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Fatty-acid composition of ovulated eggs from wild and cultured turbot (*Scophthalmus maximus*) in relation to yolk and oil globule lipids

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Abstract The fatty-acid composition of lipids from ovulated eggs of wild and cultured turbot was investigated in order to estimate the nutritional requirements during embryonic and early larval development. Lipid comprised $13.8 \pm 0.5\%$ ($n = 5$) and $13.2 \pm 0.7\%$ ($n = 7$) of the egg dry weight in wild and cultured turbot, respectively. Polyunsaturated fatty acids (PUFA) of the (n-3) series accounted for 39% of total fatty acids in total lipid of both wild and cultured fish. The predominant (n-3) PUFA was docosahexaenoic acid (22:6 n-3), which also was the most abundant fatty acid in turbot eggs and comprised 24 and 23% of the total egg fatty acids in wild and cultured fish, respectively. Phospholipids, triacylglycerols and cholesterol–wax esters of turbot eggs all exhibited a specific fatty-acid profile distinctly different from that of total lipid. The general pattern of the fatty-acid distribution in lipids of eggs from wild and cultured turbot was similar, but the relative amount of 18:2(n-6) was considerably higher and 20:1(n-9) slightly higher in cultured fish. These differences were extended to all lipid classes and probably reflect the dietary intake of certain vegetable and marine fish oils. Calculations based on light microscopical studies showed that 55 to 60% of the total lipids in cultured turbot eggs are confined to the oil globule. The size of the oil globule remained constant during embryogenesis, and a reduction in size occurred first after hatching and mainly after yolk depletion. This implies that the total amount of lipids utilised during the embryonic development is considerably less than the total lipids present in ovulated turbot eggs. Comparison of the fatty-acid composition of total lipids from eggs and vitellogenin of wild turbot reveals that egg lipids contained a lower level of saturated and a higher level of monounsaturated fatty acids. Eggs also contained wax esters, which were not detected in vitellogenin, suggesting that vitellogenin is not the only source of lipids for turbot eggs.

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Introduction

Teleost eggs are generally rich in polyunsaturated fatty acids (PUFA) of the (n-3) series, mainly eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Sargent et al. 1989). In eggs of wild marine teleost species from high latitudes, PUFA were calculated to account for 40 to 47% of the fatty acids in total lipids (Tocher and Sargent 1984; Falk-Petersen et al. 1986). The presence of a high initial content of long-chain PUFA in fish eggs has been interpreted to indicate that these fatty acids are a nutritional requirement for the embryo and yolk-sac larva (Tocher and Sargent 1984). There is strong evidence that (n-3) PUFA are crucial both to female fecundity and to embryo and early larval development, growth and survival (Watanabe and Kiron 1994; Sargent 1995). Deficiencies in (n-3) PUFA during early developmental stages are suggested to be one of the most critical aspects of the limited success in rearing of many marine teleost species.

In turbot (*Scophthalmus maximus*), it is well documented that there is an absolute dietary requirement for long-chain PUFA, particularly EPA and DHA, due to the limited capacity of turbot to synthesise these fatty acids (Owen et al. 1975; Cowey et al. 1976; Tocher and Mackinlay 1990; Linares and Henderson 1991). However, little is known about the lipid reserves available to developing embryos and the fatty-acid requirements during embryo development. Eggs of cultured turbot contain 13 to 20% lipid (dry wt)

with approximately equal amounts of phospholipid and neutral lipid (Devauchelle et al. 1988; McEvoy et al. 1993; Planas et al. 1993b). Fatty-acid analyses of these lipids showed the presence of significant amounts of PUFA, such as EPA and DHA, in addition to the more commonly encountered palmitic acid (16:0) and oleic acid (18:1 n-9) (Devauchelle et al. 1988; McEvoy et al. 1993; Planas et al. 1993a). No distinction between yolk lipids and the prominent oil globule was made in these studies. It must also be noted that all previous studies were conducted on cultured fish, while the fatty-acid composition of eggs from turbot living in their natural ecosystems under a natural feeding and physical regime has not been reported. This information is important, because the fatty-acid composition of egg lipids in cultured fish can be altered by diet (Léger et al. 1981; Watanabe et al. 1984; Leray et al. 1985; Mourente and Odriozola 1990; Harel et al. 1994). Furthermore, the fatty-acid profile of eggs from cultured chinook salmon was significantly different from eggs of wild fish of the same stock (Ashton et al. 1993). Altogether, these findings imply that care must be taken in using information derived from studies on cultured or laboratory reared fish to explain the nutritional requirements during early development. It is therefore of interest to investigate the fatty-acid composition of ovulated eggs from wild turbot in order to obtain further insight into the nutritional requirements of turbot embryos and yolk-sac larvae.

In the present study, we examined the fatty-acid composition of total lipids, phospholipids, triacylglycerols, cholesterol esters and wax esters in ovulated eggs of wild turbot from a natural habitat and in eggs of cultured turbot. Furthermore, the oil globule of the turbot egg was isolated and analysed in order to evaluate the role of the oil globule during embryo development. A further aim was to shed some light on the maternal source of turbot egg lipids by comparing the lipid content and fatty-acid composition of total lipids from wild turbot eggs with those of total lipids from vitellogenin of wild turbot, which was recently reported (Silversand and Haux 1995). Vitellogenin is a hepatically synthesised egg yolk precursor and has been proposed to be a major source of lipids for teleost eggs (Wallace 1985; Mommsen and Walsh 1988; Silversand and Haux 1995).

Materials and methods

Fish and egg sampling

Ovulated eggs were collected from wild and cultured turbot (*Scophthalmus maximus*). Wild turbot, weighing between 1 and 4 kg, were caught during the spawning period in May by gill-net in shallow water off Göteborg (58°N), Sweden. A total of 60 turbot were captured, and freshly ovulated eggs of apparently good quality (see below) were obtained from five females.

Cultured turbot, 30 females and 20 males with a body weight of 2 to 5 kg, were kept in a covered tank under natural daylight conditions at Austevoll aquaculture research station in Norway (60° N). The tank (80 m^3) was supplied with filtered sea water from a depth of 50 m with a temperature during the spawning season of 10 ± 1 °C. The cultured turbot were captured by trawling in the sea off south-western Norway (59° N) and brought to Austevoll aquaculture research station 3 yr prior to egg sampling. The fish were fed ad libitum three times a week with a formulated moist diet based on minced herring (*Clupea harengus*) and capelin (*Mallotus villosus*) (30%, w/w), ensilage of herring and capelin (25%, w/w) and dry powder (45%, w/w) made of fish-powder of herring and capelin (delipidated), extruded wheat flour and vitamins (Austevoll fish industry A/S). Total dry weight content of protein and lipids was 50 and 20%, respectively. Freshly ovulated and viable eggs were obtained from seven females during the spawning period from July–August.

