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The *in vivo* incorporation and metabolism of [1-^{14}C] linolenate (18:3n-3) in liver, brain and eyes of juveniles of rainbow trout *Oncorhynchus mykiss* L and gilthead sea bream *Sparus aurata* L.

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Abstract

Accumulation of docosahexaenoic acid (DHA; 22:6n-3) in brain and eyes during development has been demonstrated in fish but it is not clear whether liver or neural tissues themselves are of greater importance in the biosynthesis of DHA from dietary 18:3n-3. In the present study, we investigated the *in vivo* metabolism of intraperitoneally injected [1-^{14}C]18:3n-3 in liver, brains and eyes of young juvenile fish. Metabolism was followed over a 48h time-course in order to obtain dynamic information that could aid the elucidation of the roles of the different tissues in the biosynthesis and provision of DHA from dietary 18:3n-3. The study was performed in both a freshwater fish, rainbow trout *Oncorhynchus mykiss* L and a marine fish, gilthead sea bream *Sparus aurata* L to determine the effect that low or limiting Δ5-desaturase activity may have in this process. As expected, the results showed that although the sea bream incorporated more 18:3n-3 into its lipids, metabolism of the incorporated fatty acid by desaturation and elongation was generally greater in the trout. In liver, the percentages of radioactivity recovered in tetraene and pentaene products were greater in trout than in sea bream although there was no difference in hexaenes. In contrast, the recovery of radioactivity in DHA was significantly greater in brain in trout compared to sea bream. In both species, the percentage of radioactivity recovered in desaturated/elongated products was much lower in liver than in brains and eyes, but that percentage increased over the 48h time-course. In trout though, the highest percentages of desaturated products in brain and eye were observed after 12 and 24h, respectively. However in sea bream the highest percentages of desaturated products in the neural tissues were observed after 24-48h. Radioactivity was recovered in 24:5n-3 and 24:6n-3, intermediates in the Δ4-independent ("Sprecher shunt") pathway for the synthesis of DHA, in both species, especially in the brain and eyes. Overall, although the results cannot eliminate a role for liver in the biosynthesis and provision of DHA for developing neural tissues in fish, they suggest that DHA can be synthesised in fish brain and eye *in vivo*.

Abbreviations: ANOVA, analysis of variance; BHT, butylated hydroxytoluene; Chol, cholesterol; CL, cardiolipin; DHA, 4,7,10,13,16,19-docosahexaenoic acid (22:6n-3); EPA, 5,8,11,14,17-eicosapentaenoic acid (20:5n-3); FFA, free fatty acid; HUFA, highly unsaturated fatty acids ($\geq C_{20}$ with ≥3 double bonds); PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SE, steryl esters; SM, sphingomyelin; Sulf/Cer, sulfatides/cerebrosides; TAG, triacylglycerol; TNL, total neutral lipids; TPL, total polar lipids.

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Introduction

A critical functional role for n-3 polyunsaturated fatty acids (PUFA), specifically docosahexaenoic acid (DHA; 22:6n-3), in neural tissues has been established in mammals (Connor et al. 1992). DHA is specifically accumulated by mammalian brain during development (Sinclair and Crawford 1972) and nutritional depletion of DHA in brain and retinal membranes is accompanied by functional physiological defects such as reduced visual acuity and impaired learning abilities (Neuringer et al. 1994). *In vivo* production of DHA from 18:3n-3 has been demonstrated in rat brain (Dhopeshwarkar and Subramanian 1976) but other evidence suggests that much of the DHA in mammalian brains results from the accumulation of preformed DHA that may be obtained directly as maternal DHA or subsequent to metabolism of 18:3n-3 in the liver (Scott and Bazan 1989). Accumulation of DHA in brain and eyes during development has been demonstrated in turbot *Scophthalmus maximus* L. and herring *Clupea harengus* L. (Mourente et al. 1991; Mourente and Tocher 1992; Bell and Dick 1993) and nutritional deprivation of DHA has been shown to impair vision and, consequently, predation ability in juvenile herring (Sargent et al. 1993; Bell et al. 1995). However, it is uncertain if the diet of freshwater fish would normally contain sufficient preformed DHA for neural development as the level of DHA is generally low in the natural diet of freshwater fish (Bell et al. 1994). This may also be relevant in the early life stages of marine fish as, although the level of DHA in the natural diet of marine fish is much higher, there is a greatly increased requirement for DHA for proper development of neural tissue during larval stages which is believed to be potentially limiting, particularly in relation to their culture (Sargent et al. 1993). The relative importance of liver or neural tissues themselves in the biosynthesis of DHA from dietary 18:3n-3 is unclear in fish (Tocher et al. 1992).

Nutritional and biochemical studies have indicated that freshwater fish, or at least salmonid species, are capable of converting α-linolenic (18:3n-3) and linoleic (18:2n-6) acids to their highly unsaturated fatty acid (HUFA) derivatives, 20:5n-3/22:6n-3 and 20:4n-6/22:5n-6, respectively (Henderson and Tocher 1987; Sargent et al. 1989). In contrast, early studies showed that marine fish such as turbot appeared deficient in the desaturation and elongation of C_{18} PUFA and therefore require preformed HUFA in the diet (Owen et al. 1975). Evidence from studies on cultured cells confirmed that cells from marine fish were deficient in these conversions and further indicated that the deficiency was probably due to low and inadequate levels of Δ5-desaturase activity (Tocher et al. 1989; Tocher and Mackinlay 1990; Tocher and Sargent 1990a). The ability of several fish species to biosynthesize C_{20} and C_{22} HUFA *in vivo* has been determined in a number of studies using intraperitoneally injected radioactively-labelled C_{18} PUFA (Parker et al. 1979; Olsen et al. 1990; Linares and Henderson 1991; Olsen and Ringo 1992; Mourente and Tocher 1993a,b, 1994). The results of those studies largely confirmed that there was a fundamental difference between freshwater and marine fish in their ability to convert C_{18} PUFA to $C_{20/22}$ HUFA with the freshwater species studied having a significantly greater capacity for these conversions.

In our previous *in vivo* injection studies with gilthead sea bream *Sparus aurata* L. we showed that although the metabolism of [1-^{14}C]18:3n-3 to HUFA was low, there was not a total block at Δ5 desaturase and radioactivity was recovered in eicosapentaenoic acid (EPA; 20:5n-3) and other pentaenes such as 22:5 and 24:5 and small amounts were also recovered in DHA and 24:6n-3 (Mourente and Tocher 1993a,1994). The recovery of radioactivity in 24:5n-3 and 24:6n-3, particularly evident when [1-^{14}C]20:5n-3 was injected, was noteworthy as it was consistent with the presence in fish of the "Sprecher shunt" for the conversion of EPA to DHA in which EPA is elongated to 22:5n-3 and 24:5n-3 before being desaturated by a Δ6-desaturase in microsomes to 24:6n-3 with subsequent limited chain shortening to DHA, presumably in peroxisomes (Sprecher et al. 1995).

In vivo studies on PUFA metabolism in fish have concentrated on the use of small, juveniles partly due to the fact that they are rapidly growing and therefore envisaged as being highly metabolically active. However practical consid-

erations such as the high cost of isotopes, the problems associated with high doses of radioactivity (containment of ^{14}C-labelled CO_2 etc.) particularly in aqueous systems and the great dilution of isotope in *in vivo* studies, are also relevant. As a result, many of the previous *in vivo* studies utilizing intra-peritoneal injections of radiolabeled fatty acids have only analysed the whole fish (Linares and Henderson 1991; Olsen and Ringo 1992; Mourente and Tocher 1993a,b, 1994) or liver (Parker et al. 1979; Olsen et al. 1990; Henderson et al. 1995). The *in vivo* metabolism of PUFA has not been studied in neural tissues such as brains or retina in fish.

