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Lipid class and fatty acid composition of brain lipids from Atlantic herring (*Clupea harengus*) at different stages of development

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Abstract. Little is known about the changes in composition of brain lipids and fatty acids at different stages of development in fish. Wild Atlantic herring (*Clupea harengus* L.) were collected from Loch Linnhe and the Firth of Clyde, Scotland, from August 1990 to March 1991. Lipid class and fatty acid compositions of brain lipids were studied at four different stages of development: larvae at the end of the yolk sac stage, two juvenile stages and sexually mature adults. The total lipid content in brains increased during development, and larval brains contained higher proportions of neutral lipids and lower proportions of polar lipids than the brains of juvenile or adult herring. Increased proportions of polar lipids in juvenile and adult herring brains were mainly due to increased percentages of phosphatidylcholine (PC), phosphatidylethanolamine (PE), cerebrosides and sulphatides. The increase in the proportions of the glycolipid classes suggested increasing levels of myelination with development. In total lipids, saturated fatty acids generally decreased and monounsaturated fatty acids and dimethyl acetals (derived from PE-plasmalogen) increased from larvae to adults. However, the proportions of polyunsaturated fatty acids in individual phosphoglycerides were generally highest in juvenile stages, due mainly to increased 22:6n-3, and were lowest in adult fish. Relatively high percentages of 24:1 isomers were found in all the phosphoglycerides, but primarily PC, and these increased during development from larvae to adult. Fatty acids were distributed between individual phosphoglycerides with a characteristic pattern that did not change with development, although the relative amounts of individual fatty acids were altered. The variations and roles of the different lipid components of herring brain are discussed with respect to lipid compositions and functions in brains of other fishes and vertebrates.

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Introduction

Polyunsaturated fatty acids (PUFA) of the n-3 series predominate in the tissues of most fish species (Henderson and Tocher 1987, Sargent et al. 1989), and particularly in those of marine fish (Tocher and Sargent 1984, Sargent et al. 1989). This characteristic is also shared by mammalian neural and testicular tissues, which also have high levels of n-3PUFA, particularly 22:6n-3 (Sastry 1985, Poulos et al. 1973). The brains of a freshwater fish such as rainbow trout *Oncorhynchus mykiss* (Leray and Pelletier 1985, Tocher and Harvie 1988, Bell and Tocher 1989) or marine fishes such as sea bass *Dicentrarchus labrax* (Pagliarani et al. 1986), cod *Gadus morhua* (Tocher and Harvie 1988, Bell and Dick 1990) and other marine fish species (Kreps et al. 1975) have been shown to be particularly rich in n-3PUFA, mainly 20:5 and 22:6.

Recently, efforts have been made to elucidate the role (Bell and Tocher 1989, Bell and Dick 1990) and the metabolism (Tocher and Sargent 1990) of these particular fatty acids in fish neural tissue. The accumulation of 20:4n-6 and especially 22:6n-3 by the developing mammalian brain (Sinclair and Crawford 1972, Anderson and Connor 1988) and the tenacity to retain 22:6n-3 shown by adult fish (Pagliarani et al. 1986), chicks (Anderson et al. 1989), rats (Bourre et al. 1984) and monkeys (Neuringer et al. 1986) when fed diets deprived of n-3PUFA, suggested that mechanisms may exist in the brain during development to consolidate the lipid composition in adult brain. However, little is known about the changes in composition of brain lipids and fatty acids at different stages of development in fish.

In the present investigation, we studied the variations in lipids and fatty acids in the brain of wild Atlantic herring (*Clupea harengus* L.) at four stages during the life cycle. The results are discussed in relation to existing data on brain development and lipid composition in fish, and compared with the more characterized situation in higher vertebrates.

Materials and methods

Experimental fish

Four stages of development of Atlantic herring (*Clupea harengus* L.) were studied. Stage I was represented by larvae at the end of yolk sac absorption. For this stage, fertilized eggs from wild, captured, mature fish were incubated at 6 to 7 °C and then brains were sampled 15 d after hatching when the yolk sac had been completely absorbed. Wild juvenile and adult herring were trawled from Loch Linnhe and the Firth of Clyde (Scotland, UK) during the period from August 1990 to March 1991. Stages II and III were characterized as juveniles (~4 and ~7 mo-old, respectively) and Stage IV was sexually mature adults (~2 yr-old). Ages were deduced from length data according to Iles (1974). Fish were killed by decapitation and the brains dissected-out on ice, immediately frozen in liquid N_2 and stored at −80 °C until analysis. Triplicate samples (300 fish/sample for larvae; 10 fish/sample for juveniles; 5 fish/sample for adults) were taken for dry weight determination and lipid analyses.

Dry weight determination

Pre-weighed samples of brains were maintained at 110 °C for 24 h. Dry weight was determined after cooling in vacuo for at least 1 h.