Eggs from both groups of turbot were obtained by gentle stripping of ovulated females, and the collected eggs were kept on ice until lipid extraction or until frozen at − 80 °C. After sampling, the eggs from all females were examined by a dissecting microscope in order to exclude batches of eggs with morphological characteristics correlated with poor quality, e.g. loss of spherical shape, dimpled egg surface, diminished egg transparency, and/or collapsed plasma membrane (McEvoy 1984; Kjørsvik et al. 1990). To assess the viability of the sampled eggs and to verify the significance of the morphological characterisation, a sample from each batch of the cultured turbot was artificially fertilized according to the method of Jones (1972) and incubated at 14.2 ± 0.2 °C until hatching. The fertilization percentage, measured as floating eggs versus sunken, and the hatching rate were calculated. The average fertilization and hatching rates were 96 ± 4% and 88 ± 7%, respectively.

Lipid extraction

Total lipid was extracted from 1 g of eggs within 30 to 60 min after stripping. All samples were extracted and analysed in duplicate. The extraction procedure was based upon the method of Bligh and Dyer (1959) with the modifications described by Silversand and Haux (1995). Additional eggs were analysed in order to determine the total lipid content as a percentage of dry weight of ovulated turbot eggs. Approximately 3 g, in duplicate, of frozen eggs from each female were freeze-dried in an evaporator (Savant Speed Vac, USA) for 20 h. After freeze-drying, the eggs were weighed and lipid was extracted according to the procedure described for fresh eggs. Prior to lipid extraction, the freeze-dried eggs were soaked with 1.0 ml distilled water.

Phospholipids, triacylglycerols, cholesterol esters and wax esters were isolated from total lipid extracts by thin-layer chromatography (TLC) using 20 × 20 cm glass plates coated with 0.25 mm silica gel 60 H and a solvent system of hexane:diethyl ether:acetic acid (80:20:2, v/v/v) (Merck, Germany). Samples were applied to the TLC plates in a box with N_2-atmosphere to minimise oxidation. Standards were simultaneously chromatographed, and after development standard lipid classes were stained with 2'7'-dichlorofluorescein (Merck, Germany) and visualised under ultraviolet light. All TLC plates were predeveloped in chloroform:methanol (1:1, v/v) before use and dried at 110 °C. Phospholipids, triacylglycerols, cholesterol esters and wax esters were scraped off the glass plates and subjected directly to acid-catalysed transesterification. Since cholesterol esters and wax esters did not resolve clearly in the TLC solvent system used in the present study, these lipid classes were transesterified together and are referred to in the text as "cholesterol–wax esters".

Fatty-acid analysis

Fatty-acid methyl esters were prepared and analysed as described previously (Silversand and Haux 1995). Individual fatty-acid methyl esters in the samples were identified by comparison of their retention

time with the retention time of a known standard (Larodan Fine Chemicals AB, Sweden and Nu-Chek-Prep, USA). Further evidence of identity was obtained by positive identification by gas–liquid chromatography/mass spectrometry (GLC/MS) of fatty-acid methyl esters on a gas chromatograph equipped with two detectors; a flame ionisation detector and a mass selective detector (5890, 5970 quadruple mass selective detector working with electron impact ionisation at an electron energy of 70 eV, Hewlett Packard, USA) (Nilsson and Liljenberg 1991). The mass spectrometric conditions, e.g. column and temperature program, were the same as for GLC analysis except that helium was used as carrier gas. Gas–liquid chromatography/mass spectrometry was also used to positively identify fatty alcohols present in the samples.

Oil globule

In order to separate the oil globule present in the eggs, frozen eggs (3 to 4 g) from each of the sampled females were gently homogenized with a glass-Teflon homogenizer and centrifuged at $3000 \times g$ for 30 min at 20 °C. The floating oil layer was subsequently aspirated and applied to a microscope slide. Pure oil was then aspirated using a microsyringe (10 µl, Hamilton, Switzerland) with the aid of a dissecting microscope. The obtained oil was immediately extracted for lipids and transesterified according to the method described above.

The diameter of newly ovulated eggs and their oil globule from cultured females was measured by light microscopy and a micrometer in order to determine the proportion of total egg lipids confined to the oil globule. The egg and oil globule diameters were on average 1.0 and 0.18 mm, respectively, and the volume of the egg plus oil globule was calculated with the formula for a sphere $[(4/3)\pi r^3]$. By estimating the density of whole eggs to be 1.0 (since 93% of egg weight was comprised of water, data not shown) and the average density of the oil globule to be 0.9 (since the density of triacylglycerol is 0.92, cholesterol esters is 0.98 and wax esters is 0.86) (Hadley 1985), the volume was converted to weight. The dry weight content of the cultured turbot eggs was 7% in the present study, of which lipids constituted 13.2%. Taken together, these data made it possible to estimate the relative amount of oil globule lipids. Furthermore, eggs and larvae from the cultured turbot were collected at different stages of development and investigated by light microscopy in order to follow the morphological development of the embryos with special emphasis on the oil globule.

Statistical analysis

The relative amount of the fatty acids in eggs was statistically analysed by multivariate data analysis using the software program SIMCA-S (Umetri AB, Sweden). The multivariate method used was principal component analysis (PCA) (Wold et al. 1987). In the present study, all variables were scaled to zero mean and unit variance (autoscaling) before calculations.