In the present study, we have investigated the *in vivo* metabolism of intraperitoneally injected [1-^{14}C]18:3n-3 in liver, brains and eyes of young juvenile fish. In particular, we were interested in the accretion of DHA in neural tissues and so the metabolism was followed over a 48h time-course in order to obtain dynamic information that could perhaps elucidate the roles of the different tissues, i.e. liver and neural tissues themselves, in the biosynthesis and provision of DHA from dietary 18:3n-3. The study was performed in both a freshwater fish, rainbow trout, *Oncorhynchus mykiss* L. and a marine fish, gilthead sea bream to determine the effect that low or limiting Δ5-desaturase activity may have in this process.

Materials and methods

Experimental animals

Juvenile rainbow trout, *Oncorhynchus mykiss*, of approximately 3–5 g, were obtained from Piscifactoría de Benamahoma, Benamahoma, Cádiz, Spain and maintained for 1 week in the aquarium at the University of Cádiz in a 50 l rectangular glass-reinforced plastic (GRP) tank containing fresh water (temperature 20 ± 1 °C), obtained at the fish farm, which was kept well aerated and changed every two days prior to the experiment. Fish were not fed during this holding period. Juvenile gilthead sea bream, *Sparus aurata*, of 2–4 g, reared on a commercial diet, were obtained from a commercial fish farm (Cultivos Piscícolas Marinos S.A., San Fernando, Cádiz, Spain). The sea bream were maintained in a 50 l rectangular GRP tank, supplied with underground sea water (salinity 32 g l^{-1} and temperature 20 ± 1 °C) in an open circuit system with aeration. The sea bream were fed a commercial dry pelleted diet in a daily ration of 5% of total body weight until one week prior to experimentation during which time they were not fed.

Injection of radioactive fatty acids and experimental conditions

The fish were injected with radioactive isotopes and the experiment performed as described in detail previously (Mourente and Tocher 1993a,b & 1994). In the present study, twelve groups of three fish of each species were starved during the course of the experiment after injection, to ensure no dietary input of C_{20} and C_{22} HUFA and to maximise the rate of bioconversion of the injected [1-^{14}C]18:3n-3. Fish were anaesthetized by hypothermic shock (Summerfelt and Smith 1990), whereby the fish were placed in a beaker containing a 1:1 mixture of tank water and crushed ice for 1–2 min until immobilized, as it had been shown that some commonly used fish anaesthetics may interfere in fatty acid metabolism (Harrington et al. 1991). All twelve groups of three fish of each species were injected into the peritoneal cavity with 3 μCi of [1-^{14}C]18:3n-3 in 5 μl of ethanol. Separate groups of trout and sea bream were injected with 5 μl of ethanol alone to act as sham injection controls. No mortalities occurred during starvation or during the period after the fish were injected with radioactive 18:3n-3. After injection, the separate groups of fish were maintained in individual cylindrical glass aquaria containing 2 l of fresh water (trout) or sea water (bream) with aeration. Fish recovered from anaesthesia and injection within 5 min with no apparent detrimental effects. One group of three fish of each species was removed from the aquaria at 6, 12, 24 and 48 h after injection, carefully blotted with filter paper, killed by sectioning the spinal cord posterior to the brain, and the length and wet weight of the fish determined. Livers, brains and eyes were quickly dissected into ice-cold chloroform/methanol for immediate lipid analysis as described below. The same tissues were also dissected from the sham injected

control fish and the wet weight determined. A portion of the organs from the sham-injected fish were desiccated at 110 °C for 24 h, cooled *in vacuo*, and dry weights determined. The remaining portion of organs from the sham-injected fish were used for determination of lipid contents and lipid class and fatty acid compositions as described below.

Lipid extraction

Organs were homogenized in ice-cold chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene and lipid extracted essentially according to the procedure of Folch et al. (1957) as detailed previously (Tocher and Harvie 1988). The lower solvent phase was evaporated under a stream of nitrogen and lipid extracts desiccated overnight *in vacuo* before weighing. Lipid extracts were redissolved in chloroform/methanol as above at a concentration of 50 $mg.ml^{-1}$ and stored under an atmosphere of nitrogen at -20 °C until analysis. An aliquot of each extract obtained from ^{14}C-injected fish was placed in scintillation mini-vials and the solvent evaporated under a stream of nitrogen. Four ml of liquid scintillation cocktail (Ready Safe, Beckman) were 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.

Lipid class composition

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-9001PC dual wavelength flying spot scanner (Olsen and Henderson 1989).

Fatty acid composition

Fatty acid methyl esters (FAME) from total lipids were prepared by acid-catalyzed transmethylation for 16 h at 50 °C, using nonadecanoic acid (19:0) as internal standard (Christie 1989). FAME and dimethyl acetals, produced by the methylation of the alk-1-enyl chains of plasmalogen lipids, were extracted and purified as described previously (Tocher and Harvie 1988). FAME were analyzed in a Hewlett-Packard 5890A Series II gas chromatograph equipped with a chemically-bonded (PEG) Omegawax 320 fused-silica wall-coated capillary column (30m × 0.32 mm i.d.) (Supelco, Bellefonte, U.S.A.) and an on-column injector. Hydrogen was used as carrier gas with a thermal gradient from an initial 50 to 180 °C at 25 $°C.min^{-1}$ and then to a final temperature of 235 °C at 3 °C min^{-1}. Individual FAME were identified by reference to authentic standards and a well-characterized fish oil and were quantified using a PC linked to the gas chromatograph utilizing Hewlett-Packard 3365 ChemStation software. All solvents contained 0.01% BHT as an antioxidant.

Incorporation of radioactivity into PUFA

FAME were prepared and purified from total lipid as described above. Methyl esters were separated by argentation-thin-layer chromatography (TLC), using TLC plates impregnated by spraying with a solution of 2 g silver nitrate in 20 ml acetonitrile followed by activation at 110 °C for 30 min. The plates were fully developed in toluene/acetonitrile (95:5, v/v) which resolves the FAME 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 described above. Identification of labelled bands was confirmed by using authentic unlabelled standards run on parallel plates, with visualization by charring.

Materials

[1-^{14}C]18:3n-3 (approximately 50 mCi $mmol^{-1}$ and 98% pure) was obtained from NEN DuPont Investigación Técnica Industrial S.A.(ITISA), Madrid, Spain. BHT and silver nitrate were ob-

tained from Sigma Chemical Co. Ltd. (Poole, Dorset, U.K.). TLC (20 × 20 cm × 0.25 mm) and HPTLC (10 × 10 cm × 0.15 mm) plates, precoated with silica gel 60 (without fluorescent indicator), were obtained from Merck (Darmstadt, Germany). All solvents were high-performance liquid chromatography grade and were obtained from Fluka (Glossop, Derbyshire, U.K.).