Total lipid extraction

Brain lipids were extracted by homogenization in a teflon-pestle glass homogenizer in chloroform/methanol (2:1, v/v) containing 0.01% (w/v) butylated hydroxytoluene (BHT) (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 (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-13 data handling station (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 phosphoglyceride classes were prepared by acid-catalyzed transmethylation for 16 h at 50 °C (Christie 1989), using nonadecanoic acid (19:0) as internal standard, and extracted and purified as described previously (Tocher and Harvie 1988). Methyl esters were analyzed 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 an on-column injection system and hydrogen as carrier gas, with a biphasic thermal gradient from 50 to 235 °C. Individual fatty acid methyl esters were identified as described previously (Tocher and Harvie 1988) and quantified using a Shimadzu CR-3A recording integrator. All solvents contained 0.01% (w/v) BHT as antioxidant.

Statistical analysis

All results are presented as means ± SD of three samples. Differences between means were analyzed by one-way ANOVA followed by Tukey's HSD test. Differences are reported as statistically significant when $p < 0.05$.

Chemical materials

BHT and nonadecanoic acid (>99% pure) were from Sigma Chemical Co. (Poole, Dorset, UK); TLC (20 × 20 cm × 0.25 mm) and HPTLC (10 × 10 cm × 0.15 mm) plates pre-coated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany); all solvents were HPLC grade and were purchased from Rathburn Chemicals (Walkerburn, Peeblesshire, Scotland, UK).

Results

In *Clupea harengus*, brain total lipid content, as a percentage of the brain dry weight, increased from larvae to adults, significantly so between larvae and Stage II (Table 1). Total polar lipids increased from larvae to juveniles stages with a concomitant decrease in the percentage of total neutral lipids. The major polar lipid classes were phosphatidylcholine (PC) and phosphatidylethanolamine (PE), and their percentages showed significant increases

Table 1. *Clupea harengus*. Biometric data, lipid contents (as % dry wt) and lipid class compositions (as % total lipid) of brain lipid from herring at different stages. Data are means ± SD of three samples. PA/CL: phosphatidic acid/cardiolipin. Values within any one horizontal row not bearing the same superscript are significantly different at $p < 0.05$; those with same or no superscript are not significantly different

Composition	Stage			
	I	II	III	IV
Fish total length (mm)	10.9 ± 0.6^a	73.2 ± 4.9^b	86.7 ± 5.4^c	257.3 ± 5.8^d
Dry wt/brain (mg)	–	5.7 ± 0.3^a	7.5 ± 0.2^b	56.2 ± 2.1^c
Brain dry wt (%)	15.8 ± 0.2^a	18.7 ± 0.3^b	19.2 ± 0.4^b	18.3 ± 0.3^b
Lipid content	25.2 ± 4.2^a	33.4 ± 0.5^b	33.1 ± 1.4^b	37.7 ± 5.1^b
Lipid class composition				
Phosphatidylcholine	22.5 ± 0.7^a	28.9 ± 1.4^b	28.8 ± 1.9^b	25.0 ± 0.2^c
Phosphatidylethanolamine	14.9 ± 0.6^a	22.5 ± 0.6^b	21.8 ± 0.7^b	20.0 ± 0.2^c
Phosphatidylserine	6.6 ± 0.2	6.9 ± 0.4	6.5 ± 0.6	6.5 ± 0.2
Phosphatidylinositol	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.2	2.0 ± 0.0
PA/CL	3.3 ± 0.1^a	4.2 ± 0.2^b	3.8 ± 0.6^{ab}	4.4 ± 0.2^{bc}
Sphingomyelin	2.5 ± 0.2^a	1.2 ± 0.2^b	1.5 ± 0.5^{bc}	1.7 ± 0.1^{bc}
Cerebroside	0.3 ± 0.1^a	5.1 ± 0.6^b	5.3 ± 0.9^b	5.6 ± 0.2^b
Sulphatide	0.2 ± 0.1^a	2.9 ± 0.4^b	3.1 ± 1.1^b	7.2 ± 0.3^c
Total polar lipids	52.4 ± 1.7^a	73.8 ± 2.3^b	72.7 ± 1.5^b	72.4 ± 0.7^b
Cholesterol	19.0 ± 0.2^a	21.0 ± 1.3^{ab}	20.5 ± 1.9^{ab}	22.2 ± 0.9^b
Free fatty acid	3.1 ± 0.5^a	0.7 ± 0.6^b	0.9 ± 0.8^b	2.8 ± 0.2^a
Triacylglycerol	15.2 ± 0.8^a	2.6 ± 0.4^b	3.7 ± 1.0^b	1.3 ± 0.4^c
Sterol ester	10.3 ± 0.2^a	1.9 ± 0.9^b	2.2 ± 0.8^b	1.3 ± 0.4^b
Total neutral lipids	47.6 ± 1.7^a	26.2 ± 2.3^b	27.3 ± 1.5^b	27.6 ± 0.9^b