Table 1 *Scophthalmus maximus*. Relative fatty-acid composition of total lipid and phospholipids from ovulated eggs of wild and cultured turbot. Data expressed as weight % of total identified fatty acids and represent means ± SD of five wild and seven cultured fish. (*MFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids; *SFA* saturated fatty acids; – not detected or trace amounts < 0.1%)

Fatty acids	Total lipid		Phospholipid	
	Wild	Cultured	Wild	Cultured
14:0	2.7 ± 0.2	3.5 ± 0.2	1.4 ± 0.3	2.0 ± 0.3
15:0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
16:0	16.1 ± 0.3	14.9 ± 0.5	20.3 ± 1.0	19.2 ± 1.1
16:1 (n-9)	1.3 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
16:1 (n-7)	6.6 ± 1.1	5.6 ± 0.5	2.6 ± 0.4	2.2 ± 0.2
16:1 (n-5)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.0
16:2 (n-4)	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	–
16:4 (n-3)	0.6 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
17:0	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
18:0	4.0 ± 0.5	2.8 ± 0.1	5.3 ± 0.4	4.1 ± 0.2
18:1 (n-9)	15.9 ± 1.0	13.8 ± 0.5	6.9 ± 0.9	6.7 ± 0.5
18:1 (n-7)	4.9 ± 0.5	3.4 ± 0.1	3.4 ± 0.3	2.7 ± 0.1
18:1 (n-5)	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
18:2 (n-6)	1.2 ± 0.3	7.4 ± 0.7	0.8 ± 0.1	4.5 ± 0.6
18:3 (n-6)	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
18:3 (n-3)	0.5 ± 0.1	1.1 ± 0.1	0.2 ± 0.0	0.5 ± 0.0
18:4 (n-3)	0.6 ± 0.1	1.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
20:0	–	–	–	–
20:1 (n-11)	0.5 ± 0.2	0.7 ± 0.1	–	0.2 ± 0.0
20:1 (n-9)	1.6 ± 0.7	3.6 ± 0.4	1.1 ± 0.4	2.8 ± 0.4
20:1 (n-7)	0.5 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0
20:2 (n-6)	0.5 ± 0.1	0.7 ± 0.0	0.4 ± 0.1	0.6 ± 0.0
20:3 (n-6)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:3 (n-3)	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:4 (n-6)	3.0 ± 0.4	1.2 ± 0.1	4.9 ± 0.6	2.0 ± 0.2
20:4 (n-3)	0.7 ± 0.1	1.2 ± 0.1	0.4 ± 0.1	0.8 ± 0.1
20:5 (n-3)	7.8 ± 0.4	8.8 ± 0.5	9.9 ± 0.6	11.5 ± 0.7
22:0	–	–	–	–
22:1 (n-11)	0.4 ± 0.2	0.7 ± 0.2	0.2 ± 0.1	0.4 ± 0.1
22:1 (n-9)	–	0.2 ± 0.1	–	–
22:1 (n-7)	–	–	–	–
22:5 (n-3)	4.4 ± 0.3	2.7 ± 0.1	4.6 ± 0.6	2.7 ± 0.2
22:6 (n-3)	23.8 ± 1.1	23.0 ± 1.9	33.4 ± 0.5	33.3 ± 1.9
24:1 (n-9)	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.1
Total	100	100	100	100
Total SFA	23.5 ± 0.6	21.7 ± 0.7	27.8 ± 1.3	25.9 ± 1.5
Total MFA	32.5 ± 1.2	29.9 ± 1.2	16.5 ± 0.8	17.1 ± 0.9
Total PUFA	43.9 ± 1.3	48.4 ± 1.5	55.7 ± 1.0	57.0 ± 1.1
Total (n-3)	38.7 ± 1.2	38.6 ± 2.0	49.1 ± 1.1	49.5 ± 1.5
Total (n-6)	5.1 ± 0.6	9.7 ± 0.7	6.4 ± 0.7	7.5 ± 0.5
Total (n-3)/(n-6)	7.6 ± 0.9	4.0 ± 0.5	7.7 ± 0.9	6.6 ± 0.6

Results

Lipid comprised 13.8 ± 0.5% ($n = 5$) and 13.2 ± 0.7% ($n = 7$) of the egg dry weight in wild and cultured turbot, respectively. The relative fatty-acid composition of total lipid extracted from turbot eggs was dominated by PUFA, predominantly of the (n-3) series. (n-3) PUFA accounted for 38.7% of the total fatty acids in wild turbot and 38.6% in cultured turbot (Table 1). Monounsaturated fatty acids, mainly 18:1(n-9), and saturated fatty acids, mainly 16:0, comprised approximately 30 and 20%, respectively, of the total fatty acids in both groups of turbot. The major PUFA was DHA, which comprised 23.8 and 23.0% of the fatty acids in wild and cultured fish, respectively, and was also the most abundant fatty acid in both wild and cultured turbot eggs. The proportion of DHA was three times that of EPA, which constituted the second most abundant PUFA. Unidentified peaks made up less than 3% of the fatty acids in total lipid, provided that the crude

fatty-acid methyl esters were isolated with TLC prior to GLC.

Gas–liquid chromatography of the fatty-acid methyl esters from total lipids of turbot eggs without prior isolation by TLC resulted in several peaks, not identified as fatty acids, that eluted together with the fatty-acid methyl esters. These peaks were positively identified by GLC/MS as alcohols. Together these alcohols accounted for about 10% of the total peak area when the crude fatty-acid methyl esters from total lipids of turbot eggs were analysed on GLC. Three of these alcohols coeluted with fatty-acid methyl esters (18:2 n-6, 18:3 n-6 and 20:3 n-6) which is why it was obligatory to purify the fatty-acid methyl esters prior to GLC. Alcohols were present in the cholesterol–wax ester fraction but absent in phospholipids and triacylglycerols. When crude fatty-acid methyl esters from cholesterol–wax esters were analysed, alcohols constituted about 30% of total peak area. Alcohols were also found in substantial amounts in the oil globule.

Phospholipids, triacylglycerols and cholesterol–wax esters of turbot eggs all exhibited a specific fatty-acid profile distinctly different from that of total lipid (Tables 1, 2). Thus, phospholipids contained the highest

Table 2 *Scophthalmus maximus.* Relative fatty-acid composition of triacylglycerols, cholesterol–wax esters and oil globules from ovulated eggs of wild and cultured turbot. Data expressed as weight % of total identified fatty acids and represent means ± SD of five wild and seven cultured fish. (*MFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids; *SFA* saturated fatty acids; – not detected or trace amounts < 0.1%)

