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Abstract

A 28-day feeding trial was conducted for comparing the effect of different dietary phospholipid (PL) classes on the growth of post-larval turbot and on the incorporation of dietary neutral lipid fatty acids into their body lipids. Prior to the experiment the turbot were weaned for one week on a PL-free diet. The nine experimental diets were isolipidic and contained an equal amount of highly unsaturated fatty acids in the form of ethyl esters. They differed by their PL content (0, 1 or 2%) and by the PL class composition of the added soybean PL fractions.

Compared to the PL-free diet, diets enriched with phosphatidylcholine (PC) resulted in a better growth, a higher triglyceride content (% body dry matter) and increased levels of docosahexaenoic acid (% total fatty acids) in each of the examined body lipid classes (neutral lipid, phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol). The effects of the other soybean PL fractions were less explicit than those noted for soybean PC.

The results support the idea that dietary PC plays a role in the intestinal absorption of neutral lipid fatty acids. This might, at least partially, explain the superiority of PC for enhancing growth.

Abbreviations: DHA – docosahexaenoic acid (22:6n-3); EPA – eicosapentaenoic acid (20:5n-3); HUFA – highly unsaturated fatty acid; PA – phosphatidic acid; PC – phosphatidylcholine; PE – phosphatidylethanolamine; PI – phosphatidylinositol; PL – phospholipid; PS – phosphatidylserine; PUFA – polyunsaturated fatty acid.

Introduction

Considerable attention has been paid to n-3 highly unsaturated fatty acids (HUFA) in nutritional studies on marine fish larvae (Watanabe et al. 1983; Izquierdo et al. 1989; Watanabe 1993). It is usually accepted that marine fish have a poor elongation-desaturation capacity and that the provision of EPA (20:5n-3) and DHA (22:6n-3), instead of shorter chain n-3 fatty acids, is necessary to meet the requirement (Cowey et al. 1976; Sargent et al. 1993). The same applies for the fatty acids of the n-6 series, as 18:2n-6 can not be transformed in 20:4n-6 (Bell et al. 1994; Castell et al. 1994).

The provision of n-3 HUFA to marine fish larvae is mostly performed by enriching the live preys (*Brachionus* and *Artemia*) with neutral lipid rich in these HUFA (Léger et al. 1987; Dhert et al. 1990; McEvoy et al. 1996). Also the weaning diets of marine fish are supplemented with n-3 HUFA-rich lipid sources. A concentrated form consists of fatty acid ethyl esters prepared from fish oil (Coutteau et al. 1995), which were demonstrated to result in a DHA level in the fish proportional to that in the diet (Coutteau et al. 1996). Recently, we established that for a similar supply of DHA ethyl esters by the diet, the DHA level in the fish was increased by supplementing the weaning diet with phospholipids (PL), either of vegetable or of animal origin (Geurden et al. 1997b, c).

The aim of the present study was to compare the efficiency of different PL classes for enhancing the incorporation of HUFA presented in the diet as neutral lipid. For this purpose, diets having the same amount of HUFA ethyl esters were supplemented with PL fractions with very different lipid class composition and fed to newly-weaned turbot. These PL fractions were prepared from soybean PL and were consequently free of HUFA. Assuming the absence of significant elongation-desaturation activities, the capacity of each dietary PL fraction to increase the incorporation of the dietary HUFA into the fish was directly assessed by the level of these HUFA in fish lipid. Several body lipid classes (NL, PC, PE, PI) were examined since they respond differently to dietary fatty acids, as shown *in vitro* and *in vivo* (Tocher and Mackinlay 1990; Bell et al. 1994). Furthermore, the growth-promoting effect of the various experimental PL fractions was compared and eventual changes in the lipid class composition of the turbot in relation to that of the diet were examined.

Materials and methods

Fish and experimental design

Unweaned 36-day old turbot, *Scophthalmus maximus,* obtained from Ferme Marine de Douhet (France), were stocked for one week in two 1 m^3 tanks for acclimation to the laboratory conditions. During the first 4 days the larvae were weaned by gradually diminishing the number of freshly-hatched *Artemia* nauplii (EG grade, INVE Aquaculture N.V., Belgium) and increasing the amount of weaning diet, which was fed in excess for the remaining acclimation period.

At the age of 43 days, the larvae were randomly stocked in the recirculating rearing system (Coutteau et al. 1995) at a density of 67 ind. per 30 l tank. From the first day of stocking, each experimental diet was given to triplicate groups by means of automatic feeders (Charlon and Bergot 1984) delivering food every 30 min throughout the artificial 12-h light period. The feeding experiment lasted 28 days and general rearing conditions were as previously given (Geurden et al. 1997b). Temperature, salinity and ammonia-N averaged around 19 °C, 32 g $l^{-1}$and 0.3 mg l^{-1}, respectively.

Experimental diets

The nine experimental diets were prepared according to Coutteau et al. (1995). The common extruded basal diet (92.5% diet) has been described previously (Geurden et al. 1997b) and was coated with different lipid fractions (7.5% diet). These fractions contained equal amounts of the fish oil ethyl ester concentrate providing the HUFA, but differed by their PL components (Table 1). Hydrogenated coconut oil was used for rendering the diets isolipidic. All PL-sources were derived from de-oiled soybean PL, containing glycolipids as major impurity, and their PL content varied between 52 and 100% (% PL-source). The negative control diet D1 was not supplemented with PL. The other diets contained 2% total PL, except diets D2 and D7 which contained 1% PL. According to the PC-content (diet weight%), 3 categories of diets can be distinguished: PC-free (D1), PC-poor (D2-D5, <0.5% PC) and PC-rich (D6-D9, > 0.5% PC) diets. The PC-poor diet D2 contained PI-enriched PL (100% PL), diets D3, D4, D5 and D8 were supplemented with PL-fractions enriched up to about 50% of their PL-content in PA, PI, PE and PC, respectively. Diet D6 contained the original soybean PL blend. Diets D7 and D9 contained respectively 1 and 2% highly purified soybean PC.

