1991; Mourente and Tocher 1993a). The neutral lipid classes, particularly triacylglycerol, from the grey mullet were characterized by lower levels of PUFA and higher percentages of monoenes, compared to the polar lipid classes, as found previously in fish tissues (Tocher and Sargent 1984; Henderson and Tocher 1987). Therefore the lipid class and fatty acid compositions of juvenile golden grey mullet in the present study were generally similar to previous data from several fish species and tissues.

Values for the percentage incorporation of radioactivity in mullet are similar to those found for eel, *Anguilla japonica*, (Kanazawa *et al.* 1979), turbot (Linares and Henderson 1991) and gilthead sea bream (Mourente and Tocher 1993a). Similarly, the values obtained for the relative incorporation of ^{14}C-PUFA into total polar and neutral lipids in the present study with mullet were generally comparable with the values reported for the turbot (Linares and Henderson 1991) and gilthead sea bream (Mourente and Tocher 1993a). In the turbot, the incorporation of all four PUFA, particularly C_{20} PUFA, was predominantly into polar lipid classes (Linares and Henderson 1991), but with sea bream, although the C_{20} PUFA were incorporated primarily into polar lipids, greater percentages of C_{18} PUFA were incorporated into neutral lipid classes (Mourente and Tocher 1993a). With mullet, there was a similar pattern, with generally greater incorporation of C_{20} PUFA into polar lipids, except that there was a very high incorporation of ^{14}C-LA into polar lipids. In the marine fish species studied by Kanazawa *et al.* (1979), there was no consistent pattern for the distribution of radioactivity between neural and polar lipids, whereas, in the freshwater species studied, the incorporation into the neutral lipids always exceeded that into polar lipids (Kanazawa *et al.* 1979). It was shown recently that the relative distribution of injected ^{14}C-PUFA between neutral and polar lipids was highly dependent upon the specific tissue analyzed (Olsen *et al.* 1990). However, LA is present at high levels in cyanobacteria, green and brown algae and plants (Harwood and Russell 1984; Kayama *et al.* 1989), possibly common foods of grey mullet at the juvenile stage in the wild (Albertini-Berhaut 1973; Albertini-Berhaut 1974; Oren 1981). Thus, the selectivity of the incorporation of ^{14}C-LA into polar lipids in mullet, in comparison with the incorporation of ^{14}C-LA into polar lipids in turbot and sea bream, may be influenced by the normal dietary input of a herbivorous fish. Juvenile turbot or gilthead sea bream are carnivorous fish (Jones 1970; Colman 1972), and so may encounter far lower levels of LA in their natural diet (Sargent *et al.* 1989), perhaps explaining the differences in LA incorporation. The selective incorporation of specific PUFA into particular glycerophospholipi classes implies specificity in the enzyme mechanisms involved in *de novo* synthesis and/or the deacylation-reacylation reac-

tions of glycerophospholipid turnover, including CDP-diacylglycerol phosphotransferases and lysophospholipid acyltransferases (Bell *et al.* 1986).

The specific incorporation and retention of AA in PI in mullet is in agreement with previous analytical studies of both marine (Bell *et al.* 1983; Tocher and Sargent 1984) and freshwater fish tissues (Henderson and Tocher 1987), *in vivo* incorporation studies including turbot (Linares and Henderson 1991) and gilthead sea bream (Mourente and Tocher 1993a) and *in vitro* studies on plaice (*Pleuronectes platessa*) neutrophils (Tocher and Sargent 1986), rainbow trout astrocytes (Tocher and Sargent 1990a) and mixed brain cells (Tocher *et al.* 1991), and established cell lines from various fish species (Tocher 1990; Tocher and Dick 1990; Tocher and Mackinlay 1990). In mammals, AA is the precursor of a wide range of highly biologically active derivatives collectively termed eicosanoids (Smith 1989). The weight of evidence indicates that AA is the predominant eicosanoid precursor in both marine and freshwater fish, despite the preponderance of EPA in fish tissues (Henderson *et al.* 1985; Tocher and Sargent 1987; Henderson and Tocher 1987; Tocher *et al.* 1991). Although no direct evidence exists, it has been proposed that PI may, in come way, play a role in the provision of eicosanoid precursor in n − 3 PUFA-rich tissues or species (Bell *et al.* 1983; Tocher and Sargent 1984).