Fatty acids	Triacylglycerol		Cholesterol–wax esters		Oil globule	
	Wild	Cultured	Wild	Cultured	Wild	Cultured
14:0	5.2 ± 1.7	6.8 ± 1.2	0.2 ± 0.1	0.2 ± 0.1	2.8 ± 0.4	4.1 ± 1.0
15:0	0.7 ± 0.2	0.5 ± 0.0	–	–	0.4 ± 0.0	0.3 ± 0.0
16:0	19.6 ± 1.1	15.3 ± 0.8	1.6 ± 0.3	1.2 ± 0.2	12.0 ± 0.3	10.3 ± 0.5
16:1 (n-9)	2.2 ± 0.3	1.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	1.6 ± 0.1	1.1 ± 0.2
16:1 (n-7)	11.6 ± 1.9	10.6 ± 0.8	8.6 ± 1.0	7.0 ± 1.1	11.4 ± 1.4	9.8 ± 0.8
16:1 (n-5)	0.4 ± 0.1	0.4 ± 0.0	–	–	0.3 ± 0.0	0.3 ± 0.0
16:2 (n-4)	0.1 ± 0.1	0.4 ± 0.0	–	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.0
16:4 (n-3)	0.8 ± 0.1	0.6 ± 0.1	1.1 ± 0.1	0.5 ± 0.0	0.8 ± 0.0	0.3 ± 0.0
17:0	0.4 ± 0.1	0.2 ± 0.0	–	–	0.3 ± 0.1	0.2 + 0.1
18:0	4.5 ± 0.8	2.0 ± 0.2	1.0 ± 0.2	0.4 ± 0.1	3.1 ± 0.6	1.5 ± 0.2
18:1 (n-9)	21.1 ± 3.0	20.1 ± 0.8	29.0 ± 1.4	25.1 ± 2.3	24.7 ± 1.7	23.3 ± 1.1
18:1 (n-7)	7.3 ± 0.8	4.6 ± 0.3	5.0 ± 0.5	2.8 ± 0.3	6.6 ± 0.2	4.0 ± 0.2
18:1 (n-5)	0.5 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
18:2 (n-6)	1.2 ± 0.3	7.8 ± 0.5	2.4 ± 0.6	15.5 ± 1.3	1.7 ± 0.4	11.4 ± 0.8
18:3 (n-6)	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
18:3 (n-3)	0.4 ± 0.1	1.0 ± 0.1	1.1 ± 0.3	2.9 ± 0.2	0.7 ± 0.1	1.9 ± 0.2
18:4 (n-3)	0.7 ± 0.2	1.6 ± 0.3	1.2 ± 0.3	2.6 ± 0.4	0.9 ± 0.2	1.9 ± 0.3
20:0	–	–	–	–	0.2 ± 0.0	–
20:1 (n-11)	1.1 ± 0.5	1.4 ± 0.3	0.4 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.8 ± 0.3
20:1 (n-9)	2.6 ± 1.2	5.4 ± 0.8	1.8 ± 0.9	3.3 ± 0.5	1.5 ± 0.1	4.0 ± 0.7
20:1 (n-7)	0.6 ± 0.2	0.2 ± 0.0	0.7 ± 0.3	0.3 ± 0.0	0.5 ± 0.2	0.2 ± 0.1
20:2 (n-6)	0.4 ± 0.1	0.6 ± 0.0	0.9 ± 0.2	0.9 ± 0.1	0.5 ± 0.1	0.6 ± 0.0
20:3 (n-6)	–	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
20:3 (n-3)	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.1	0.3 ± 0.0
20:4 (n-6)	1.1 ± 0.2	0.4 ± 0.1	1.8 ± 0.5	0.4 ± 0.1	1.4 ± 0.2	0.3 ± 0.0
20:4 (n-3)	0.5 ± 0.1	1.0 ± 0.2	1.4 ± 0.3	2.4 ± 0.3	0.8 ± 0.1	1.4 ± 0.1
20:5 (n-3)	3.4 ± 0.3	4.1 ± 0.3	9.8 ± 0.8	9.3 ± 1.1	6.0 ± 0.2	5.7 ± 0.5
22:0	–	–	–	–	–	–
22:1 (n-11)	0.5 ± 0.4	1.1 ± 0.4	0.3 ± 0.2	0.5 ± 0.1	0.3 ± 0.0	0.6 ± 0.1
22:1 (n-9)	–	0.2 ± 0.1	–	–	–	0.1 ± 0.0
22:1 (n-7)	–	–	–	–	–	–
22:5 (n-3)	3.0 ± 0.6	1.9 ± 0.2	5.4 ± 0.5	2.8 ± 0.4	4.1 ± 0.4	2.1 ± 0.1
22:6 (n-3)	9.4 ± 0.5	8.8 ± 1.2	24.4 ± 2.9	19.2 ± 3.7	15.7 ± 1.1	12.3 ± 1.5
24:1 (n-9)	0.2 ± 0.0	0.1 ± 0.1	–	–	0.1 ± 0.0	0.1 ± 0.1
Total	100	100	100	100	100	100
Total SFA	30.5 ± 2.1	24.9 ± 1.1	2.8 ± 0.5	1.8 ± 0.3	18.9 ± 0.8	16.5 ± 0.8
Total MFA	48.1 ± 2.9	46.2 ± 1.5	46.5 ± 1.8	40.1 ± 4.1	47.7 ± 1.1	44.6 ± 2.0
Total PUFA	21.4 ± 1.3	28.9 ± 1.9	50.7 ± 2.2	58.0 ± 4.4	33.5 ± 0.6	38.9 ± 2.0
Total (n-3)	18.4 ± 1.0	19.4 ± 3.1	44.9 ± 3.1	40.3 ± 5.3	29.3 ± 0.3	25.8 ± 2.1
Total (n-6)	2.9 ± 0.4	9.2 ± 0.5	5.8 ± 1.0	17.5 ± 1.3	4.0 ± 0.7	12.7 ± 0.8
Total (n-3)/(n-6)	6.5 ± 0.6	2.1 ± 0.3	7.7 ± 1.9	2.3 ± 0.5	7.4 ± 1.5	2.0 ± 0.2

level of PUFA and the lowest level of monounsaturated fatty acids. The high degree of PUFA in phospholipids was mainly due to the preponderance of DHA, which accounted for 33.4 and 33.3% of the fatty acids in wild and cultured fish respectively, and the low level of monounsaturated fatty acids in phospholipids was mainly due to a relatively low level of 18:1(n-9). Triacylglycerols, compared to phospholipids, contained considerably less PUFA, such as DHA, EPA and 20:4(n-6), and were instead dominated by 16:0 and 18:1(n-9) (Table 2). The cholesterol–wax ester fraction was unique among the lipid classes in that saturated fatty acids were almost absent (Table 2).

The pattern of fatty-acid distribution in total lipid of the oil globule from wild and cultured turbot eggs was different from that of total egg lipids (Table 2). The oil globule was dominated by monounsaturated fatty acids followed by PUFA. The lower level of PUFA in the oil globule, compared to the total egg lipids, was mainly due to a lower percentage of DHA. Thin-layer chromatography revealed that the oil globule of turbot eggs contained triacylglycerols and cholesterol–wax esters, while phospholipids were absent (data not shown).