The diet particle size was 500-800 μm. A smaller-sized (300-500 μm) diet with identical lipid composition as diet D1 served as weaning diet. The PL composition of the diets shown in Table 1 is calculated from analytical data of the PL-source (Vandemoortele N.V., Belgium; Lucas Meyer GmbH, Germany; INRA Laboratoire de Biochimie et Technologie des Protéines, France) and does not take into account the 0.2% PL included in the basal diet (Geurden et al. 1997b).

Sampling

The final survival was calculated from daily mortality percentages based on the actual number of fish in each tank. For the initial wet weight determination 60 fish were sampled. The intermediate growth data were based on a weekly sample of 5 fish taken randomly in each of the 3 replicate dietary treatment groups and final weight on a sample of 25 fish from each group. The fish were starved for 16 h prior to sampling, dried on a paper towel, weighed individually and frozen at −40 °C. Dry weight was determined on freeze-dried samples, corrected for residual water content obtained by placing subsamples (100 mg) of homogenized freeze-dried fish in an oven (60 °C) for 24 h. The

Table 1. Formulation of the variable part of the coated lipid fractions[1] and the phospholipid (PL) composition of the experimental diets[2]

Diet	D1	D2	D3	D4	D5	D6	D7	D8	D9
Major diet PL[3]	-	PI	PA	PI	PE	SL	PC	PC	PC
Coated lipid fraction (% diet)									
PL-source[4]	-	1.0	3.7	3.5	2.5	3.2	1.0	3.1	2.1
Hydrogenated oil[5]	4.4	3.4	0.7	1.0	1.9	1.3	3.4	1.4	2.4
Fish oil EE[6]	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
PL content (mg g^{-1} diet)									
PC	-	0.3	1.1	1.7	4.9	6.6	9.8	12.0	19.6
PE	-	2.0	4.4	5.0	9.6	6.3	0.1	6.5	0.2
PI	-	6.4	4.1	11.2	2.9	4.5	-	0.6	-
PA	-	0.8	10.2	1.8	0.8	2.0	-	-	-
Total PL[7]	-	10	20	20	20	20	10	20	20

[1] The total coated fraction comprised 7.5% of the diet weight. Additional components were: 0.5% emulsifier (INVE Aquaculture N.V., Belgium), 0.02% Vit E (dl-alfa-tocopherol acetate, Roche N.V., Belgium) and 0.01% ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinolin, Sigma, E-8260); [2] Theoretical values, calculated from weight% composition PL-source; [3] PI: phosphatidylinositol, PA: phosphatidic acid, PE: phosphatidylethanolamine, SL: soybean lecithin with PC and PE as major PL classes, PC: phosphatidylcholine; [4] All PL-sources are derived from de-oiled soybean PL. D2: purified PI-enriched soybean PL (100% PL) (INRA, Laboratoire de Biochimie et Technologie des Protéines, Nantes, France); D3, D4, D5, D8: de-oiled soybean lecithin enriched in PA (54% PL), PI (57% PL), PE (79% PL) and PC (66% PL), respectively, and D6: de-oiled soybean lecithin (63% PL, Vamothin F1) (Vandemoortele N.V., Belgium), D7, D9: highly purified soybean PC (Epikuron 200, 97% PC) (Lucas Meyer GmbH, Germany); [5] Hydrogenated coconut oil, Cocos 32/43 (Vandemoortele N.V., Belgium); [6] Concentrate of ethyl esters containing approximately 50% n-3 HUFA (INVE Aquaculture N.V., Belgium); [7] Total includes lyso PC and N-acyl PE.

homogenized fish powder was pooled according to the dietary treatment groups and stored under vacuum at −40 °C for further analysis.

Lipid analysis

The fatty acid composition of the diets was determined by a direct esterification following Lepage and Roy (1984), using 23:0 as internal standard. Turbot whole body lipid was extracted from triplicate subsamples according to Folch et al. (1957). The lipid extracts were dried under nitrogen, weighed, dissolved at a concentration of 10 mg ml^{-1} chloroform / methanol (2:1, v/v plus 0.01%, w/v butyl hydroxytoluene) and stored at −20 °C.

The lipid composition of the tissues was analyzed using unidimensional, double development, high-performance thin-layer chromatography (HPTLC) according to Olsen and Henderson (1989). The 10 × 10 cm silica gel 60 plates were predeveloped in hexane: diethylether (1:1) for the removal of impurities whereafter they were dried for 30 min at 110 °C. Approximately 30 μg of lipid extract was spotted on the 2 mm spot sites. The polar development system consisted of methyl acetate: isopropanol: chloroform: methanol: 0.25% aqueous KCl (25:25:25:10:9) and the subsequent neutral system of hexane: diethylether: acetic acid (85:15:1.5). After development, the plates were dried under vacuum, sprayed with 3% (w/v) cupric acetate in 8% (v/v) phosphoric acid and charred for 20 min at 160 °C. The plates were scanned by a Sharp JX-325 color scanner (reflection mode). The optical density reading of the scanner was calibrated according to a grey-value reference standard (Kodak). The scanner was connected to image analysis software (ImageMaster, Pharmacia Biotech, the Netherlands) which performed the peak integration (surface × optical density). Identification of peaks was based on standards (Avanti Polar Lipids, Inc., USA).