Statistical analysis

All results are presented as means ± SD (n=3) unless otherwise stated. The data were checked for homogeneity of the variances by the Bartlett test and, where necessary, the data were arcsin-transformed before further statistical analysis. Biometric data for trout and sea bream were analyzed by the Students t-test. Tissue lipid class and fatty acid composition data (Tables 2 and 3) were analyzed by two-way analysis of variance (ANOVA) (species and tissue) followed, where appropriate, by Tukey's multiple comparison test. The incorporation and metabolism of isotope data (Tables 5-7) were analysed by two-way ANOVA (species and time after injection) followed, where appropriate, by Tukey's multiple comparison test. The data in Tables 5-7 for each species were also individually analysed by one-way ANOVA (time after injection) followed, where appropriate, by Tukey's multiple comparison test. In all cases, differences between means were reported as significant if $p < 0.05$.

Results

Fish of approximately the same size and stage of development (young juveniles) were used in this experiment and the biometric data showed that the trout and sea bream were very similar other than the trout having significantly larger livers (Table 1). The lipid class compositions of the organs showed that there was no difference between the species in the proportions of total polar and total neutral lipids (Tables 2,4). However there were some differences between trout and sea bream in the relative proportions of individual lipid classes, particularly the neutral classes. In contrast, and as expected, there were a great many differences between the lipid class compositions of the different organs with high total polar lipid, phosphatidylethanolamine (PE), phosphatidylserine (PS) and cholesterol, and low triacylglycerol (TAG) being characteristic of brain (Tables 2,4). Indeed, it was noteworthy that the brains of the trout and sea bream were very similar both in lipid content and lipid class composition. In contrast, trout eye and sea bream liver were rich in TAG and total neutral lipid. The fatty acid compositions of the same organs were generally very similar in the trout and sea bream other than perhaps higher 18:2n-6, 20:5n-3, total (n-6)PUFA, total (n-3)PUFA, and total PUFA in trout (Tables 3,4). There were generally more organ/tissue specific differences in the fatty acid compositions but, as with lipid class compositions, these were highly dependent upon species as indicated by the significant interactions in two-way analysis of variance (ANOVA) which made generalisations difficult (Tables 3,4).

The amount of radioactivity recovered in the total lipid from liver decreased with time after injection, significantly so in the case of trout, when analysed by one-way analysis of variance (ANOVA) (Table 5). Similarly, the amount of radioactivity recovered in the total lipid from brain and eye also decreased with time after injection, again significantly in the case of trout,

Table 1. Biometric data (length and wet weight) and weights of organs of rainbow trout and sea bream

Parameter	Trout	Sea bream
Length of fish (cm)	7.7 ± 0.8	6.4 ± 0.3
Wet weight of fish (g)	4.6 ± 1.8	2.9 ± 0.6
Brain – wet wt. (mg)	44.0 ± 12.0	40.9 ± 3.4
– dry wt. (mg)	9.3 ± 2.4	8.7 ± 1.8
– dry wt. (%)	21.2 ± 0.7	21.2 ± 2.7
Liver – wet wt. (mg)	87.8 ± 6.7	26.7 ± 3.5*
– dry wt. (mg)	24.0 ± 1.2	7.6 ± 1.7*
– dry wt. (%)	27.3 ± 1.2	28.0 ± 2.6
Eye – wet wt. (mg)	119.4 ± 44.2	96.4 ± 9.9
– dry wt. (mg)	21.9 ± 9.0	15.0 ± 3.0
– dry wt. (%)	18.1 ± 0.8	15.4 ± 1.4*

Data are means ± SD (n = 6 for trout, n = 10 for sea bream). Differences between the two groups of means were significant ($p < 0.05$) where indicated (*).

Table 2. Total lipid contents (% of dry weight) and lipid class compositions (percentage of total lipid) of organs from rainbow trout and sea bream

	Rainbow trout			Sea bream		
Lipid	Brain	Liver	Eye	Brain	Liver	Eye
Total lipid	29.8 ± 2.7	21.8 ± 2.4	19.7 ± 2.5	31.1 ± 2.3	35.0 ± 3.0	9.8 ± 0.6
PC	22.9 ± 0.5	24.6 ± 0.6	16.7 ± 0.9	20.4 ± 0.4	17.1 ± 0.2	21.1 ± 2.1
PE	22.0 ± 1.4	10.6 ± 0.1	11.6 ± 1.6	22.6 ± 0.5	9.4 ± 0.3	16.2 ± 1.0
PS	8.8 ± 0.7	5.0 ± 0.5	3.5 ± 1.1	10.3 ± 0.3	3.2 ± 0.3	6.4 ± 1.1
PI	2.2 ± 0.1	5.3 ± 0.4	1.9 ± 0.3	2.5 ± 0.1	3.7 ± 0.5	2.7 ± 0.1
PA/CL	2.3 ± 0.0	3.4 ± 0.1	1.8 ± 0.3	3.0 ± 0.1	4.1 ± 0.2	2.5 ± 0.3
SM	1.5 ± 0.0	2.4 ± 0.3	1.4 ± 0.1	1.2 ± 0.0	2.0 ± 0.1	2.7 ± 0.0
Sul/Cer	5.8 ± 0.9	1.0 ± 0.2	0.7 ± 0.2	6.2 ± 0.8	1.2 ± 1.0	2.7 ± 0.5
TPL	65.5 ± 3.1	52.3 ± 1.3	37.6 ± 3.2	66.2 ± 1.2	40.7 ± 0.2	54.3 ± 4.0
Chol	20.3 ± 0.7	11.0 ± 0.3	11.2 ± 1.4	23.7 ± 1.7	11.9 ± 0.3	19.2 ± 1.2
TAG	7.2 ± 4.1	23.1 ± 3.3	46.1 ± 4.0	2.7 ± 1.1	34.9 ± 1.0	15.0 ± 0.3
SE	2.8 ± 0.2	11.2 ± 3.3	3.7 ± 0.5	4.9 ± 0.4	11.4 ± 0.3	9.8 ± 2.5
FFA	4.2 ± 0.3	2.1 ± 0.8	1.4 ± 0.1	2.5 ± 0.5	1.1 ± 0.2	1.7 ± 0.0
TNL	34.5 ± 3.1	47.7 ± 1.3	62.4 ± 3.2	33.8 ± 1.2	59.3 ± 0.2	45.7 ± 4.0

Results are means ± SD (n = 8 for trout and n =3 for sea bream); Chol, cholesterol; CL, cardiolipin; FFA, free fatty acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SE, steryl ester; SM, sphingomyelin; Sul/Cer, Sulfatides/cerebrosides; TAG, triacylglycerol; TNL, total neutral lipids; TPL, total polar lipids.

when analysed by one-way ANOVA (Tables 6,7). Two-way ANOVA showed that there was significantly more radioactivity from [1-^{14}C]18:3n-3 incorporated into the total lipid of all organs in sea bream compared to trout (Table 8). However, because the levels of radioactivity recovered in tissues from sea bream were much greater than those from trout, the statistically non-significant sea bream data carried proportionally more weight in two-way ANOVA (where the combined trout and sea bream data at each time point is compared over the time course), and so the effect of time after injection was only significant in two-way ANOVA with brain (Table 8). The two-way ANOVA was similarly affected when looking at the effect of time after injection on the proportions of radioactivity recovered in individual fatty acid fractions. The combined trout and sea bream data are compared at each time point and the quantitative differences between trout and sea bream data at each point were often greater than the differences due to time after injection and so two-way ANOVA was not ideal for looking at the effect of time after injection. For this reason, two-way ANOVA was supported by one-way ANOVA specifically for the effects of time after injection in each idividual species.