The fatty-acid profiles of the total lipid, the different lipid classes and the oil globules from cultured turbot eggs were in many ways similar to those of eggs from wild turbot. However, there were several differences evident in the relative fatty-acid distribution in the lipids of cultured and wild turbot eggs. These differences were extended to all lipid classes (Tables 1, 2). The most prominent difference was the greater proportion of linoleic acid (18:2 n-6) in the cultured turbot eggs. The higher level of linoleic acid was observed in total lipid as well as in phospholipids, triacylglycerols, cholesterol–wax esters and oil globules. In all these lipid fractions, the percentage of linoleic acid was approximately six-fold higher in lipids of cultured turbot eggs compared to corresponding lipids in wild turbot eggs. The proportion of linoleic acid was particularly high in the cholesterol–wax ester fraction of cultured turbot eggs, where it constituted 15.5% of total fatty-acid composition. Eggs from cultured turbot also contained somewhat greater proportions of 18:3(n-3), 18:4(n-3), 20:1(n-9), 20:4(n-3) and 22:1(n-11) than did eggs from wild turbot. On the other hand, the percentage of the 16 and 18 carbon saturated and monounsaturated fatty acids, e.g. 16:0, 16:1(n-7), 18:0, 18:1(n-9) and 18:1(n-7), was lower in cultured turbot eggs, as were 20:4(n-6) and 22:5(n-3). Cholesterol–wax esters and oil globules of cultured turbot eggs also contained lower levels of DHA.

The fatty-acid compositions of total lipid, phospholipids, triacylglycerols and cholesterol–wax esters of the eggs from each individual fish were subjected to multivariate data analysis. Fig. 1A shows the two component PC model obtained when the relative fatty-acid composition of total lipids of eggs from the 12 investigated turbot were analysed by PCA. The first two PC

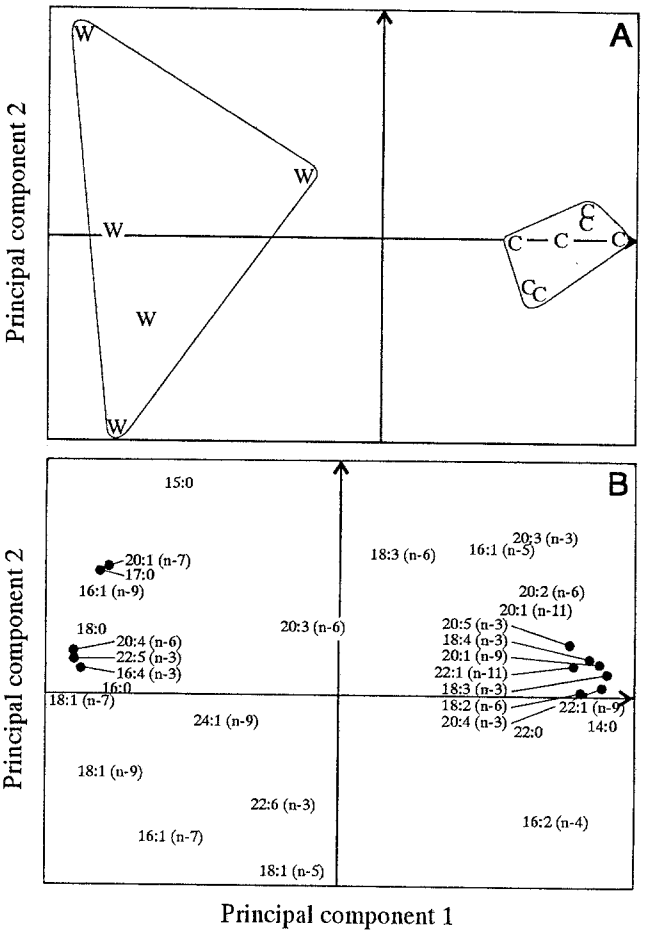

Fig. 1 *Scophthalmus maximus.* **A** Plot of the scores along first vs second principal component of the relative fatty-acid composition of total lipid from eggs of wild and cultured turbot with **B** the corresponding loading plot. The intercept between the two coordinate-axes in the plots corresponds to zero. A total of 32 fatty acids in 12 turbot were used for analysis. Principal Components 1 and 2 accounted for 59 and 7% of total variance, respectively. (*W* wild turbot; *C* cultured turbot)

together accounted for 66% (PC 1 for 59% and PC 2 for 7%) of the total variance in the data. The PCA shows that the fatty-acid composition was similar between individuals within each group of turbot, i.e. wild and cultured, and that each group was distinct. Furthermore, cultured turbot were more closely grouped in the PC model than the eggs of wild turbot, demonstrating that the eggs of cultured turbot exhibited less individual fatty acid variation. This was elucidated by encircling the two groups of fish in the PC plot. The eggs of wild and cultured turbot were mainly separated along PC 1. The loading plot (Fig. 1B) shows how the different fatty acids influenced the distribution of the samples in the corresponding PC plot. Since the largest difference between wild and cultured eggs was along PC 1, the fatty acids most responsible for this distribution were those far to the left and far to the right in the loading plot. Fig. 2 shows the two component PC