For the preparation of the fatty acid methyl esters of fish PC, PE, PI and NL, approximately 2.5 mg of lipid was applied on 20 × 20 cm glass plates coated with silica gel 60. The classes were separated in the same polar solvent mixture as for HPTLC. The spots were visualized under UV light after spraying the plates with 0.1% 2′–7′ dichlorofluorescein in 97%

Table 2. Fatty acid composition of the experimental diets (mg g^{-1} dry diet) [1]

Diet	D1	D2	D3	D4	D5	D6	D7	D8	D9
Major diet PL	-	PI	PA	PI	PE	SL	PC	PC	PC
14:0	14.7	12.7	9.1	9.5	10.5	9.8	12.8	9.5	10.8
16:0	14.5	13.8	15.7	13.5	13.3	14.8	12.6	13.2	15.9
16:1n-7	0.6	0.5	0.6	0.6	0.5	0.6	0.5	0.6	0.7
18:0	11.2	10.2	8.6	9.0	8.4	9.2	10.5	8.3	10.4
18:1n-9	6.4	6.1	7.8	8.0	7.7	8.1	6.1	7.9	7.8
18:1n-7	0.6	0.6	0.9	1.0	1.0	0.9	0.9	1.0	0.8
18:2n-6	4.2	6.9	15.5	12.3	12.5	14.6	8.5	15.6	13.0
18:3n-3	0.4	0.6	1.8	1.3	1.2	1.8	0.9	1.5	2.0
20:0	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2
20:1n-9	0.6	0.5	0.5	0.6	0.6	0.6	0.6	0.6	0.5
20:4n-6	0.3	0.3	0.3	0.4	0.4	0.4	0.3	0.4	0.3
20:5n-3	5.5	5.3	5.4	5.3	5.4	5.5	5.1	5.2	5.3
22:5n-6	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.3	0.2
22:5n-3	1.2	1.2	1.2	1.1	1.2	1.3	1.1	1.2	1.2
22:6n-3	12.4	12.2	11.9	11.4	12.0	12.4	11.5	11.6	11.9
Saturates	40.6	37.0	33.8	32.4	32.6	34.1	36.3	31.4	37.4
Monoenes	8.4	8.0	10.1	10.3	9.9	10.5	8.3	10.2	10.2
n-6 PUFA	4.9	7.5	16.0	12.8	13.2	15.2	9.1	16.3	13.5
n-3 PUFA	20.1	19.9	21.2	20.1	20.4	21.8	19.3	20.2	20.4
n-3 / n-6	4.1	2.7	1.3	1.6	1.6	1.4	2.1	1.2	1.5

[1] Data represent means of duplicate esterifications and sums include minor fatty acids not listed in the table.

ethanol. The classes were scraped off and transmethylated overnight at 50 °C (Christie 1982). The resulting methyl esters were analyzed by on column injection in a Chrompack CP 9001 gas chromatograph (GC) with H_2 as carrier gas. The temperature was programmed to raise from 85 (injection) to 150 °C at 30 °C per min, from 150 to 152 °C at 0.1 °C per min, from 152 to 172 °C at 0.65 °C per min and further from 172 to 187 °C at 25 °C per min. The final temperature of 187 °C was maintained for 7 min. The GC was equipped with a 2.5 m methyl deactivated precolumn connected to a 50 m × 0.32 mm i.d. polar capillary column (BPX70, SGE, Australia). Peak identification was based on reference standards (Nu-Check Prep. Inc, USA).

Statistical analysis

Survival, weight, fish total lipid content and fish lipid class and HUFA composition were subjected to a one-way analysis of variance (ANOVA) for evaluating the effect of the dietary PL composition. Only the HUFA were considered in the ANOVA as these fatty acids were supplied in equal amounts by the different diets. For testing the effects of diet, lipid class and sampling period a three-way ANOVA was performed on the HUFA (log transformed) detected in NL, PC and PE for both day 7 and day 28. HUFA levels in PI were available only for day 28 and were compared in a separate one-way ANOVA. When a significant ($p<0.05$) difference was evidenced by the ANOVA, data were further analyzed by Duncan 's multiple range test (Statistica, StatSoft Inc.).

Results

Dietary fatty acid composition

Table 2 summarizes the fatty acid composition of the diets. They all contained similar amounts of essential HUFA, related to the equal amount of fish oil ethyl esters. DHA accounted for 1.1-1.2%, EPA for 0.5% and 20:4n-6 for 0.3-0.4% of diet dry weight. Variations between the diets were noted for 18:2n-6 and 18:3n-3, depending on the soybean PL added to the diets. The amount of saturated fatty acids varied according to the level of hydrogenated coconut oil. The

Table 3. Survival (%), weight (mg), lipid content and lipid class composition of turbot at the start (initial) and at the end of the 28-day feeding trial[1]

Diet	Initial	D1	D2	D3	D4	D5	D6	D7	D8	D9
Major diet PL		-	PI	PA	PI	PE	SL	PC	PC	PC
Survival (%)		87	90	93	87	91	90	85	92	99
Wet weight (day 7)	210	364	404	374	420	391	448	397	440	443
Wet weight (day 28)	210	930^{d}	110^{cd}	1030^{cd}	1230^{bc}	1210^{bc}	1240^{bc}	1410^{ab}	1310^{abc}	1460^{a}
Dry weight, DW (day 28)	36.4	161^{d}	188^{cd}	176^{cd}	214^{bc}	215^{bc}	223^{b}	254^{a}	242^{ab}	268^{a}
Total lipid (% DW)	10.3	10.7^{d}	12.4^{b}	12.6^{b}	13.4^{ab}	11.7^{c}	13.6^{a}	13.8^{a}	13.8^{a}	14.2^{a}
Total lipid composition of turbot whole body (% total lipid)										
Total neutral lipid	47.9	48.0^{c}	52.7^{a}	51.0^{b}	49.3^{b}	49.5^{b}	52.5^{a}	51.4^{b}	53.1^{a}	53.4^{a}
Triglyceride	20.9	22.0^{d}	27.0^{ab}	22.9^{c}	26.3^{b}	24.8^{b}	27.5^{a}	25.5^{b}	27.9^{a}	28.1^{a}
Cholesterol	14.6	13.2^{b}	13.1^{b}	14.4^{a}	11.9^{c}	12.2^{c}	11.9^{c}	12.2^{c}	12.2^{c}	11.8^{c}
Free fatty acid	2.2	2.1	2.1	2.3	1.9	3.0	2.9	3.0	3.0	2.5
Sterol ester	10.2	10.7	10.6	11.4	9.3	9.5	10.2	10.7	10.0	11.0
Total polar lipid[2]	52.1	52.0^{a}	47.3^{c}	49.0^{b}	50.7^{b}	50.5^{b}	47.5^{c}	48.6^{b}	46.9^{c}	46.5^{c}
Polar lipid composition of turbot whole body (% total identified PL)										
Phosphatidylcholine[3]	50.9	50.3	49.2	51.6	49.8	49.0	51.0	48.9	49.9	50.2
Phosphatidylethanolamine	26.3	26.0	26.0	26.2	25.1	26.3	25.8	25.7	27.5	27.6
Phosphatidylserine	9.4	10.6	11.2	9.9	10.4	10.8	10.2	11.1	9.5	9.4
Phosphatidyinositol	7.2	7.0	7.7	6.9	8.4	7.8	6.9	8.1	7.0	7.6
Phosphatidic acid[4]	6.2	6.1	5.9	5.5	6.3	6.1	6.1	6.2	6.2	5.2