The percentage of radioactivity recovered unmetabolized as 18:3n-3 was significantly lower in trout liver than in sea bream liver and decreased with time (Tables 5,8). The percentages of radioactivity recovered as elongated and desaturated products tended to increase with increasing time after injection, particularly 20:3, 20:4, 24:5 and

Table 3. Fatty acid compositions (percentage of wt) of total lipid of organs from rainbow trout and sea bream

	Rainbow trout			Sea bream		
Fatty acid	Brain	Liver	Eye	Brain	Liver	Eye
14:0	3.7 ± 0.7	0.8 ± 0.0	1.8 ± 0.3	3.2 ± 0.4	6.7 ± 1.2	3.4 ± 0.4
16:0	18.2 ± 0.6	17.0 ± 0.5	15.6 ± 0.5	16.5 ± 0.7	14.5 ± 0.6	14.8 ± 0.8
18:0	9.2 ± 0.2	5.8 ± 0.0	6.0 ± 0.4	12.3 ± 0.3	5.9 ± 0.6	9.8 ± 0.1
Total sat	32.9 ± 0.9	28.7 ± 0.4	27.5 ± 0.3	34.4 ± 0.3	29.2 ± 1.3	30.3 ± 0.3
16:1(n-7)	2.5 ± 0.1	2.6 ± 0.2	4.7 ± 0.3	2.7 ± 0.1	3.3 ± 0.7	3.0 ± 0.2
18:1(n-9)	13.6 ± 0.5	9.7 ± 1.0	14.9 ± 0.5	14.2 ± 0.3	9.9 ± 1.6	11.5 ± 0.7
18:1(n-7)	2.5 ± 0.0	2.2 ± 0.1	3.1 ± 0.1	1.6 ± 0.2	2.1 ± 0.5	2.2 ± 0.2
20:1(n-9)	0.8 ± 0.1	0.9 ± 0.1	1.8 ± 0.1	0.7 ± 0.0	2.6 ± 1.1	1.3 ± 0.2
20:1(n-7)	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
22:1	0.6 ± 0.3	0.7 ± 0.1	1.4 ± 0.1	0.6 ± 0.0	1.8 ± 0.4	0.9 ± 0.2
24:1	2.6 ± 0.3	1.8 ± 0.6	0.7 ± 0.1	2.2 ± 0.2	1.1 ± 0.1	1.0 ± 0.0
Total mono	22.8 ± 0.9	18.1 ± 0.6	27.1 ± 0.9	22.1 ± 0.6	21.1 ± 4.6	20.1 ± 1.6
18:2(n-6)	0.9 ± 0.3	4.2 ± 0.6	6.4 ± 0.3	1.2 ± 0.3	3.1 ± 0.9	1.9 ± 0.3
20:2(n-6)	0.3 ± 0.2	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.0
20:3(n-6)	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0
20:4(n-6)	0.9 ± 0.0	2.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	1.9 ± 0.3	1.2 ± 0.0
22:5(n-6)	1.1 ± 0.3	0.8 ± 0.2	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.1
Total (n-6)	5.5 ± 0.6	10.6 ± 0.8	10.1 ± 0.2	4.0 ± 0.3	7.9 ± 0.4	5.0 ± 0.2
18:3(n-3)	0.1 ± 0.0	0.5 ± 0.0	0.8 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
18:4(n-3)	0.3 ± 0.1	0.4 ± 0.1	0.9 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
20:3(n-3)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:4(n-3)	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
20:5(n-3)	3.9 ± 0.1	5.1 ± 0.2	4.9 ± 0.2	2.7 ± 0.1	3.0 ± 0.1	2.7 ± 0.1
22:5(n-3)	2.0 ± 0.1	1.6 ± 0.1	1.6 ± 0.0	1.0 ± 0.1	1.4 ± 0.3	1.1 ± 0.0
22:6(n-3)	24.3 ± 0.3	28.1 ± 0.8	19.0 ± 1.9	23.8 ± 1.2	23.2 ± 3.9	26.3 ± 0.5
Total (n-3)	32.5 ± 0.5	37.5 ± 0.5	29.1 ± 1.7	30.0 ± 0.9	32.6 ± 3.5	33.5 ± 0.2
Total DMA	2.9 ± 0.2	nd	1.1 ± 0.1	3.6 ± 0.2	nd	3.6 ± 0.4
Total PUFA	38.1 ± 1.1	48.0 ± 0.3	39.3 ± 1.5	34.1 ± 0.6	40.5 ± 3.2	38.5 ± 0.0

Results are means ± SD (n = 3). n.d., none detected. DMA, dimethylacetals; PUFA, polyunsaturated fatty acids.

22:6, with the highest percentages in all products in liver found 48 h after injection. In addition, the percentages of radioactivity recovered as the elongation product, 20:3n-3, and all tetraene and pentaene products were greater in trout than in sea bream (Tables 5,8). All of the above differences were highly significant except there was no difference between sea bream and trout in the proportion of radioactivity recovered in 22:6 and 24:6 in the liver (Table 8).

The incorporation of [1-^{14}C]18:3n-3 in total lipid from brain of trout and sea bream was approximately 10- and 26-fold lower than the incorporation into liver 6 hours after injection (Table 6). However, the retention of radioactivity in the brain was relatively greater as the amount of radioactivity recovered in brain lipid was only 3- and 14-fold lower in trout and sea bream, respectively, after 48 h. In contrast to liver, there was no significant difference in the percentage of radioactivity recovered unmetabolized (i.e. as 18:3) in brain between sea bream and trout (Tables 6,8). Significantly higher percentages of radioactivity were recovered in 22:6 in trout brain whereas the amounts of radioactivity recovered in 18:4, 20:5, 24:5 and 24:6 were significantly greater in sea bream brain (Tables 6,8). There was a difference between the two species in the time course after injection, with peak values for most metabolites occurring 12h after injection in trout brain but after 24 or 48h in sea bream (Table 6). Interestingly, it was the percentages of

Table 4. Results (F-values) of two-way analysis of variance for the data in Tables 2 and 3