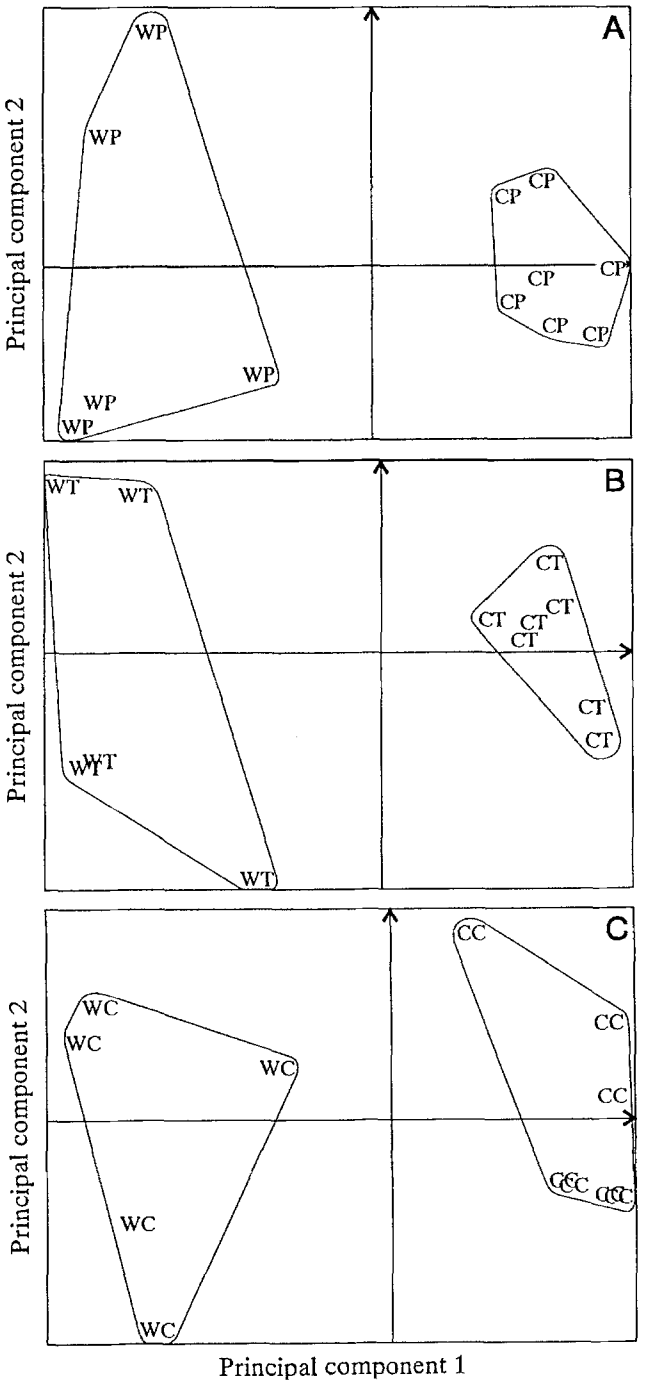

Fig. 2 *Scophthalmus maximus.* Plot of the scores along first vs second principal component of the relative fatty-acid composition of phospholipids, triacylglycerols and cholesterol–wax esters from eggs of wild (*W*) and cultured (*C*) turbot. The intercept between the two coordinate-axes in the principal component plot corresponds to zero. **A** Score plot of the first two principal components for fatty-acid composition of phospholipids (*P*) accounting for 56 and 10% of total variance, respectively. **B** Score plot of the first two principal components for fatty-acid composition of triacylglycerols (*T*) accounting for 54 and 11% of total variance, respectively. **C** Score plot of the first two principal components for fatty-acid composition of cholesterol–wax esters (*C*) accounting for 49 and 15% of total variance, respectively

models obtained when the relative fatty-acid composition of phospholipids, triacylglycerols and cholesterol–wax esters from wild and cultured turbot eggs were subjected to PCA. As for the distribution in the PC model obtained when the fatty-acid composition of total egg lipids was analysed, the wild and cultured turbot were grouped when each of the lipid classes was modelled. These groups were clearly separated from each other, mainly along PC 1.

Calculations based on light microscopical observations of cultured turbot eggs in the present study show that 55 to 60% of the total lipids in cultured turbot eggs are confined to the oil globule. These calculations are based on average egg and oil globule volumes of 0.52 µl and 3 nl, respectively. Furthermore, the size of the oil globule remained constant during embryogenesis, and a reduction in size occurred first after hatching and mainly after yolk depletion (Fig. 3).

Discussion

The present study shows that total lipid extracted from eggs of wild turbot was highly unsaturated. The richness of PUFA, particularly EPA and DHA, indicates that turbot embryos and yolk-sac larvae have a nutritional requirement for these fatty acids. The great proportion of DHA in lipids, predominantly in phospholipids, of turbot eggs is consistent with earlier findings on marine fish eggs (Tocher and Sargent 1984; Falk-Petersen et al. 1986) and has been suggested to be related to a specific requirement for DHA during the development of neural tissues, such as brain and retina (Sargent 1995). Neural tissues are highly enriched in DHA (Tocher and Harvie 1988), and during the early developmental stages in fish considerable growth and development of these tissues occurs, as reflected by the great proportion of neural tissues in the total body mass of the embryo and yolk-sac larva (Fig. 3).

The general pattern of the fatty-acid distribution in lipids of eggs from cultured turbot was similar to the distribution in lipids of wild turbot eggs. However, the relative amount of specific fatty acids differed markedly between eggs of cultured and wild fish. The fatty-acid composition of total lipid, phospholipids, triacylglycerols as well as cholesterol–wax esters fell into two distinct groups, eggs of wild and cultured fish, as demonstrated by PCA. The main advantage with PCA is that the combined influences of all fatty acids are used simultaneously for the comparison of the eggs from all investigated females (Ulvund and Grahl-Nielsen 1988). The main difference between egg lipids of cultured and wild turbot was the markedly greater proportion of linoleic acid in egg lipids of the cultured broodstock. Linoleic acid is scarce in the marine environment, and an unnatural deposition of this fatty acid in body lipids, including egg lipids, is typical of fish given a formulated