[1] Mean values (n=3 for rearing results and total lipid, n=2 for lipid class compositions) in a same row sharing a common superscript are not significantly different ($p>0.05$). The coefficient of variation for each mean value never exceeded 5%; [2] Total polar lipids include unidentified glycolipids and pigments; [3] PC includes a small amount of lyso PC and sphingomyelin (< 2% of total PL); [4] PA includes cardiolipin.

total lipid content of the diets was $12.5 \pm 0.3\%$ of diet dry weight.

Growth, lipid content and lipid class composition of turbot

The principal rearing characteristics of the turbot are shown in Table 3. Final survival was high (85-99%), without significant difference among the various treatment groups. The wet body weight of the turbot increased by a factor 1.7-2.1 after 7 days. After 28 days this factor was comprised between 4.4 and 6.9, depending on the diet. As compared to the PL-free diet D1, all PL-supplemented diets, except diets D2 and D3, resulted in a significantly higher final weight. The best growth was obtained with the diets containing the highest levels of PC (D7, D8, D9), without difference between a 1 (D7) or 2% (D9) dietary PC supply. Turbot fed the PC-rich phospholipid fractions also contained more total lipid per unit of dry matter and more triglycerides in their body lipids than those fed the PL-free diet. When calculating the amount of triglycerides per individual fish, it was found that fish fed the PC-rich diet D9 had almost three-fold the amount of triglycerides found in fish fed the PL-free diet D1 (11 mg vs. 4 mg per fish). Regarding the fish polar lipid components, PC and PE were the major PL classes together accounting for about 75% of total body PL. The polar lipid class composition of the fish, expressed as a percentage of total body PL, was very uniform in all dietary groups and did not reflect the differences in polar lipid composition of the diets.

Fatty acid composition of initial turbot

Table 4 details the fatty acid (FA) profiles in neutral lipid (NL), PC, PE and PI of whole turbot at the start of the experiment. Initial PC and PI were the most saturated with 16:0 and 18:0 as most abundant saturated FA, respectively. PE was the less saturated and con-

Table 4. Fatty acid composition of major lipid classes of turbot whole body at the beginning of the feeding trial (% fatty acids)

	PL class initial turbot			
	NL	PC	PE	PI
14:0	4.1	3.5	0.5	1.4
16:0	11.4	27.4	8.1	6.2
16:1n-7	4.7	1.1	0.6	1.1
18:0	4.3	5.0	10.3	29.6
18:1n-9	19.3	18.6	12.2	8.2
18:1n-7	6.1	6.6	7.6	3.5
18:2n-6	5.5	5.8	3.2	1.9
18:3n-3	15.1	6.4	3.5	2.2
18:4n-3	2.0	0.5	0.5	0.2
20:0	0.2	0.2	0.2	0.1
20:1n-9	0.8	0.5	0.7	0.4
20:1n-7	0.2	0.1	0.2	0.1
20:2n-6	0.5	0.7	0.6	0.5
20:3n-6	0.2	0.2	0.2	0.3
20:4n-6	1.4	1.7	3.6	16.8
20:3n-3	1.8	1.1	1.8	0.6
20:4n-3	0.9	0.7	0.7	0.4
22:0	0.2	0.2	tr	0.1
20:5n-3	8.6	7.7	10.0	9.3
22:1n-9	0.1	0.1	-	0.3
22:1n-7	0.7	0.1	-	0.4
22:4n-6	0.1	0.1	0.4	0.1
22:3n-3	0.1	tr	0.1	-
22:5n-6	0.2	0.2	0.8	0.2
22:4n-3	0.1	-	-	-
24:0	0.1	-	-	0.1
22:5n-3	2.3	1.9	6.0	3.7
24:1n-9	0.1	0.4	-	0.1
22:6n-3	4.8	6.2	25.6	10.0
Saturates	21.9	37.7	21.0	38.9
Monoenes	34.3	29.1	22.4	14.8
n-6 PUFA	8.1	8.8	8.6	19.9
n-3 PUFA	35.7	24.4	47.9	26.3
n-3 / n-6	4.4	2.8	5.5	1.3

[1] Values represent means of the duplicate lipid extracts. Sums include minor fatty acids not listed in the table, tr <0.1, -: not detected.

tained 18:0 as major saturated FA. The highest level of monoenoic FA was found in NL and PC, the lowest in PI. NL and PI had similar proportions of total PUFA, but with a very distinct n-3/n-6 ratio of 4.4 and 1.3, respectively. This ratio was lowered in the turbot PI fraction due to the important accumulation of n-6 HUFA, more specifically 20:4n-6. The high amount of n-3 PUFA in the NL of the initial fish was mainly due to 18:3n-3, accounting for 15% in NL vs 2.2-6.4% in the polar lipid classes and was characterized by a very low DHA/EPA ratio (0.6). The DHA/EPA ratio was the highest in PE (2.6) where DHA accounted for 26% of total FA.