Data	Species	Organ	Interaction	Multiple range test
Table 2				
Total lipid	ns	77.2 [c]	35.4 [c]	brain,liver-eye
Phosphatidylcholine	ns	ns	ns	
Phosphatidylethanolamine	8.2 [a]	242.3 [c]	13.5 [c]	brain-liver-eye
Phosphatidylserine	ns	4.7 [a]	ns	brain-liver
Phosphatidylinositol	ns	107.1 [c]	27.2 [c]	brain,liver-eye
Phosphatidic acid/cardiolipin	55.1 [c]	100.5 [c]	ns	brain-liver-eye
sphingomyelin	9.8 [b]	67.4 [c]	74.4 [c]	brain-liver,eye
sulfatides/cerebrosides	7.3 [a]	92.5 [c]	ns	brain-liver,eye
Total polar lipid	ns	118.5 [c]	46.4 [c]	brain-liver,eye
Cholesterol	62.2 [c]	147.9 [c]	16.8 [c]	brain-liver-eye
Triacylglycerol	36.9 [c]	161.2 [c]	391.7 [c]	brain-liver,eye
Steryl ester	11.9 [b]	28.7 [c]	4.6 [a]	brain-liver-eye
Free fatty acid	16.8 [b]	36.7 [c]	9.0 [c]	brain-liver,eye
Total neutral lipid	ns	118.5 [c]	46.4 [c]	brain-liver,eye
Table 3				
14:0	5.5 [a]	62.8 [c]	40.9 [c]	brain,liver-eye
16:0	31.9 [c]	19.1 [c]	ns	brain-liver,eye
18:0	222.7 [c]	330.3 [c]	52.7 [c]	brain-liver-eye
Total sat	23.6 [c]	91.4 [c]	4.1 [a]	brain-liver,eye
16:1(n-7)	ns	22.0 [c]	21.2 [c]	brain,liver-eye
18:1(n-9)	ns	37.3	9.4 [b]	brain,eye-liver
18:1(n-7)	30.9 [c]	10.6 [b]	5.5 [a]	brain,liver-eye
20:1(n-9)	ns	18.8 [c]	31.8 [c]	brain-liver,eye
20:1(n-7)	ns	ns	11.4 [b]	
22:1	ns	14.2 [c]	19.4 [c]	brain-liver,eye
24:1	ns	42.3 [c]	4.5 [a]	brain-liver-eye
Total mono	ns	5.9 [a]	8.8 [b]	liver-eye
18:2(n-6)	55.1 [c]	65.2 [c]	35.8 [c]	brain-liver,eye
20:2(n-6)	ns	ns	ns	
20:3(n-6)	ns	8.6 [b]	ns	brain-liver
20:4(n-6)	9.1 [a]	75.8 [c]	9.6 [b]	brain-eye,liver
22:5(n-6)	44.9 [c]	ns	9.6 [b]	
Total (n-6)	195.1 [c]	139.8 [c]	22.7 [c]	brain-liver-eye
18:3(n-3)	ns	174.3 [c]	54.3 [c]	brain-liver-eye
18:4(n-3)	24.7 [c]	38.6 [c]	24.7 [c]	brain,liver-eye
20:4(n-3)	13.2 [b]	10.3 [b]	13.2 [c]	brain-liver,eye
20:5(n-3)	756.2 [c]	43.7 [c]	22.7 [c]	brain-liver-eye
22:5(n-3)	74.7 [c]	ns	11.1 [b]	
22:6(n-3)	ns	ns	16.2 [c]	
Total (n-3)	ns	ns	ns	
Total dimethylacetals	122.9 [c]	405.4	59.9 [c]	brain-liver-eye
Total polyunsaturated	32.1 [c]	43.6 [c]	7.1 [b]	brain-liver-eye

Significance levels are [a], $p < 0.05$; [b], $p < 0.001$; [c], $p < 0.001$; ns, not significant ($p > 0.05$). In the results for the multiple range test (Tukey HSD) for the differences between organs, where organs are separated by a dash the data are significantly different ($p < 0.05$). Where organs are separated by a comma, the data are not significant and if an organ is not shown it is not significantly different to either other organ.

22:5, 24:5 and 24:6, intermediates in the pathway between 20:5 and 22:6, which showed peak levels at 24h in sea bream. Similarly recovery of radioactivity as 24:6, the immediate precursor of 22:6, had its peak value at 6h, in trout brain. The differences between trout and sea bream were most clearly observed in brain compared to liver (and eye) as evinced by the fact that the interactions between species and time after injection were consistently significant indicating that much of

Table 5. Incorporation of radioactivity from injected [1-^{14}C]18:3n-3 into total lipid (nmol mg total lipid^{-1}) of liver from rainbow trout and sea bream and metabolism by desaturation/elongation at 6, 12, 24 and 48 h after injection

	Rainbow trout				Sea bream			
Time after injection (h)	6	12	24	48	6	12	24	48
Incorporation (nmol mg^{-1} TL)	0.8 ± 0.2^{a}	0.6 ± 0.1^{ab}	0.3 ± 0.1^{b}	0.1 ± 0.0^{c}	3.3 ± 2.5	2.3 ± 1.0	1.2 ± 1.1	0.8 ± 0.3
18:3(n-3)	88.3 ± 0.7^{a}	89.6 ± 1.4^{a}	82.9 ± 2.6^{b}	79.4 ± 2.4^{b}	94.8 ± 1.4	93.8 ± 1.5	93.7 ± 2.1	90.8 ± 4.3
20:3(n-3)	3.4 ± 0.3^{b}	2.5 ± 0.8^{b}	6.8 ± 2.0^{a}	5.1 ± 1.1^{ab}	0.9 ± 0.3^{b}	1.1 ± 0.5^{ab}	1.3 ± 0.4^{ab}	2.0 ± 0.5^{a}
18:4(n-3)	2.6 ± 0.1^{ab}	1.7 ± 0.3^{b}	2.3 ± 0.2^{ab}	3.2 ± 0.8^{a}	1.7 ± 0.1^{a}	0.7 ± 0.4^{b}	1.3 ± 0.6^{ab}	1.7 ± 0.1^{a}
20:4(n-3)	2.4 ± 0.3^{b}	2.7 ± 0.2^{b}	3.4 ± 0.5^{ab}	4.3 ± 0.7^{a}	0.5 ± 0.2	0.5 ± 0.1	1.2 ± 0.7	1.1 ± 0.4
20:5(n-3)	0.9 ± 0.3^{ab}	0.5 ± 0.1^{b}	0.9 ± 0.2^{ab}	1.4 ± 0.5^{a}	0.5 ± 0.1^{ab}	0.3 ± 0.1^{b}	0.5 ± 0.1^{ab}	0.7 ± 0.1^{a}
22:5(n-3)	0.7 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	1.8 ± 0.9	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 0.2
24:5(n-3)	1.0 ± 0.1^{b}	1.1 ± 0.3^{b}	1.6 ± 0.2^{ab}	2.8 ± 1.2^{a}	0.4 ± 0.2^{ab}	0.2 ± 0.1^{b}	0.4 ± 0.1^{ab}	0.7 ± 0.2^{a}
22:6(n-3)	0.6 ± 0.1^{b}	0.7 ± 0.0^{b}	0.7 ± 0.1^{b}	1.4 ± 0.5^{a}	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	1.3 ± 0.5
24:6(n-3)	0.4 ± 0.2	0.5 ± 0.1	0.4 ± 0.2	0.9 ± 0.3	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	1.1 ± 0.6

Results are percentages of total radioactivity recovered and are shown as means ± SD (n = 3). Values with different superscript letters within a given row for each individual species are significantly different ($p < 0.05$). TL, total lipid.

Table 6. Incorporation of radioactivity from injected [1-^{14}C]18:3n-3 into total lipid (pmol mg total lipid^{-1}) of brain from rainbow trout and sea bream and metabolism by desaturation/elongation at 6, 12, 24 and 48 h after injection