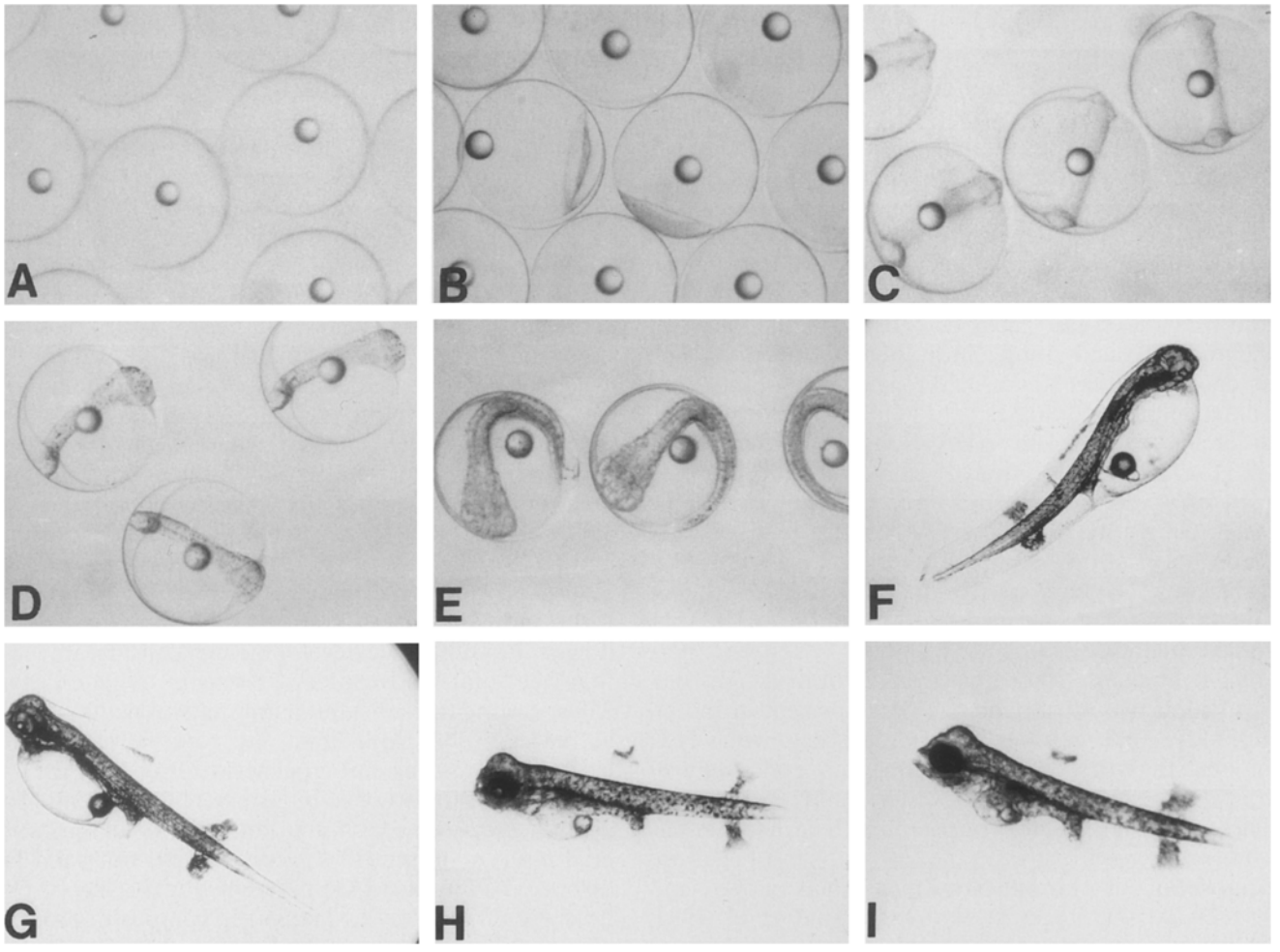

Fig. 3 *Scophthalmus maximus*. Photomicrographs of turbot eggs and larvae during development at 14° C. **A** Freshly ovulated eggs. **B–I** Eggs and larvae at different times after fertilization. **B** 18 h, **C** 2 d, **D** 3 d, **E** 4 d, **F** 5 d, **G** 6 d, **H** 7 d, **I** 8 d

diet containing vegetable oils (Watanabe et al. 1984; Sargent et al. 1989; Harel et al. 1994). The high content of linoleic acid in lipids of cultured turbot eggs in the present study was most likely caused by the wheat flour in the formulated diet. Lipids of cultured turbot eggs also contained higher levels of the long-chain monoenoic fatty acids, 20:1(n-9) and 22:1(n-11), compared to eggs from the wild stock. The higher percentage of these fatty acids indicates the presence of lipids from calanoid copepod feeding fish, such as herring and capelin, in the diet. Fish feeding on copepods store 20:1(n-9) and 22:1(n-11), and an abundance of these fatty acids is characteristic of virtually all commercial fish oils produced from northern Atlantic fisheries (Sargent 1995). Thus, the different levels of the fatty acids mentioned above, between eggs of wild and cultured turbot, were most probably caused by a difference in the dietary fatty-acid composition. However, neither the exact fatty-acid composition of the natural diet consumed by the wild fish, nor the fatty-acid composition of the formulated diet given to the cultured turbot, was known, and it must therefore be emphasised that the differences observed may not be entirely due to the diet. Finally, although the fatty-acid profile of eggs from wild and cultured turbot differed, total lipid, phospholipids and triacylglycerols of eggs from both groups of turbot contained the same great proportion of (n-3) PUFA, mainly DHA. Similar results were obtained for wild and cultured chinook salmon (Ashton et al. 1993). Thus, the apparent conservation of (n-3) PUFA, particularly DHA, by turbot females during the accumulation of egg lipids may be indicative of the essentiality of these fatty acids during early ontogeny.

Comparison of the data in the present study with previous findings on turbot eggs is complicated by the differences in the approaches taken. Planas et al. (1993a) reported the fatty-acid composition of total lipid extracted from cultured turbot eggs 1 d after fertilization, which may partly explain the greater proportion of 18:0 and 18:1(n-9) and the considerably lower

level of DHA compared to both groups of turbot in the present study. McEvoy et al. (1993) fractionated the lipids extracted from freshly ovulated eggs into total neutral lipids and phospholipids prior to fatty-acid analysis and observed a markedly greater proportion of 18:1(n-9) in phospholipids. Turbot eggs in earlier studies exhibited a much larger individual variation in total lipid content (Devauchelle et al. 1988; McEvoy et al. 1993), and the fatty-acid profile was also much less consistent among individual females (McEvoy et al. 1993). The apparent discrepancies between the present and previous studies may be caused by differences in dietary fatty-acid composition, but other factors such as developmental stages of the eggs, egg quality, and analytical methodologies used may contribute to these differences as well. The observation in the present study that the fatty alcohols derived from wax esters coeluted with fatty-acid methyl esters upon GLC shows that the fatty-acid methyl esters must be isolated by TLC after transesterification before GLC. It is not clear if this isolation procedure was conducted in earlier studies, and this may be one further explanation for the observed differences.