Table 2. *Clupea harengus*. Fatty acid composition (percentage weight) of total lipids from the brain of herring at different stages. Data are means ± SD of three samples. SD = 0.0 implies SD < 0.05. DMA: dimethyl acetal; PUFA: polyunsaturated fatty acid. Totals include fatty acids consistently present at < 0.5% but not included in table. Values within any one horizontal row not bearing the same superscript are significantly different at $p < 0.05$; those with same or no superscript are not significantly different

Fatty acid	Stage			
	I	II	III	IV
14:0	1.6 ± 0.2^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.6 ± 0.1^{c}
15:0	0.5 ± 0.1^{a}	0.1 ± 0.0^{b}	0.3 ± 0.2^{ab}	0.7 ± 0.4^{ab}
16:0DMA	0.1 ± 0.0^{a}	0.3 ± 0.0^{b}	0.5 ± 0.0^{c}	1.3 ± 0.1^{d}
16:0	21.8 ± 0.3^{a}	13.6 ± 0.1^{b}	15.9 ± 0.3^{c}	12.9 ± 0.7^{b}
16:1n-7	3.9 ± 0.3^{a}	3.3 ± 0.0^{ab}	3.2 ± 0.0^{b}	7.4 ± 2.3^{c}
C16PUFA *	2.0 ± 0.2^{a}	1.3 ± 0.1^{b}	0.5 ± 0.1^{c}	1.0 ± 0.5^{bc}
18:0DMA	0.1 ± 0.0^{a}	1.3 ± 0.1^{b}	2.2 ± 0.2^{c}	2.3 ± 0.6^{c}
18:1DMA **	0.4 ± 0.1^{a}	3.2 ± 0.1^{b}	2.7 ± 0.2^{b}	4.5 ± 0.6^{c}
18:0	9.2 ± 0.1^{a}	6.1 ± 0.1^{b}	6.5 ± 0.4^{b}	5.1 ± 0.2^{c}
18:1n-9	9.2 ± 0.1^{a}	16.9 ± 0.1^{b}	18.5 ± 0.4^{c}	21.1 ± 1.4^{d}
18:1n-7	4.4 ± 0.1^{a}	3.3 ± 0.0^{b}	3.6 ± 0.1^{c}	3.7 ± 0.3^{c}
18:2n-6	1.0 ± 0.1^{a}	0.6 ± 0.2^{b}	0.2 ± 0.1^{c}	0.2 ± 0.1^{c}
20:1 **	1.0 ± 0.0^{a}	0.6 ± 0.0^{b}	0.8 ± 0.0^{c}	1.3 ± 0.1^{d}
20:4n-6	1.4 ± 0.1^{a}	0.7 ± 0.0^{b}	0.8 ± 0.0^{c}	1.1 ± 0.1^{d}
20:5n-3	7.9 ± 0.3^{a}	4.6 ± 0.2^{b}	5.9 ± 0.4^{c}	5.2 ± 0.4^{c}
22:1 **	0.7 ± 0.2	0.6 ± 0.0	1.0 ± 0.1	1.0 ± 0.2
22:5n-3	1.5 ± 0.1	1.4 ± 0.1	2.7 ± 1.2	1.3 ± 0.1
22:6n-3	22.5 ± 0.9^{a}	25.9 ± 0.8^{b}	26.7 ± 0.5^{b}	15.2 ± 1.8^{c}
24:0	0.1 ± 0.0^{a}	0.5 ± 0.1^{b}	1.1 ± 0.2^{c}	1.7 ± 0.1^{d}
24:1 **	0.9 ± 0.0^{a}	2.8 ± 0.2^{b}	5.0 ± 1.3^{c}	7.1 ± 0.4^{d}
Total saturates ***	33.2 ± 0.5^{a}	20.6 ± 0.2^{b}	24.2 ± 0.5^{c}	22.1 ± 0.9^{d}
Total monoenes	20.2 ± 0.5^{a}	27.6 ± 0.1^{b}	32.1 ± 1.1^{c}	41.6 ± 3.0^{d}
Total DMA	0.6 ± 0.1^{a}	4.8 ± 0.5^{b}	5.4 ± 0.5^{b}	8.1 ± 0.6^{c}
Total n-6PUFA ****	4.7 ± 0.1^{a}	2.2 ± 0.2^{b}	1.6 ± 0.3^{c}	1.9 ± 0.3^{bc}
Total n-3PUFA *****	34.1 ± 0.8^{a}	33.7 ± 0.5^{a}	36.9 ± 0.9^{b}	22.7 ± 2.2^{c}