	Rainbow trout				Sea bream			
Time after injection (h)	6	12	24	48	6	12	24	48
Incorporation (pmol mg^{-1} TL)	83 ± 12^{a}	58 ± 6^{b}	40 ± 9^{bc}	33 ± 2^{c}	127 ± 39	67 ± 10	70 ± 39	57 ± 2
18:3(n-3)	67.6 ± 0.4	58.7 ± 12.1	75.6 ± 1.8	64.1 ± 7.9	80.9 ± 12.5	70.9 ± 9.1	61.1 ± 15.8	64.6 ± 5.1
20:3(n-3)	6.4 ± 1.4	8.3 ± 0.6	7.7 ± 3.6	13.7 ± 4.7	13.3 ± 0.5^{a}	6.1 ± 1.1^{bc}	7.5 ± 0.3^{b}	5.5 ± 0.4^{c}
18:4(n-3)	2.6 ± 0.3^{ab}	4.3 ± 1.5^{a}	2.0 ± 0.2^{b}	3.2 ± 0.3^{ab}	2.4 ± 0.1^{b}	5.0 ± 2.2^{ab}	5.0 ± 1.8^{ab}	5.7 ± 0.8^{a}
20:4(n-3)	2.7 ± 0.4^{c}	4.3 ± 0.1^{b}	2.0 ± 0.5^{c}	5.5 ± 0.1^{a}	2.6 ± 0.6	3.2 ± 0.2	3.8 ± 0.6	4.3 ± 1.6
20:5(n-3)	2.7 ± 0.8^{ab}	4.2 ± 1.3^{a}	1.3 ± 0.4^{b}	2.6 ± 1.2^{ab}	3.1 ± 0.6	3.0 ± 0.9	4.4 ± 1.3	4.7 ± 0.7
22:5(n-3)	2.4 ± 0.5^{b}	6.3 ± 1.1^{a}	0.9 ± 0.4^{b}	2.7 ± 1.4^{b}	1.3 ± 0.3^{b}	2.6 ± 0.6^{ab}	4.7 ± 1.7^{a}	2.9 ± 0.2^{ab}
24:5(n-3)	1.7 ± 0.4^{ab}	2.2 ± 0.5^{a}	0.8 ± 0.5^{b}	1.7 ± 0.1^{ab}	1.4 ± 0.6^{b}	3.4 ± 0.6^{ab}	4.9 ± 1.5^{a}	3.0 ± 0.2^{ab}
22:6(n-3)	10.5 ± 2.0	12.6 ± 7.6	8.8 ± 6.4	5.1 ± 1.0	2.8 ± 0.1^{b}	3.8 ± 0.9^{ab}	4.7 ± 1.2^{ab}	5.2 ± 0.7^{a}
24:6(n-3)	3.6 ± 1.4^{a}	2.4 ± 0.6^{ab}	1.2 ± 0.8^{b}	1.7 ± 0.1^{ab}	2.1 ± 0.6^{b}	2.3 ± 0.2^{ab}	3.8 ± 1.4^{a}	3.8 ± 0.1^{ab}

Results are percentages of total radioactivity recovered and are shown as means ± SD (n = 3). Values with different superscript letters within a given row for each individual species are significantly different ($p < 0.05$). TL, total lipid.

Table 7. Incorporation of radioactivity from injected [1-^{14}C]18:3n-3 into total lipid (pmol mg total lipid^{-1}) of eye from rainbow trout and sea bream and metabolism by desaturation/elongation at 6, 12, 24 and 48 h after injection

	Rainbow trout				Sea bream			
Time after injection (h)	6	12	24	48	6	12	24	48
Incorporation (pmol mg^{-1} TL)	47 ± 12^{a}	35 ± 3^{ab}	38 ± 2^{ab}	26 ± 1^{b}	141 ± 90	256 ± 175	175 ± 147	88 ± 14
18:3(n-3)	78.7 ± 1.1^{a}	80.7 ± 1.2^{a}	65.9 ± 0.4^{b}	80.3 ± 1.9^{a}	79.1 ± 12.5	83.8 ± 11.5	64.3 ± 15.1	71.2 ± 0.8
20:3(n-3)	4.8 ± 1.6^{bc}	7.6 ± 0.8^{a}	7.0 ± 0.1^{ab}	4.3 ± 0.4^{c}	3.0 ± 1.1^{b}	2.4 ± 0.9^{b}	6.2 ± 0.9^{a}	5.6 ± 0.9^{a}
18:4(n-3)	2.0 ± 0.6^{b}	1.8 ± 0.3^{b}	4.5 ± 0.6^{a}	2.2 ± 0.4^{b}	2.4 ± 0.2	3.0 ± 1.9	5.1 ± 1.4	3.3 ± 0.4
20:4(n-3)	1.7 ± 0.5^{c}	2.3 ± 0.5^{c}	5.6 ± 0.1^{a}	3.7 ± 0.6^{b}	2.7 ± 0.7^{ab}	1.8 ± 0.4^{b}	4.3 ± 1.0^{a}	2.6 ± 0.6^{ab}
20:5(n-3)	2.0 ± 1.4	1.9 ± 0.4	2.9 ± 0.4	1.9 ± 0.8	2.4 ± 1.1	2.4 ± 1.4	2.6 ± 0.5	3.6 ± 0.5
22:5(n-3)	1.8 ± 0.8	1.3 ± 0.4	1.6 ± 0.1	1.4 ± 0.8	3.0 ± 1.4	1.6 ± 0.8	2.9 ± 0.5	2.7 ± 0.1
4:5(n-3)	2.2 ± 1.7	1.1 ± 0.3	2.7 ± 0.1	1.5 ± 0.2	2.5 ± 0.9^{ab}	1.4 ± 0.7^{b}	2.8 ± 1.0^{ab}	3.8 ± 0.1^{a}
22:6(n-3)	5.6 ± 1.8^{ab}	2.5 ± 0.3^{c}	7.3 ± 0.1^{a}	3.7 ± 0.3^{b}	2.7 ± 0.8^{b}	2.1 ± 0.8^{b}	7.8 ± 2.6^{a}	4.2 ± 1.0^{ab}
24:6(n-3)	1.2 ± 1.0^{b}	0.9 ± 0.3^{b}	2.8 ± 0.3^{a}	0.9 ± 0.1^{b}	2.2 ± 1.1^{b}	1.8 ± 0.7^{b}	4.0 ± 0.9^{a}	3.2 ± 0.1^{ab}

Results are percentages of total radioactivity recovered and are shown as means ± SD (n = 3). Values with different superscript letters within a given row for each individual species are significantly different ($p < 0.05$). TL, total lipid.

Table 8. Results (F-values) of two-way analysis of variance for the data in Tables 5-7

Data	Species	Time after injection	Interaction	Multiple range test
Table 5				
Incorporation	11.7 [b]	ns	ns	
18:3(n-3)	77.2 [c]	11.2 [c]	3.4 [a]	48-6,12
20:3(n-3)	74.3 [c]	8.3 [b]	5.3 [b]	6,12-24 and 12-48
18:4(n-3)	32.3 [c]	10.7 [c]	ns	6-12,24-48
20:4(n-3)	172.4[c]	10.6 [c]	ns	6-24,48 and 12-48
20:5(n-3)	20.2 [c]	7.9 [b]	ns	12-48
22:5(n-3)	25.8 [c]	5.4[b]	ns	48-6,12
24:5(n-3)	41.1 [c]	7.4 [b]	ns	48-6,12
22:6(n-3)	ns	10.3 [b]	ns	48-6,12,24
24:6(n-3)	ns	5.5 [b]	ns	48-6,24
Table 6				
Incorporation	10.1 [b]	9.8 [b]	ns	6-12,24,48
18:3(n-3)	ns	ns	ns	
20:3(n-3)	ns	ns	11.9 [c]	
18:4(n-3)	9.6 [b]	4.2[a]	ns	6-12
20:4(n-3)	ns	13.2 [c]	6.2 [b]	48-6,12,24
20:5(n-3)	8.0 [a]	ns	5.9 [b]	
22:5(n-3)	ns	8.0[b]	16.8 [c]	12-6,24,48
24:5(n-3)	32.4 [c]	4.7 [a]	11.0 [c]	6-12,24
22:6(n-3)	11.8 [b]	ns	ns	
24:6(n-3)	5.4 [a]	ns	8.3 [b]	
Table 7				
Incorporation	13.1 [b]	ns	ns	
18:3(n-3)	ns	5.0 [a]	ns	24-6,12
20:3(n-3)	18.1 [c]	8.5 [b]	12.6 [c]	24-6,12,48
18:4(n-3)	4.8 [a]	10.2 [c]	ns	24-6,12,48
20:4(n-3)	ns	29.6[c]	4.5[a]	24-6,12 and 48-12,24
20:5(n-3)	ns	ns	ns	
22:5(n-3)	11.7[b]	ns	ns	
24:5(n-3)	5.1 [a]	4.2 [a]	ns	12-24,48
22:6(n-3)	ns	18.7 [c]	ns	24-6,12,48
24:6(n-3)	23.6 [c]	10.4 [c]	ns	24-6,12,48