Major changes in the fatty acid profiles of turbot due to the feeding

The fatty acid profiles of the body lipid classes of turbot, fed the diets for 7 and 28 days, are summarized in Tables 5 and 6, respectively. The NL fraction showed the strongest dietary response. In NL, saturated fatty acids increased from 22 to 31-36% after 7 days and to 32-42% after 28 days. The amounts of monoenes dropped from 34 to 25-31% after 7 days, down to 21-28% in final fish. Total n-6 PUFA in NL varied between 6 and 21% according to the diet. Despite the doubling of DHA, the percentage of total n-3 PUFA decreased due to the important decline of 18:3n-3 and, to a lesser extent, of 20:5n-3. The fatty acid groups in whole body PC reacted similarly as in NL, but not as drastically. In PE and PI the proportions of the major fatty acid groups remained more constant.

The PL-deprived turbot stored in all body lipid classes a higher amount of monoenoic and of saturated FA and lower amounts of n-6 and n-3 PUFA than the PL-fed fish (Tables 5 and 6). The levels of monoenoic FA and of n-3 PUFA in the fish were not in accordance with the dietary levels, as they were similar in the PL-free and the PL-supplemented diets. The higher level of 18:2n-6 in the PL-fed fish reflected the higher dietary level. However, when comparing the 18:2n-6 levels among the PL-supplemented groups, an effect of the PL class composition of the diet was noted. For instance, in spite of the similar amounts of 18:2n-6 in the PA-rich diet D3 and in the PC-rich diet D8, fish receiving diet D8 had incorporated a higher amount of 18:2n-6. In all body classes, increased levels of 18:2n-6 were accompanied by increased levels of 20:2n-6. In the NL and PC fractions of the final fish, the dead-end elongation product 20:2n-6 accounted often for more than 4 times the level of 20:4n-6.

Effect of dietary PL class composition on HUFA incorporation in turbot

Tables 7 and 8 give the results of the 3-way analysis of variance for evaluating the effect of the diet (D), the sampling period (P) and the lipid class (C) on the retention of the dietary HUFA in the fish. The most

Table 5. Selected fatty acid composition of major lipid classes of whole turbot fed the experimental diets for 7 days. The HUFA values in each lipid class were subjected to a one-way analysis of variance[1]

Diet	D1	D2	D3	D4	D5	D6	D7	D8	D9
Major diet PL	-	PI	PA	PI	PE	SL	PC	PC	PC
Turbot neutral lipid (% fatty acids NL)									
Saturates	36.3	31.6	32.0	33.1	31.5	31.5	30.9	30.8	30.6
Monoenes	31.4	29.6	29.5	27.6	29.6	26.8	28.7	26.0	25.1
n-6 PUFA	8.6	9.8	10.9	12.0	12.1	12.5	12.0	16.0	16.4
18:2n-6	6. 4	7.4	8.4	9.6	9.7	10.1	9.2	12.5	12.9
20:2n-6	0.6	0.6	0.8	0.9	0.9	0.9	0.9	1.2	1.3
20:4n-6	1.0	1.1	1.1	0.9	0.9	0.9	1.1	0.9	0.9
n-3 PUFA	23.8	28.9	27.6	27.3	26.8	29.3	28.5	27.2	28.0
18:3n-3	7.2	7.6	7.8	6.5	7.7	7.4	8.1	5.8	6.7
20:5n-3	5.3^{c}	6.6^{a}	6.0^{b}	6.3^{b}	6.1^{b}	6.7^{a}	6.8^{a}	6.3^{ab}	6.5^{ab}
22:5n-3	2.2^{b}	3.0^{a}	2.4^{b}	2.7^{a}	2.5^{b}	2.8a	3.0^{a}	2.7^{a}	2.7^{a}
22:6n-3	6.4^{d}	8.8^{ab}	8.5^{b}	9.3^{a}	7.7^{c}	9.5^{a}	8.4^{b}	9.6^{a}	9.7^{a}
Turbot phosphatidylcholine (% fatty acids PC)									
Saturates	41.8	38.9	40.0	39.0	39.1	38.8	38.2	38.1	37.9
Monoenes	28.0	25.1	26.0	23.7	23.5	22.7	24.7	21.6	22.0
n-6 PUFA	9.7	10.8	12.2	13.7	14.5	15.2	12.6	17.2	16.6
18:2n-6	7.2	8.1	9.6	10.9	11.6	12.0	9.0	14.8	13.2
20:2n-6	1.1	1.0	1.2	1.3	1.6	1.5	1.3	1.7	1.5
20:4n-6	0.9	1.0	0.9	0.9	1.0	1.1	0.8	0.8	0.9
n-3 PUFA	20.0	25.2	21.8	23.6	22.9	23.3	24.5	23.1	23.5
18:3n-3	2.9	2.6	2.5	2.4	2.4	2.8	2.6	2.2	2.3
20:5n-3	6.1^{b}	6.9^{a}	6.2^{b}	6.7^{a}	6.2^{b}	6.8^{a}	6.8^{a}	6.6^{a}	6.4^{ab}
22:5n-3	1.9^{c}	2.5^{a}	2.2^{b}	2.3^{b}	2.2^{b}	2.3^{b}	2.2^{b}	2.2^{b}	2.3^{b}
22:6n-3	8.0^{d}	11.1^{a}	8.8^{c}	10.9^{a}	10.0^{b}	10.9^{a}	11.4^{a}	11.0^{a}	11.1^{a}
Turbot phosphatidylethanolamine (% fatty acids PE)									
Saturates	26.2	22.8	23.8	22.3	26.1	23.6	22.4	24.5	23.2
Monoenes	19.8	19.8	18.1	18.6	17.2	17.0	16.8	15.7	16.0
n-6 PUFA	8.8	9.2	11.4	12.9	11.8	12.1	11.5	14.0	15.0
18:2n-6	4.5	5.3	6.2	8.7	7.6	7.2	6.8	9.6	9.7
20:2n-6	0.6	0.8	0.8	1.1	1.0	1.0	1.0	1.2	1.3
20:4n-6	2.3	2.1	2.5	2.1	2.3	2.1	2.3	2.1	2.2
n-3 PUFA	45.2	48.1	46.6	46.2	45.0	47.3	49.3	45.7	45.8
18:3n-3	2.4	2.8	2.1	2.6	1.9	2.1	2.9	2.0	2.2
20:5n-3	9.3^{a}	9.0^{a}	9.1^{a}	7.8^{b}	8.5^{ab}	7.9^{b}	9.0^{a}	7.9^{b}	7.8^{b}
22:5n-3	5.5	5.1	5.7	5.0	5.0	5.1	5.5	5.0	5.0
22:6n-3	26.4^{c}	28.2^{b}	28.4^{b}	28.5^{b}	27.9^{b}	30.2^{a}	29.7^{a}	29.4^{a}	30.0^{a}

[1] Footnote as given in Table 4. Only HUFA are considered by the ANOVA as they are supplied in equal amounts by the different diets. Mean HUFA values sharing a common letter are not significantly different ($p>0.05$).