The lipids of turbot eggs are present in two distinct forms; the yolk lipids and the lipids present in the oil globule. Lipid extractions and analysis with TLC showed that the oil globule consisted exclusively of triacylglycerols, cholesterol esters and wax esters. These observations are consistent with earlier findings on other teleosts, showing that oil globules of eggs are comprised of non-polar lipids (Nakagawa and Tsuchiya 1971; Léger et al. 1981; Brind et al. 1982; Eldridge et al. 1983; Moodie et al. 1989). Estimations made in the present study indicate that a considerable amount (55 to 60%) of the lipids in turbot eggs are confined to the oil globule. These calculations are based on an average oil globule volume of 3 nl which is in good agreement with an average oil globule volume of 4 nl previously reported for cultured turbot eggs (Rønnestad et al. 1992). Oil globules also make up a considerable amount (> 50%) of the egg lipids in striped bass (Eldridge et al. 1983), walleye (Moodie et al. 1989) and a cyprinodontid fish (Brind et al. 1982). Considering that phospholipids appear to constitute 40 to 50% of the total lipids present in turbot eggs (Devauchelle et al. 1988; McEvoy et al. 1993; Planas et al. 1993b) and that no phospholipids are present in the oil globule, it is reasonable to suggest that the yolk lipids of turbot eggs to a large extent consist of phospholipids. This is in good agreement with earlier studies showing that phospholipids dominate the yolk lipids in both eggs with oil globules (Nakagawa and Tsuchiya 1971; Eldridge et al. 1983; Moodie et al. 1989) and in eggs without oil globule (Tocher and Sargent 1984; Falk-Petersen et al. 1986). Lipids of turbot egg yolk were not analysed in the present study, because the yolk could not be completely separated from the oil globule. Previously presented centrifugation methods for the separation of yolk and oil globules from fish eggs were not useful for turbot (Léger et al. 1981; Eldridge et al. 1983; Moodie et al. 1989). Thus, after homogenization and centrifugation of the eggs, numerous lipid droplets, i.e. an emulsion, were found in the yolk fraction, even when the fraction was repeatedly stirred and recentrifuged. These droplets were apparent when the centrifuged yolk fractions were observed by a dissecting microscope. However, the finding that the lipid class composition differed so drastically between total egg lipids and oil globule confirmed that our technique successfully separated oil globule from yolk lipids, i.e. that a pure fraction of oil globule was obtained.

The finding that the size of the oil globule in cultured turbot eggs in the present study remains constant during embryogenesis, and a reduction in size occurs first after hatching and mainly after yolk depletion, confirms earlier reports on turbot (Rønnestad et al. 1992) and other teleosts (Brind et al. 1982; Eldridge et al. 1983; Moodie et al. 1989). The fact that the oil globule is not metabolised during embryogenesis in turbot implies that the total amount of lipids utilised during the embryonic development is considerably less than the total lipid content present in ovulated eggs. Thus, despite the substantial amounts of neutral lipids in the egg, only yolk lipids are metabolised to any larger extent during embryogenesis. Altogether, the approach that the fatty-acid requirements of fish embryos are indicated by the fatty-acid composition of the initial lipid reserves present in ovulated eggs is not valid for turbot. The fatty-acid requirements for the turbot embryo are more likely to match the composition of the phospholipids of the egg. This means that the requirement of (n-3) PUFA, mainly DHA, is considerably greater than previously indicated by analysis of total lipids in turbot eggs.

Although the maternal source of egg lipids has never been identified for fish, it seems reasonable to assume that the hepatically synthesised egg yolk precursor, vitellogenin, plays a fundamental role in the process of lipid accumulation in the growing teleost oocyte. This assumption is based on three criteria, (1) large amounts of vitellogenin are incorporated into growing oocytes of teleosts (Tyler 1993; Hyllner et al. 1994), (2) teleost vitellogenin is a lipoprotein containing 16 to 21% lipids (dry wt) (Hori et al. 1979; Norberg and Haux 1985; Norberg 1995; Silversand and Haux 1995) and (3) these lipids are characterised by a high content of (n-3) PUFA (Silversand and Haux 1995), which is also a characteristic feature of teleost egg lipids. In fact, the fatty-acid composition of total lipid from vitellogenin of wild cod (Silversand and Haux 1995) is strikingly similar to the composition of total lipids from wild cod eggs (Klungsøyr et al. 1989). However, comparison of the fatty-acid composition of total lipids from wild turbot eggs in the present study with the composition of total lipids from vitellogenin of wild turbot (Silversand and Haux 1995) reveals several

differences. Egg lipids contained a higher percentage of monounsaturated fatty acids (33 vs 23%), mainly 18:1(n-9), and a lower percentage of saturated fatty acids (24 vs 32%), mainly 16:0, compared to lipids of vitellogenin. Furthermore, turbot eggs contained wax esters which were not detected in vitellogenin from turbot. These findings indicate that vitellogenin is not the only source of lipids for turbot eggs and other sources must be considered. Lipids have been suggested to accumulate in growing teleost oocytes by the uptake of circulating lipoproteins other than vitellogenin (Léger et al. 1981; Black and Skinner 1987; Nagler and Idler 1990; Wallaert and Babin 1992) or by endogenous lipogenesis within the oocytes (Wiegand and Idler 1982). In opaline gourami (*Trichogaster cosby*), a species that accumulates large amounts of wax esters in the egg oil globule, Sand et al. (1969) reported that wax esters were synthesised within the eggs. Thus, the discrepancies in the apparent relationship between the lipids of vitellogenin and eggs between cod and turbot may be correlated to the fact that turbot eggs contain an oil globule which is not present in cod eggs. Lipids of teleost vitellogenin are characterised by a high content of phospholipids (Hori et al. 1979; Norberg and Haux 1985; Norberg 1995) and (n-3) PUFA (Silversand and Haux 1995), which also has been indicated for the egg yolk of turbot in the present study. Taken together, it is tempting to hypothesise that yolk lipids are derived from vitellogenin, and the lipids of the oil globule originate from other lipoproteins or by autosynthesis in the oocyte. This hypothesis highlights the importance of vitellogenin and the lipid composition of this lipoprotein for teleost embryonic development. Furthermore, this interpretation is different from the earlier view of Léger et al. (1981), where it was suggested that lipoproteins other than vitellogenin may enter the oocytes and serve as the major source of yolk lipids, whereas lipids from vitellogenin were preferentially deposited in the oil globule in rainbow trout.

In conclusion, the requirement for fatty acids during embryogenesis is not reflected by the fatty-acid profile of total lipids but rather by the profile of egg phospholipids. This implies that the need for (n-3) PUFA is considerably greater than that which can be deduced from analysis of total egg lipids. Although the relative content of specific fatty acids differed between eggs of wild and cultured turbot, there were no apparent signs of nutritional imbalance affecting the reproductive performance in cultured turbot. The cultured turbot in the present study were fed an artificial diet containing lipid based on marine oil, known to be particularly rich in (n-3) PUFA, and the dietary level of fatty acids allowed high fertilization and hatching percentages as well as normal growth and development of the embryo and yolk-sac larva.

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