Significance levels are [a], $p < 0.05$; [b], $p < 0.001$; [c], $p < 0.001$; ns, not significant ($p > 0.05$). In the results for the multiple range test (Tukey HSD) for the differences between time points, where time points (6,12,24 and 48h) are separated by a dash the data are significantly different ($p < 0.05$). Where time points are separated by a comma, the data are not significant and where a time point does not appear it is not significantly different to any other time point.

the variability in the time course was highly dependent upon the species (Table 8). This was not the case in liver (and eye) where the variability in the data masked any interaction.

The incorporation of [1-^{14}C]18:3n-3 into total lipid from eyes of trout and sea bream was lower than the incorporation into liver and more similar to that into brain (Table 7). In general, the recovery of radioactivity in eye total lipid decreased with time after injection, albeit after an initial increase at 12h in sea bream, although the large variation observed in sea bream made the trend non-significant (Tables 7,8). As with brain, there was no significant difference in the percentage of radioactivity recovered unmetabolized in eye between sea bream and trout and there was no obvious trends in the time course. Significantly higher percentages of radioactivity were recov-

ered in 20:3 in trout eye whereas the amounts of radioactivity recovered in 18:4, 22:5, 24:5 and 24:6 were significantly greater in sea bream eye (Tables 7,8). Peak values for most desaturated metabolites in eye occurred 24h after injection in both trout and sea bream, except for 20:5 and 24:5 in sea bream which showed peak values after 48h (Table 7).

Discussion

The absolute amounts of radioactive fatty acid recovered in the tissues can be calculated using the data contained in Tables 1 and 2 and 5-7. The liver, brain and eyes from trout contained from 4160 to 520, 232 to 92 and 202 to 112 pmol of radioactive fatty acid, respectively. The comparative figures for sea bream liver, brain and eyes were 8910 to 2160, 343 to 154 and 211 to 132 pmol. Therefore, the uptake and incorporation of injected 18:3n-3 by the sea bream organs was apparently greater than in trout. However, as the same amount of radioactivity was injected into both species and the trout were slightly larger fish, this difference in absolute amount of radioactivity incorporated per tissue could be partly due to the greater dilution of the isotope in the trout. However, it was interesting that the difference in absolute incorporation varied with the tissue, with the liver most affected with a two-fold difference compared to only 1.5-fold with the brain and almost identical incorporation into the eyes of sea bream and trout. The loss of radioactivity with time after injection also varied with tissue. In the liver, radioactivity decreased 8-fold in trout and 4-fold in sea bream by 48h but in the brain and eyes the radioactivity was only halved in 48h. This may reflect the livers role in the processing and export of lipid to extra-hepatic tissues but may also reflect greater oxidation of fatty acids in the liver and/or greater retention of n-3PUFA in brain and eyes. Similarly, the proportion of radioactivity recovered in DHA was higher in brain than in eyes and this may also reflect a greater retention/biosynthesis in brain than eye although it may also be a reflection of the fact that only a relatively small proportion of the eye is neural retina.

The brain and eyes of both trout and sea bream were also characterized by proportionally more of the incorporated radioactivity being recovered as desaturation products (combined tetra-, penta- and hexaenes) compared to liver. This may indicate greater conversion of 18:3n-3 and biosynthesis of DHA in neural tissues (brain and retina) but could also represent import of the desaturated products, rapidly exported from liver. Probably the major difference between the sea bream and the trout in relation to the desaturation products of 18:3n-3 was the significantly greater proportion of radioactivity recovered in DHA in trout brain. In contrast, the significant trends in recovery of radioactivity in tetra- and pentaenes was for sea bream to have the higher levels in brain and eyes. Higher recoveries of radioactivity in tetraenes in sea bream was expected but for this to also be the case in pentaenes may appear inconsistent with a low level of Δ5 desaturase in marine fish (Tocher et al. 1989; Tocher and Mackinlay 1990; Tocher and Sargent 1990a). However, the data for sea bream obtained in the present study is generally consistent with that found in the earlier injection studies albeit that these were on whole fish and organs were not studied (Mourente and Tocher 1993a, 1994). An average of the values obtained from the liver/brain/eye data in the present study would approximate to the whole fish data obtained previously considering the greater radioactivity that is associated with liver compared to brain and eyes (Mourente and Tocher 1993a, 1994).

In contrast, the data obtained with rainbow trout in the present study appears to show a generally lower flux through the desaturase/elongase pathways compared to an earlier study on trout (Owen et al. 1975). Recently though, it was shown that no radioactivity was recovered in penta- or hexaene products in liver lipids in the highly carnivorous, freshwater pike (*Esox lucius*) injected with [1-^{14}C]18:3n-3 (Henderson et al. 1995). However, in similar studies on tilapia (*Oreochromis nilotica*) (Olsen et al. 1990) and Arctic charr (*Salvelinus alpinus*) (Olsen and Ringo 1992), the flux through the desaturase/elongase pathways was greater than in the present study. These differences are very likely due to dietary effects as the fatty acid desaturases are known to be affected by dietary factors in mammals (Brenner 1981). Although not well defined,

the effects of diet on desaturation/elongation of fatty acids have been reported in fish. The conversion of intraperitoneally-injected [1-^{14}C]18:3n-3 to 20:5n-3 and 22:6n-3 was low in tilapia fed a commercial diet containing fish oil and greatly stimulated by feeding a diet containing 18:2n-6 as virtually the only PUFA (Olsen et al. 1990). Similarly, Arctic charr on a commercial diet containing fish oil showed relatively low levels of desaturation of injected [1-^{14}C]18:3n-3, whereas the highest levels of desaturation activity were observed in fish fed diets containing 1% 18:2n-6 and 1% 18:3n-3 as the sole PUFA (Olsen and Ringo 1992). Recently, it was shown that the conversion of [1-^{14}C]18:3n-3 to DHA in rainbow trout hepatocytes was doubled in fish fed a diet deficient in n-3PUFA compared to fish fed a diet containing fish oil (Buzzi et al. 1996a). However, there are no data in the literature on the effects of dietary factors on the desaturation/elongation pathway in marine fish and so the experiment was performed using the procedure adopted earlier for marine fish (Linares and Henderson 1991; Mourente and Tocher 1993a,b, 1994). Clearly, the results from the present study, where trout fed a commercial diet containing fish oil and then starved for 1 week prior to injection displayed a relatively low level of desaturase/elongase activity, showed that it would be useful to investigate and compare the activities of the fatty acyl desaturation/elongation pathways in both freshwater fish and marine fish fed diets rich in C_{18} PUFA.