Table 6. Selected fatty acid composition of major lipid classes of whole turbot at the end of the 28-day feeding trial. The HUFA values in each lipid class were subjected to a one-way analysis of variance [1]

Diet	D1	D2	D3	D4	D5	D6	D7	D8	D9
Major diet PL	-	PI	PA	PI	PE	SL	PC	PC	PC
Turbot neutral lipid (% fatty acids NL)									
Saturates	41.5	37.6	35.3	35.0	34.8	33.0	36.4	32.0	35.0
Monoenes	28.2	26.8	25.6	24.3	23.2	22.4	24.1	21.0	20.8
n-6 PUFA	6.2	11.3	14.9	16.9	17.6	17.9	14.8	21.3	19.3
18:2n-6	3.8	8.6	12.2	13.9	14.7	15.0	11.7	18.0	16.4
20:2n-6	0.9	1.0	1.6	1.9	1.9	1.8	1.1	2.3	1.9
20:4n-6	0.6	0.5	0.5	0.5	0.3	0.5	0.4	0.5	0.4
n-3 PUFA	24.0	24.3	24.2	23.8	24.4	26.7	24.7	25.8	24.9
18:3n-3	6.2	3.2	4.3	3.1	3.5	3.9	3.4	3.3	3.7
20:5n-3	4.9	5.1	4.9	5.1	5.0	5.3	5.0	5.4	5.1
22:5n-3	3.1^{b}	3.5^{a}	3.3^{ab}	3.8^{a}	3.5^{a}	3.6^{a}	3.6^{a}	3.5^{a}	3.4^{a}
22:6n-3	8.2^{d}	11.4^{b}	10.0^{c}	10.9^{b}	11.4^{b}	12.4^{a}	11.6^{b}	12.3^{a}	11.8^{ab}
Turbot phosphatidylcholine (% fatty acids PC)									
Saturates	40.8	39.1	39.9	38.1	37.8	36.9	36.6	35.3	36.0
Monoenes	31.8	28.6	28.5	27.3	25.1	24.4	27.5	21.9	22.2
n-6 PUFA	10.0	11.6	13.7	15.0	17.9	17.1	15.7	20.0	19.7
18:2n-6	7.5	8.5	10.7	11.9	14.5	13.8	12.5	16.2	16.3
20:2n-6	1.0	1.3	1.6	1.8	2.2	1.9	1.5	2.6	2.0
20:4n-6	0.5	0.5	0.4	0.5	0.5	0.6	0.6	0.5	0.5
n-3 PUFA	17.0	20.7	16.9	19.5	19.4	21.6	20.2	22.7	22.1
18:3n-3	1.0	1.0	1.1	1.0	1.1	1.6	1.1	1.3	1.3
20:5n-3	4.0^{b}	4.9^{a}	4.0^{b}	4.3^{b}	4.6^{ab}	4.8^{a}	4.7^{a}	5.1^{a}	5.0^{a}
22:5n-3	2.0	2.3	2.0	2.4	2.2	2.4	2.2	2.3	2.1
22:6n-3	9.5	11.7	9.4	11.3	11.1	11.9	11.9	13.4	12.9
Turbot phosphatidylethanolamine (% fatty acids PE)									
Saturates	25.1	26.7	25.8	24.6	24.3	25.3	24.8	24.0	25.0
Monoenes	20.8	16.6	16.9	15.4	15.6	14.4	15.9	14.7	14.9
n-6 PUFA	10.8	9.1	10.0	11.1	10.4	11.7	10.3	12.0	12.5
18:2n-6	5.8	5.6	6.7	7.1	6.6	7.7	6.8	8.7	8.7
20:2n-6	0.9	1.0	1.1	1.5	1.3	1.4	1.2	1.6	1.6
20:4n-6	1.4	1.3	1.4	1.5	1.6	1.5	1.5	1.5	1.4
n-3 PUFA	43.3	47.6	47.3	49.0	49.8	48.6	49.0	49.3	47.4
18:3n-3	1.1	0.7	0.8	0.7	0.6	0.6	0.9	0.6	1.0
20:5n-3	7.1^{a}	6.9^{a}	7.1^{a}	6.5^{b}	6.9^{a}	6.2^{b}	6.1^{b}	6.0^{b}	6.0^{b}
22:5n-3	4.8^{b}	5.1^{a}	5.3^{a}	5.3^{a}	5.4^{a}	5.1^{a}	4.9^{ab}	5.3^{a}	5.0^{a}
22:6n-3	28.4^{e}	33.0^{c}	32.3^{d}	34.9^{a}	34.8^{a}	34.8^{a}	34.0^{b}	35.2^{a}	33.9^{b}
Turbot phosphatidylinositol (% fatty acids PI)									
Saturates	45.4	42.2	40.6	42.1	40.0	41.5	40.8	39.3	40.3
Monoenes	19.2	16.0	16.7	14.9	16.4	12.1	15.7	14.7	13.4
n-6 PUFA	10.3	12.8	13.4	14.0	14.5	16.1	14.1	15.4	15.5
18:2n-6	2.0	2.7	3.9	4.0	4.3	4.7	3.5	4.6	4.4
20:2n-6	0.3	0.4	0.9	0.9	1.0	1.2	0.6	1.1	1.0
20:4n-6	7.2^{c}	8.1^{b}	7.8^{b}	8.4^{ab}	8.6^{a}	8.8^{a}	8.6^{a}	9.1^{a}	8.7^{a}
n-3 PUFA	25.1	29.0	28.3	29.0	29.1	30.4	29.4	30.6	30.8
18:3n-3	0.4	0.4	0.7	0.5	0.5	0.8	0.4	0.6	0.5
20:5n-3	6.4^{b}	7.1^{a}	6.7^{ab}	7.1^{a}	7.2^{a}	7.4^{a}	7.3^{a}	7.5^{a}	7.6^{a}
22:5n-3	3.7^{b}	4.2^{a}	4.0^{a}	4.1^{a}	4.2^{a}	4.2^{a}	4.2^{a}	4.5^{a}	4.5^{a}
22:6n-3	13.7^{c}	15.8^{b}	16.0^{b}	16.5^{b}	16.4^{b}	17.3^{a}	16.1^{b}	17.6^{a}	17.1^{a}