* Predominantly 16:2 and 16:3; ** predominantly n-9 isomer; *** includes 20:0; **** includes 20:2n-6, 22:4n-6 and 22:5n-6; ***** includes 18:3n-3, 18:4n-3, 20:3n-3 and 20:4n-3

from larvae to juveniles. In adults, PC and PE showed intermediate percentages that were significantly different from the percentages in both larvae and juveniles. In contrast, the proportions of phosphatidylserine (PS) and phosphatidylinositol (PI) remained relatively constant throughout development. The percentages of the glycolipids, cerebrosides and sulphatides increased significantly from larvae to juvenile stages and, in the case of sulphatides, there was a further significant increase from Stage III to adults. The major neutral lipid classes in larval brains were cholesterol, triacylglycerol (TAG) and sterol esters (SE). The proportion of cholesterol was almost constant over the four stages, but the proportions of TAG and SE each decreased significantly from larvae to the juvenile stages and further decreased (not significantly in the case of SE) from Stage III to adults.

In total lipid, the proportions of total saturates (primarily 16:0 and 18:0) were significantly lower and total monoenes (primarily 18:1n-9, 24:1 isomers and, in adults, 16:1n-7) were significantly greater in juvenile and adult

Table 3. *Clupea harengus*. Phosphatidylcholine fatty acid compositions (percentage weight) from the brain of herring at different stages. Further details as in legend and footnotes to Table 2

Fatty acid	Stage			
	I	II	III	IV
14:0	1.9 ± 0.1^{a}	0.3 ± 0.1^{b}	0.3 ± 0.2^{b}	0.8 ± 0.0^{c}
15:0	0.7 ± 0.1^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}
16:0	34.9 ± 1.8^{a}	20.1 ± 0.3^{b}	23.5 ± 1.0^{c}	19.3 ± 0.4^{b}
16:1n-7	5.4 ± 0.4^{a}	2.9 ± 0.3^{b}	4.2 ± 0.4^{c}	7.2 ± 0.1^{d}
C16PUFA *	1.3 ± 0.1^{a}	0.4 ± 0.0^{b}	0.1 ± 0.0^{c}	0.5 ± 0.0^{b}
18:0	2.7 ± 0.2^{a}	5.1 ± 1.1^{b}	2.9 ± 1.1^{ab}	2.3 ± 0.0^{ab}
18:1n-9	11.5 ± 0.3^{a}	19.1 ± 1.3^{b}	22.5 ± 0.7^{c}	21.9 ± 0.3^{c}
18:1n-7	3.6 ± 0.1^{a}	2.9 ± 0.1^{b}	3.1 ± 0.1^{b}	2.7 ± 0.2^{b}
18:2n-6	0.9 ± 0.1^{a}	0.2 ± 0.1^{b}	0.1 ± 0.0^{b}	0.3 ± 0.0^{bc}
20:4n-6	0.9 ± 0.0^{a}	0.5 ± 0.0^{b}	0.4 ± 0.0^{b}	0.7 ± 0.1^{c}
20:5n-3	7.8 ± 0.2^{a}	4.9 ± 0.3^{b}	4.4 ± 0.1^{b}	4.3 ± 0.1^{bc}
22:1 **	0.6 ± 0.0^{a}	1.3 ± 0.2^{b}	1.2 ± 0.3^{b}	1.6 ± 0.0^{b}
22:5n-3	0.9 ± 0.1^{a}	1.5 ± 0.2^{b}	1.2 ± 0.4^{ab}	0.9 ± 0.1^{ab}
22:6n-3	15.2 ± 0.8^{a}	22.7 ± 2.1^{b}	21.3 ± 0.1^{b}	13.4 ± 0.2^{c}
24:1 **	0.3 ± 0.0^{a}	6.2 ± 0.5^{b}	6.2 ± 0.1^{b}	12.8 ± 0.2^{c}
Total saturates ***	40.8 ± 2.1^{a}	26.6 ± 0.9^{b}	27.5 ± 1.3^{b}	26.9 ± 0.5^{b}
Total monoenes	21.4 ± 0.6^{a}	33.4 ± 3.1^{b}	38.5 ± 0.6^{c}	48.4 ± 0.7^{d}
Total n-6PUFA ****	3.1 ± 0.1^{a}	1.9 ± 0.1^{b}	1.6 ± 0.4^{b}	1.3 ± 0.1^{b}
Total n-3PUFA *****	25.5 ± 0.8^{a}	34.4 ± 2.4^{b}	27.6 ± 0.7^{c}	19.7 ± 0.4^{d}

stages than in larvae (Table 2). Total dimethyl acetals (DMA; derived from alkenyl-linked lipids) increased by over 10-fold from larvae to juvenile stages and by a further 1.5-fold from juvenile to adult. The percentages of total n-3PUFA, predominantly 20:5 and 22:6, were relatively constant during the larval and early juvenile stages but decreased significantly in adults, mainly due to a decrease in the percentage of 22:6n-3 from Stage III to adults. The percentages of total n-6PUFA were higher in larvae than in juveniles and adults.