In the present study, radioactivity from injected ^{14}C-LA and LNA was recovered mainly in CPL, EPL and triacylglycerol in grey mullet, although these two C_{18} PUFA were not major components of any lipid class. Radioactivity from injected ^{14}C-LA and LNA was also extensively esterified into lipids in juvenile turbot (Linares and Henderson 1991) and gilthead sea bream (Mourente and Tocher 1993a). The percentages of radioactivity recovered in more unsaturated fatty acids in total lipids of grey mullet injected with ^{14}C-LA or ^{14}C-LNA were 7.4% and 14.1%, respectively. This indicates that the conversion of dietary C_{18} PUFA to HUFA in grey mullet is relatively low. However, all the desaturases in grey mullet showed greater activity towards n − 3 PUFA precursors compared with the equivalent n − 6 PUFA precursors. The Δ6-desaturase showed the highest level of activity with 7.4% of ^{14}C-LA and 14.1% of ^{14}C-LNA being desaturated. In the case of the Δ4-desaturase, 6.1% of injected ^{14}C-AA was desaturated to 22:5n − 6, whereas 10.5% of injected ^{14}C-EPA was desaturated to DHA and 24:6n − 3. The Δ5-desaturase activities were the lowest, with only 1.2% and 7.3% of Δ5 products obtained from ^{14}C-LA and ^{14}C-LNA, respectively. The levels of Δ5- and Δ4-desaturase activities towards n − 3 substrates were higher in mullet than those found in sea bream where 5.5% of injected ^{14}C-LNA and 6.6% of injected ^{14}C-EPA were desaturated (Mourente and Tocher 1993a). The levels of these activities in turbot appear to be higher than in both sea bream or mullet although incomplete resolution of fatty acid fractions in the turbot data makes direct comparison difficult (Linares and Henderson 1991). These data indicate that juvenile grey mullet fit the pattern suggested for marine fish with low levels of Δ5-desaturase activity necessitating the provision of n − 3 HUFA in the diet, despite this species being more herbivorous compared to other marine fish studied. The presence of radioactivity in C_{24} HUFA in fish injected with labelled EPA was also noted in sea bream and is consistent with the hypothesis, postulated by Voss *et al.* (1991), that desaturation of 22:5n − 3 in rat liver is achieved by elongation to 24:5n − 3 followed by Δ6-desaturation to 24:6n − 3 and limited peroxisomal β-oxidation to DHA.

Fish usually have to be starved for a considerable period of time in order to substantially deplete neutral lipid levels. In the present study, wild-caught grey mullet, starved for one week, had lower tissues reserves of $C_{20/22}$ HUFA in body neutral lipid stores (triacylglycerols and steryl esters) than found in previous experiments with cultured turbot or gilthead sea bream (Linares and Henderson 1991; Mourente and Tocher 1993a). It is unlikely that $C_{20/22}$ HUFA in neutral lipid would significantly affect Δ6-, Δ5- and Δ4-desaturase activities after a 7 day fast (Brenner 1981). However, in a previous study with Arctic charr (*Salvelinus alpinus*), the rates of bioconversion of C_{18} and C_{20} PUFA *via* the desaturase/elongase pathway were lower when measured in fed fish compared with starved fish (Olsen and Ringo 1992).

In conclusion, the results of the present study, investigating the metabolism of labelled PUFA *in vivo*, indicate that juvenile golden grey mullet have only limited ability to convert C_{18} PUFA to $C_{20/22}$ HUFA. Therefore, the results suggest that grey mullet require the provision of preformed EPA, and probably DHA, in the diet. The primary impairment in the desaturase/elongase pathway was at the Δ5-desaturase, although the Δ4-desaturase activity was also relatively low. The defect in Δ5-desaturase activity combined with the finding that AA is selectively incorporated and retained in membrane PI suggests that, in addition to EPA and DHA, grey mullet may also have a requirement for preformed AA in the diet. The impairment in Δ5-desaturase activity was similar to that found in turbot both *in vivo* (Henderson and Linares 1991) and *in vitro* (Tocher *et al.* 1989; Tocher and Mackinley 1990) and in juvenile gilthead sea bream *in vivo* (Mourente and Tocher 1993a). In addition, golden grey mullet are euryhaline, eurythermic (tending to warm waters), resistant to oxygen deficiencies and catadromous (Bograd 1961; Oren 1981), similar to gilthead sea bream (Chervinski 1984; Mourente and Tocher 1992b). However, grey mullet are herbivorous at the juvenile stage (Albertini-Berhaut 1973; Albertini-Berhaut 1974; Oren 1981). Therefore, it is noteworthy that three marine fish species with various biological and ecological differences, including different feeding habits, displayed the same pattern of PUFA metabolism. The evidence is consistent with the hypothesis that low Δ5-desaturase activity is a general characteristic of marine fish which prevails in spite of habitat, behaviour and feeding habit (carnivorous/herbivorous).

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