[1] Footnotes as given in Table 5.

Table 7. Mean squares[1] for main effects and interactions given by the 3-way ANOVA on HUFA levels in turbot lipid classes (NL, PC and PE) sampled at two different periods (day 7 and day 28 of the trial)

HUFA	Main effects				Interactions			Error
	Diet (D)	Period (P)	Class (C)		D × P	D × C	P × C	df = 54
(df =8)	(df =1)	(df =2)		(df =8)	(df =16)	(df =2)		
20:4n-6	0.23	166.9***	217.0***		0.39	0.50	4.62***	0.212
20:5n-3	0.69	201.2***	105.5***		0.45	1.81**	4.47**	0.019
22:5n-3	0.38**	3.1***	123.8***		0.12	0.17	4.42***	0.010
22:6n-3	10.8***	76.5***	1475.0***		0.39	1.07*	5.22***	0.046

[1]Mean squares are based on log-transformed values and are multiplied by a common factor 100. The F value is based on the residual variance DxPxC (df = 16). Error: analytical error between duplicate analyses. Total degrees of freedom (df) = 108. ∗, ∗∗, ∗∗∗ denotes a significance at $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

Table 8. Duncan's multiple range test ($p<0.05$) for the main effects in the 3-way ANOVA from Table 7. Data represent mean HUFA values (% total fatty acids) averaged over all the observations (n = number of observations) [1]

	Diet (n=12)									Lipid class (n=36)			Sampling (n=54)	
	D1	D2	D3	D4	D5	D6	D7	D8	D9	NL	PC	PE	day7	day28
20:4n-6	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.0	0.7^b	0.7^b	1.8^a	1.4^a	0.8^b
20:5n-3	6.1	6.5	6.2	6.1	6.2	6.3	6.4	6.2	6.1	5.7^b	5.6^b	7.5^a	7.1^a	5.4^b
22:5n-3	2.3^b	3.6^a	3.5^a	3.6^a	3.5^a	3.6^a	3.5^a	3.5^a	3.4^a	3.1^b	2.2^c	5.2^a	3.4^b	3.6^a
22:6n-3	14.5^e	17.3^c	16.2^d	17.6^b	17.1^c	18.3^a	17.8^b	18.5^a	18.2^a	9.9^c	10.9^b	31.1^a	15.9^b	18.7^a

[1] For each effect, data in a row sharing a common superscript are not significantly different.

significant dietary effect (D) on HUFA levels was detected for DHA (Table 7). The DHA values, averaged over the 3 lipid classes at the 2 periods, were 12 to 30% higher in PL-fed fish than in PL-deprived fish (Table 8). In addition, significant differences were seen between the PL-supplemented diets with as ranking order: D3 < (D2, D5) < (D4, D7) < (D6, D8, D9), denoting a higher DHA incorporation in fish fed the PC-rich diets. The other HUFA (22:5n-3, 20:5n-3 and 20:4n-6) did not distinguish the diets as explicitly as did DHA. The level of 22:5n-3 was significantly lower in fish fed the PL-free diet, without any further distinction between the dietary PL-fractions. Based on the EPA levels, the diet effect was not significant, whereas an important diet x class (D x C) interaction ($p<0.01$) was observed, indicating different responses of the lipid classes in turbot to the dietary EPA supply. Indeed, in PC and NL the EPA levels were higher in most groups fed the PL-supplemented diets than in fish fed the PL-free diet, in agreement with findings for DHA, whereas a reverse pattern was found in the fish PE. Based on the accumulation of 20:4n-6 no diet effect was noted in the 3 considered body lipid classes. In contrast, in body PI, higher levels of 20:4n-6 were observed in fish fed the PC-rich diets (Table 6).

Aside from the variations of the HUFA levels related to the dietary effects, HUFA levels also varied according to the sampling period and the body lipid class (Table 7). The whole body PE fraction contained a significantly higher level of each considered HUFA than PC or NL. Regarding the sampling time, the average levels of DHA and 22:5n-3 incresed, whereas EPA and 20:4n-6 decreased between day 7 and day 28 (Table 8).

Discussion

This study confirms the positive effect of phospholipids (PL) on growth observed in foregoing studies on larvae and post larvae of different fish species (Kanazawa et al. 1981, 1983a, b; Geurden et al. 1997a, b). Clear differences according to the polar head group of the dietary PL were also evident, with a superiority of PC over the other PL classes. Some of the PC fractions were highly purified as compared to the other PL fractions, which contained noticeable amounts of gly-

colipids. It seems however unlikely that the observed growth differences could be explained by a negative effect of glycolipids. The superiority of PC over PI was also noticed when comparing the groups fed diets D2 (PI-rich) and D7 (PC-rich), which were both supplemented with purified PL fractions.