In PC, the proportions of total saturates and total monoenes were greater than in total lipids (Table 3). Saturates in PC decreased significantly from larvae to juveniles but then remained constant, whereas monoenes increased significantly through all stages. As with total lipid, the increased proportions of monoenes was mainly due to increased percentages of 16:1n-7, 18:1n-9, and 24:1 isomers. The proportion of total n-3PUFA was greatest in Stage II juveniles but declined from then to adults (Table 3). The changes in n-3PUFA were mainly due to fluctuations in the percentage of 22:6n-3.

PE was more unsaturated than PC (Table 4). The proportion of total n-3PUFA was greatest in larvae and then decreased significantly between larvae and the adult stage. The decreased percentage of n-3PUFA in Stage III juveniles and adults was due to decreased 22:6n-3, whereas 20:5n-3 decreased from larvae to Stage II juveniles and then remained relatively constant. The proportions of total DMA remained constant from larvae to Stage II, but thereafter increased significantly throughout development to adults. The proportion of total saturates showed a downward trend from larvae to adults, whereas the proportion of total monoenes tended to increase.

Table 4. *Clupea harengus*. Phosphatidylethanolamine fatty acid composition (percentage weight) from brain of herring at different stages. tr: <0.05%. Further details as in legend and footnotes to Table 2

Fatty acid	Stage			
	I	II	III	IV
14:0	0.3±0.1	0.3±0.0	tr	0.2±0.0
15:0	0.2±0.0	0.2±0.0	tr	0.2±0.0
16:0DMA	0.7±0.1^{a}	1.0±0.2^{a}	1.1±0.4^{a}	2.9±0.1^{b}
16:0	13.1±1.2^{a}	5.5±2.8^{bc}	8.2±0.4^{b}	5.7±0.1^{c}
16:1n-7	1.0±0.1^{a}	0.9±0.3^{a}	1.8±0.4^{b}	3.7±0.1^{c}
C16PUFA*	0.8±0.0	1.0±0.1	0.8±0.1	0.8±0.1
18:0DMA	0.9±0.2^{a}	1.6±0.7^{ab}	4.2±1.7^{bc}	6.8±0.1^{c}
18:1DMA	1.9±0.5^{a}	1.1±0.4^{a}	3.4±0.9^{b}	10.1±0.2^{c}
18:0	7.8±0.1^{a}	8.2±1.5^{a}	8.7±0.6^{a}	4.8±0.1^{b}
18:1n-9	6.5±0.2^{a}	10.3±3.9^{a}	19.8±1.2^{b}	23.7±1.1^{c}
18:1n-7	7.1±0.2^{a}	3.1±0.6^{b}	2.9±0.9^{b}	3.4±0.2^{b}
18:2n-6	0.8±0.1^{a}	0.1±0.1^{b}	0.3±0.0^{c}	0.4±0.2^{bc}
20:1**	0.8±0.2	0.7±0.1	0.5±0.0	0.5±0.1
20:3n-6	0.2±0.1	0.5±0.2	0.6±0.3	tr
20:4n-6	0.9±0.1^{a}	0.6±0.0^{b}	0.7±0.1^{ab}	1.2±0.0^{c}
20:5n-3	7.9±0.2^{a}	5.9±0.3^{b}	5.3±0.6^{b}	5.6±0.2^{b}
22:1**	0.6±0.1^{a}	0.4±0.0^{b}	0.3±0.0^{b}	0.7±0.1^{a}
22:4n-6	0.2±0.1^{a}	0.7±0.2^{b}	0.5±0.3^{ab}	tr
22:5n-6	0.5±0.2	0.5±0.1	0.4±0.1	tr
22:5n-3	1.9±0.1^{a}	2.9±0.7^{a}	1.8±0.5^{ab}	1.3±0.0^{b}
22:6n-3	34.7±1.0^{a}	31.2±0.9^{b}	28.8±1.6^{b}	17.9±0.3^{c}
24:0	0.2±0.1	0.6±0.3	0.3±0.2	0.3±0.0
24:1**	0.6±0.0^{a}	1.4±0.6^{ab}	0.8±0.3^{a}	1.9±0.1^{b}
Total saturates***	21.7±0.9^{a}	14.8±4.1^{ab}	17.3±0.9^{a}	11.9±0.2^{b}
Total monoenes	16.6±0.5^{a}	16.8±4.3^{a}	26.2±0.8^{b}	33.9±1.0^{c}
Total DMA	3.5±1.0^{a}	3.7±0.6^{a}	8.7±3.4^{a}	19.9±0.3^{b}
Total n-6PUFA	2.6±0.5	2.4±0.1	2.5±0.8	1.8±0.5
Total n-3PUFA*****	47.2±1.2^{a}	41.3±1.4^{b}	37.7±2.3^{b}	26.3±0.2^{c}

PS presented the most unsaturated pattern of all the phosphoglycerides (Table 5). The proportion of total n-3PUFA increased significantly from larvae to Stage II, with both 20:5n-3 and 22:6n-3 increasing significantly during this period. Total n-3PUFA then decreased over the later stages due to decreases in the percentage of 22:6n-3, whereas 20:5n-3 remained constant. The proportions of total n-6PUFA and saturates remained relatively constant during development. However, the proportion of total monoenes increased significantly from Stages II to III and from Stage III to adult.