Studies with cultured fish cells support the fact that nutrition affects the desaturase pathways. The activity of the desaturase pathway towards [1-^{14}C]18:3n-3 in rainbow trout brain astrocytes in primary culture, initially grown in medium containing relatively n-3-deficient serum then in lipid-free medium, was 4-5 fold greater than in newly isolated mixed brain cells from rainbow trout fed a fish oil-containing diet (Tocher and Sargent 1990b, 1992). Brain astrocytes from a marine fish, the turbot, in primary culture under the conditions described above for trout astrocytes showed very little desaturase activity in comparison to the trout astrocytes (Tocher and Sargent 1990b; Tocher 1993). Similarly, comparison of established cell lines from trout (RTG-2) and turbot (TF) removed from nutritional influences and cultured under identical conditions clearly demonstrated the differences in their ability to desaturate and elongate 18:3n-3.
Therefore, in experiments using fresh preparations of tissues/cells or *in vivo* experiments on fed fish, the activities of the desaturation/elongation pathway in rainbow trout are suppressed, making the data more similar to marine fish.

One aim of the present study was to determine whether DHA in neural tissue such as the brain was primarily a product of *in situ* metabolism in brain or due to the import of preformed DHA, exported by the liver and ultimately derived from the diet, either as DHA or a precursor such as 18:3n-3. The data showed that the time-courses obtained for liver, brain and eyes were different, particularly in rainbow trout. In both trout and sea bream, the liver time-course is consistent with a pulse (injection) of radioactivity incorporated by the first time point (6h), with subsequent and consistent loss of radioactivity from the tissue due to turnover of lipids, export and oxidation etc. This was also observed in sea bream in whole fish over a longer time-course of 2-8 days (Mourente and Tocher 1994). The recovery of radioactivity in individual fatty acid fractions in liver indicated that, as time after injection increased, a greater proportion of the radioactivity remaining was associated with longer chain, more unsaturated metabolic products. This is probably the result of continuing desaturase/elongase activity and/or slightly greater retention of the metabolic products, but of course in absolute terms the desaturation/elongation products decreased with time after injection too. In contrast, in the longer time-course of 2-8 days there was no increase in the relative proportion of radioactivity recovered in metabolic products (Mourente and Tocher 1994).

The data for incorporation of radioactivity into total lipid in the brain and eyes showed a very similar pattern to liver in that there was a general decrease in recovery with time after injection. In sea bream eyes, there was a suggestion of further net import of radioactivity after about 12-24h, but the data were not statistically significant. Therefore, there is no evidence for net import of total radioactivity into brains or eyes after 6h post-injection. In contrast, the data for desaturated/elongated products of injected [1-^{14}C]18:3n-3 showed

that there was an increase in the relative amounts of these metabolites over 6-12h in brain and 12-24h in eye, particularly in trout. Together with the time-course data for the liver, this could be consistent with a net import of metabolic products, produced in and then exported from the liver, in brain and eyes over 6 to 24h. However, it is unclear why the relative proportion of desaturated/elongated products should then decrease at 48h. The most likely alternative explanation, namely *in situ* biosynthesis in the tissue and greater retention of longer-chain more unsaturated products, is equally unable to explain the decrease at 48h.

Recently it was shown that rainbow trout hepatocytes can be very active in the biosynthesis of DHA with up to 50% of applied 18:3n-3 being converted to DHA in cells from fish on an olive oil diet (Buzzi et al. 1996a). However, it has also been demonstrated that brain cells from fish are also active in the incorporation and metabolism of PUFA. Tocher and Sargent (1990b) showed that [1-^{14}C]18:3n-3 was desaturated and elongated in cultured brain astrocytes from rainbow trout with approximately $^{1}/_{3}$ of radioactivity recovered as desaturated products including 7% as pentaenes (6% EPA), but only less than 1% as DHA. When [1-^{14}C]20:5n-3 was supplied, up to 19% of the EPA was elongated and desaturated (Tocher and Sargent 1990b). With cultured astrocytes from a marine fish, turbot, very little of incorporated [1-^{14}C]18:3n-3 was metabolized to penta/hexaenes (0.7% of total radioactivity recovered) although there was very active elongation (Tocher 1993). It had been shown previously that mixed brain cell suspensions from turbot rapidly incorporated both [1-^{14}C]18:3n-3 and [1-^{14}C]22:6n-3, reaching maxima in 2-4h, and there was no significant difference between the two fatty acids (Tocher et al. 1992). The mixed brain cell suspensions from turbot were able to metabolize 18:3n-3 by desaturation/elongation, with a maximum of approximately 7.5% of total radioactivity recovered in desaturation products, including 20:5n-3 and 22:6n-3 (1.4 and 1.3%, respectively). Up to 10% of [1-^{14}C]20:5n-3 incorporated by turbot mixed brain cell suspensions was converted to DHA (Tocher et al. 1992). Therefore, there is evidence that both liver and brain itself can contribute to the DHA present in fish brain.

The relative importance of *in situ* conversion of 18:3n-3 to DHA in brain and retina versus the supply of preformed DHA to the neural tissues during development is still largely unresolved in mammals (Cook 1991). Uptake and incorporation of preformed DHA has been demonstrated in rat brain (Martin et al. 1994) and frog and rat retina (Chen and Anderson 1993; Martin et al. 1994). However, synthesis of DHA from 18:3n-3 has been well characterized in both rat brain (Green and Yavin 1993) and frog retina (Wang and Anderson 1993). Neurons themselves cannot synthesize DHA from 18:3n-3, but the cerebral endothelium primarily produces and releases EPA and astrocytes produce and release DHA (Moore et al. 1990,1991). Therefore, it would appear that both liver and the neural tissues themselves can contribute to DHA in neural tissue in other animals. Thus, if preformed DHA is available the cerebral endothelium will take it up and transfer it to brain (Moore 1993). An additional pathway can utilize 18:3n-3 and EPA with the cerebral endothelium taking them up and targeting them to the brain, performing some desaturation/elongation which astrocytes would complete (Moore 1993).

In the present study radioactivity was recovered in the C_{24} products, 24:5n-3 and 24:6n-3, in both rainbow trout and sea bream and in all three tissues although the levels found in the brain and eye were far greater than in the liver. Previously, in studies on whole fish, we had shown that radioactivity was recovered in the C_{24} metabolites in sea bream and golden grey mullet injected with [1-^{14}C]20:5n-5 but only traces were found after injection with [1-^{14}C]18:3n-3 (Mourente and Tocher 1993a,b,1994). In the present study, the proportion of radioactivity recovered in the two C_{24} metabolites followed an almost identical pattern and followed, almost exactly, the pattern observed in the immediate precursor fatty acid, 22:5n-3, and the product, DHA. The presence of the C_{24} metabolites in brain and eye are possibly the best evidence for *in situ* desaturation and elongation in these tissues, at least from 20:5n-3, as it is possibly unlikely that these metabolites would be exported from the liver. The recovery of radioactivity in C_{24} metabolites has been considered as being consistent with the operation in fish of the "Sprecher shunt" pathway for the biosynthesis of DHA as described in rats

(Sprecher et al. 1995). Recently, more direct evidence for the operation of this pathway in rainbow trout hepatocytes has been published (Buzzi et al. 1996b).

In conclusion, although the results of the present study cannot eliminate a role for liver in the biosynthesis and provision of DHA for developing neural tissues in fish, they suggest that DHA can be synthesized in fish brain and eye *in vivo*.

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