PI exhibited the highest proportions of 20:4n-6 and 20:5n-3, which both initially decreased from larvae to Stage II juveniles and then gradually increased in the later stages (Table 6). The proportions of 22:6n-3 in PI varied, but were generally lower than in the other phosphoglyceride classes. Total saturates and monoenes also varied without any pattern throughout development.

Discussion and conclusions

The specimens of *Clupea harengus* at all stages of development in this study were wild fish. Furthermore, the fish were analyzed soon after capture and were not fed with artificial diets. Therefore, the lipid and fatty acid compo-

Table 5. *Clupea harengus*. Phosphatidylserine fatty acid compositions (percentage weight) from the brain of herring at different stages. tr: <0.05. Further details as in legend and footnotes to Table 2

Fatty acid	Stage			
	I	II	III	IV
14:0	0.6±0.1^{a}	0.1±0.0^{b}	0.1±0.0^{b}	tr
15:0	0.3±0.1^{a}	0.1±0.0^{b}	0.1±0.0^{b}	0.3±0.3^{ab}
16:0	8.9±1.4^{a}	4.3±0.2^{b}	5.6±0.9^{bc}	4.9±0.2^{c}
16:1n-7	0.6±0.1^{a}	0.8±0.1^{a}	0.9±0.2^{a}	2.2±0.3^{b}
C16PUFA*	1.8±0.5^{a}	0.4±0.1^{b}	0.1±0.0^{c}	tr
18:0	15.1±0.9^{a}	14.7±1.2^{a}	17.9±2.1^{ab}	13.6±0.4^{b}
18:1n-9	4.1±0.3^{a}	6.1±0.3^{b}	10.1±1.4^{c}	17.8±0.5^{d}
18:1n-7	5.6±0.5^{a}	3.4±0.7^{bc}	2.5±0.3^{b}	4.0±0.6^{c}
18:2n-6	0.6±0.1^{a}	0.2±0.1^{b}	0.1±0.0^{b}	0.4±0.3^{ab}
20:1**	0.8±0.1^{a}	0.3±0.0^{b}	0.5±0.0^{c}	0.9±0.3^{a}
20:4n-6	0.4±0.1	0.6±0.3	0.5±0.2	0.8±0.3
20:5n-3	3.5±0.2	4.6±1.4	4.4±0.8	4.6±0.3
22:1**	0.5±0.1^{a}	0.2±0.0^{b}	0.8±0.1^{c}	1.5±0.5^{c}
22:5n-6	0.4±0.3	0.4±0.0	0.6±0.4	tr
22:5n-3	3.4±0.3^{a}	4.8±0.3^{b}	5.2±0.5^{bc}	5.4±0.2^{c}
22:6n-3	36.2±2.0^{a}	46.5±0.3^{b}	42.1±5.4^{ab}	32.7±1.8^{a}
24:0	tr	tr	0.4±0.3	0.6±0.0
24:1**	1.0±0.3^{ab}	0.7±0.1^{a}	2.6±0.4^{b}	5.2±0.1^{c}
Total saturates***	25.3±2.3^{a}	19.8±1.1^{b}	25.0±3.3^{ab}	20.7±0.4^{b}
Total monoenes	12.6±0.4^{a}	11.6±0.9^{b}	17.5±2.8^{a}	31.7±1.3^{c}
Total n-6PUFA****	3.1±1.0	1.9±0.4	2.3±0.7	1.8±0.9
Total n-3PUFA*****	45.1±1.7^{a}	57.3±1.3^{b}	52.6±6.8^{ab}	43.3±2.6^{a}

Table 6. *Clupea harengus*. Phosphatidylinositol fatty acid compositions (percentage weight) from the brain of herring at different stages. tr: <0.5%. Further details as in legend and footnotes to Table 2

Fatty acid	Stage			
	I	II	III	IV
14:0	1.0±0.2^{a}	0.4±0.1^{b}	0.3±0.1^{b}	0.2±0.1^{b}
15:0	0.6±0.1^{a}	0.3±0.0^{b}	0.2±0.0^{c}	1.0±0.2^{d}
16:0	9.4±2.3^{ab}	13.6±1.4^{b}	7.4±1.2^{a}	9.9±1.7^{a}
16:1n-7	0.9±0.1^{a}	2.6±0.4^{b}	1.8±0.7^{ab}	1.0±0.2^{a}
C16PUFA*	1.1±0.1^{a}	0.6±0.1^{b}	0.4±0.1^{b}	0.1±0.0^{c}
18:0	22.2±4.4	19.4±0.4	16.1±2.5	18.8±1.7
18:1n-9	5.3±0.7^{a}	7.9±1.2^{b}	8.8±2.2^{abc}	11.8±0.8^{c}
18:1n-7	7.0±0.3^{a}	5.4±0.7^{b}	3.4±0.6^{c}	6.9±0.4^{a}
18:2n-6	0.5±0.2^{a}	0.3±0.1^{ab}	0.1±0.1^{b}	tr
18:3n-3	0.6±0.3^{a}	0.1±0.0^{b}	tr	0.6±0.4^{a}
20:1**	0.7±0.2	1.1±0.2	0.5±0.2	0.9±0.4
20:4n-6	9.8±0.9^{a}	5.1±0.9^{b}	4.2±0.6^{b}	8.3±0.9^{a}
20:5n-3	11.9±1.2^{ab}	9.7±2.2^{a}	13.9±1.4^{b}	19.0±1.4^{c}
22:1**	0.9±0.3	0.7±0.1	0.4±0.1	0.3±0.2
22:5n-3	0.9±0.1^{a}	1.6±0.3^{b}	2.7±0.9^{b}	1.3±0.2^{b}
22:6n-3	11.8±0.4^{a}	16.1±1.9^{b}	16.9±1.7^{b}	14.5±2.5^{ab}
24:1**	0.5±0.2^{a}	2.4±0.9^{b}	0.8±0.5^{ab}	1.2±0.2^{b}
Total saturates***	33.6±6.0^{ab}	34.1±2.5^{a}	24.4±3.8^{b}	30.1±0.4^{ab}
Total monoenes	15.3±1.9^{a}	20.1±1.7^{b}	15.8±3.2^{a}	22.2±0.9^{b}
Total n-6PUFA****	11.7±0.8^{a}	7.9±0.9^{bc}	5.5±1.2^{b}	8.3±0.9^{c}
Total n-3PUFA*****	26.2±1.5^{a}	29.0±0.5^{b}	33.9±2.2^{bc}	35.7±2.7^{c}

sitions of the brains represent those of wild Atlantic herring.

The lipid class composition of brains from larvae showed surprisingly high levels of neutral lipid classes such as TAG and SE. However, the eyes and the rest of the body of larvae also showed high levels of neutral lipid classes (M. V. Bell personal communication). This may be a characteristic of this stage of development, when the yolk sac has just been absorbed with the absorbed lipid deposited, rather non-specifically, in tissues as neutral lipids. By the time of yolk-sac absorption, the yolk-sac lipids in herring are predominantly neutral lipids (Tocher et al. 1985). However, high amounts (>8%) of TAG have been reported in rainbow trout brain and retina (Tocher and Harvie 1988).

In juveniles and adults, polar lipid classes predominated and cholesterol accounted for over 75% of total neutral lipids. The percentages of total polar lipids in juvenile and adult herring brains were very similar to those of trout and cod brains (Tocher and Harvie 1988, Tocher and Sargent 1990) and those of brains from sea bass that had been fed on a cod-liver-oil diet (Pagliarani et al. 1986). The most significant trend in lipid-class composition in herring brain at different stages of development was the increase in the glycolipids, with cerebrosides increasing >18-fold and the sulphatides increasing >35-fold from larvae to adults, indicative of myelination processes (Kreps et al. 1975, Sastry 1985). The proportions of glycolipids in adult herring brain were considerably greater than those found in trout and cod brain (Tocher and Harvie 1988).

The most prominent feature of the fatty acid composition in brain lipids was the increase in the proportions of DMA and monoenes such as 16:1n-7, 18:1n-9 and 24:1 isomers accompanied by decreasing saturates and n-3PUFA as the fish developed and matured. The increase in DMA was confined entirely to PE, of course, whereas the increased monoenes were observed in all the phosphoglyceride classes, although increased 16:1n-7 and 24:1 isomers were most prominant in PC. Increased DMA are the result of increased synthesis and accumulation of PE-plasmalogens. PE-plasmalogens are implicated in myelin membrane composition (Sastry 1985) and, therefore, like the glycolipids, their increase in herring brain lipids during development probably reflects the myelination processes occurring up to adult stage.

Nervonic acid (24:1) is relatively abundant in SM of fish brains (Kreps et al. 1975). However, the present study confirms the presence of relatively high levels of 24:1 in phosphoglycerides, especially PC, from fish brain, previously reported for carp (Natarajan et al. 1985), trout and cod (Tocher and Harvie 1988, Bell and Tocher 1989). The present study shows that, although 24:1 is present in larval brains, the bulk of 24:1 in herring brain is accumulated during development and maturation of the fish. The proportion of 24:1 isomers in total lipids and PC of adult herring brain was up to 5 times higher than the proportions of 24:1 found in total lipid and PC in sea bass, trout or cod brains (Pagliarani et al. 1986, Tocher and Harvie 1988). The presence of significant levels of 24:1 in brain phosphoglycerides has not been reported in mammals, and the role(s) that molecular species of phosphoglycerides containing 24:1 may have in fish neural tissues remains to be elucidated.

In the total lipid of adult herring brain the proportions of 20:5n-3, 22:6n-3 and total n-3PUFA were lower than those in trout and cod brain (Tocher and Harvie 1988). However, the distribution of the individual PUFA, and fatty acids in general, between the different phosphoglycerides in herring is similar to that in other fish species and mammals (Tocher and Harvie 1988). Specifically, high 18:0 and enrichment of C_{22} PUFA in PS, high 16:0 and lower PUFA in PC, intermediate levels of 22:6n-3 in PE, and high 18:0 and C_{20} PUFA (primarily 20:4n-6 and 20:5n-3) in PI, found in the present study, were also observed in trout and cod brain (Tocher and Harvie 1988). However, the relative percentages of individual fatty acids in fish brain phosphoglycerides are very variable, depending upon species. For instance, the percentage of 20:4n-6 in PI from adult herring brain was lower than that found in brain PI in carp (Natarajan et al. 1985), similar to that in rainbow trout (Tocher and Harvie 1988), and higher than that in cod (Tocher and Harvie 1988, Bell and Dick 1990). In contrast, the percentage of 20:5n-3 in PI from adult herring brain was ~10 times higher than that in PI from carp brain (Natarajan et al. 1985), but similar to the percentages of 20:5n-3 in PI from rainbow trout and cod brains (Tocher and Harvie 1988).

Signal transduction processes in neural tissues are associated with the rapid turn-over of inositol-phosphoglycerides, in which 20:4n-6 is the only major PUFA in mammals (Crawford 1990). As noted earlier, PI in fish brain is characterized by relatively high levels of 20:5n-3, as well as 20:4n-6. The effect this may have on signal transduction processes in fish is unknown and warrants further study (Tocher and Harvie 1988, Bell and Tocher 1989, Bell and Dick 1990). Furthermore, the effects that substantial amounts of the eicosanoid precursor 20:5n-3 have on eicosanoid status and metabolism in fish brains is of considerable interest (Tocher et al. 1991).

Docosahexaenoic acid (22:6n-3) is generally the major PUFA in all vertebrate brains, but its level varies (Sastry 1985). The proportion of 22:6n-3 in phosphoglyceride classes from adult herring brain were comparable but, in general, less than those found in brains from cod and trout (Tocher and Harvie 1988), carp (Natarajan et al. 1985), and various species of marine teleosts (Kreps et al. 1975). Large amounts of 22:6n-3 are required during brain development in mammals, more specifically during a critical period that coincides with synaptogenesis and the biogenesis of photoreceptor membranes (Bazan 1990). However, the functional importance of high levels of 22:6n-3 in excitable membranes of neural tissues is poorly understood. The possible roles of 22:6n-3 in neural tissues may include effects on the biophysical properties of membranes, modulation of lipid-protein interactions and membrane-bound enzymes, and a role as precursor for functionally important lipoxygenase products (Neuringer et al. 1988, Bazan 1990).

In the phosphoglycerides from developing herring brain, some accumulation of 22:6n-3 was observed over the early stages (mainly larvae to Stage II juveniles). This

is consistent with the accumulation of 22:6n-3 observed during early brain development in avians (Anderson et al. 1989) and mammals (Bourre et al. 1984, Neuringer et al. 1986, Neuringer et al. 1988, Bazan 1990). The myelination processes may also be largely complete by early juvenile stage, as the greatest increase in glycolipids was between larval and Stage II juvenile herring. However, the processes of myelination, or perhaps myelin maturation, may continue for some considerable time after this, since PE-plasmalogens and 24:1 were still lower in Stage III juveniles than they were in adults. Therefore, myelination in herring may span a longer period than in rats (3 wk postnatal) or pigs (5 wk postnatal) (Hargreaves and Clandinin 1990).

Overall, there appears to be a general pattern for the lipid and fatty acid composition of adult herring brain. The major phosphoglycerides such as PC, PE and PS are less rich in 22:6n-3 than trout or cod (Tocher and Harvie 1988) or other species of marine teleosts (Kreps et al. 1975). In contrast, herring brain had higher levels of DMA in PE (i.e., PE-plasmalogens) and 24:1 isomers in phosphoglycerides generally, but especially PC, and, along with the higher levels of glycolipids, this may reflect a greater degree of myelination in this species compared to trout and cod. These characteristics of adult herring brain lipids are more similar to the characteristics of elasmobranch (Kreps 1981) and higher vertebrate (Sastry 1985) brain lipids, possibly suggesting a higher degree of brain evolution in herring compared to other teleost fish species (Kreps et al. 1975, Kreps 1981).

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