The superiority of PC over the other dietary PL classes is in general accordance with previous data on larvae or post-larvae of other fish species (Takeuchi et al. 1992; Kanazawa 1993). In contrast, PI has been reported to be as efficient as PC in a study on larval ayu *Plecoglossus altivelis* (Kanazawa et al. 1985). The larval response of common carp *Cyprinus carpio* to PC and PI was distinct, i.e. PC stimulated the initial growth, but induced deformities and a subsequent mortality, while PI prevented the deformities and ensured a high survival (Geurden et al. 1997a, 1998). The growth differences presently observed between the turbot in relation to the dietary PL-supply are smaller than in carp larvae. This discrepancy in response, as well as the absence of a PL effect on the survival of the turbot, is probably explained by the more advanced developmental stage of weaned turbot as compared to first-feeding carp (210 mg vs. 2 mg initial body weight, respectively).

Another distinctive characteristic of the PL-fed fish is the significantly higher deposition of triglycerides in their whole body lipid. Based on the high recovery of labelled fatty acids, Linares and Henderson (1991) suggested a very active synthesis of triglycerides in turbot. The increased ratio of 'storage to membrane lipid' following a dietary PL supplementation was also noticed in newly-weaned European sea bass (Geurden et al. 1997c). But, as shown in the present study, the PL class profile of the fish, expressed as a percentage of total polar lipid, remained unaffected by the PL class composition of the diet.

The specific distribution of the various fatty acids in each of the examined lipid classes agrees with data reported for the whole body (Linares and Henderson 1991) and for separate tissues of the same species (Bell et al. 1985, 1994, 1995). The high n-3/n-6 and EPA/DHA ratios at the beginning of the experiment are typical of newly-weaned fish and reflect the previous *Artemia* feeding (Coutteau et al. 1996; Geurden et al. 1997b, c). Whereas some specific dietary fatty acid responses were noted in the separate fish lipid classes, general tendencies affecting all classes were seen during the course of the experiment, such as the increase of 18:2n-6 relative to 18:3n-3 and the increase of DHA relative to EPA. The levels of 18:2n-6 in the fish were related to the dietary levels, being higher in the PL-supplemented diets than in the PL-free diet. However, the example of diet D3 (PA-rich) which provided the same amount of 18:2n-6 as diet D8 (PC-rich), but which in the fish resulted in a considerably lower 18:2n-6 level than the PC-rich diet, indicates a stimulating influence of dietary PC on the fatty acid incorporation. The increasing deposition of 20:2n-6, a 'dead-end' product, with increasing 18:2n-6 levels in diet and fish agrees with the data of Bell et al. (1994) in juvenile turbot and indicates an inhibition of the delta-6-desaturase activity (Tocher 1993), possibly by the high HUFA level in the diet. In contrast, when culturing turbot cells in a medium poor in n-3 HUFA, 18:2n-6 was more desaturated than elongated (Tocher and Mackinlay 1990).

Following our introductory hypothesis, the comparison of HUFA levels in fish fed diets containing an equal amount of HUFA, presented as neutral lipid, should indicate the capacity of the different dietary PL classes for enhancing the absorption of neutral lipid fatty acids. In fact, the outcome varied according to the HUFA, i.e. DHA allowed the best discrimination and separated the PL-free, the PC-poor and the PC-rich diets; 22:5n-3 distinguished the PL-free diet from the other diets; EPA gave results dependent on the fish class and 20:4n-6 was discriminant only in fish PI. The utilization of the body level of a specific fatty acid as a quantitative indicator of absorption implies a minimal degradation or synthesis of this fatty acid. These conditions seem valid for DHA. Bell et al. (1995) showed a DHA increase proportional to dietary levels in turbot PC and PE, without simultaneous EPA increase. Low retroconversion of DHA to EPA in turbot was also reported by Tocher and Mackinlay (1990) and Mourente et al. (1991). Furthermore, the synthesis of DHA from EPA, via 24:5 and 24:6n-3 (Mourente and Tocher 1994), seems unlikely when considering the inhibition of the delta-6 desaturase, suggested before by the accumulation of 20:2n-6 in the fish and the high DHA/EPA ratio in the diets. In freshwater fish, n-3 HUFA addition to the diet completely retro-inhibited the conversion of 18:3n-3 into DHA (Léger et al. 1981; Olsen et al. 1990) and a similar situation might be assumed in present turbot fed diets which are relatively rich in n-3 HUFA.

The higher level of 22:5n-3 in fish fed PL could be due either to an increased EPA elongation in PL-fed fish, or, more likely, to an enhanced absorption of this fatty acid from the diet, in analogy with the above findings for DHA. The validity of EPA as a 'marker' of

fatty acid uptake is dubious. The general decrease of this fatty acid throughout the experiment might have hindered an improved accumulation by PL. However, in NL and PC, EPA had decreased less in the PL-fed fish, in agreement with the expected PL effect. But, this was not the case in PE where EPA levels appeared inversely related to DHA levels. Antagonistic relations between DHA and EPA levels in marine fish PE have been reported before by Watanabe (1993). The low response of fish 20:4n-6 to the PL supplementation is possibly explained by the low analytical precision linked to the small amount in the fish. But, in fish PI, where 20:4n-6 reached high levels, there was an increased incorporation of this fatty acid related to the dietary PC supply. Moreover the latter increase was not associated to an inverse variation of EPA in fish PI, as found by Bell et al. (1995) in turbot fed diets with different 20:4n-6 contents, but was accompanied by an increase of all n-3 HUFA.

The observed relationship between the amount of PC in the diet, fish growth and fish DHA content could lead to several interpretations. For instance, the higher DHA in fish fed PC-rich diets might be looked at as a trivial consequence of their higher final weight. This is however contradicted by the higher amount of DHA in the 400-450 mg fish, fed the PC-diets for one week, compared to the 950 mg PL-deprived fish at the end of the trial. The other way around, the better growth of the PL-fed fish is unlikely the consequence of a better absorption of DHA preventing an essential fatty acid deficiency, taking into account the important amount of DHA in all diets (1.2% diet dry weight), which should be sufficient to satisfy the requirement of postlarval turbot (Léger et al. 1979; Le Millinaire et al. 1983). Moreover, the considerable increase of DHA in the body neutral lipids in all groups during the course of the experiment suggests the fulfillment of the DHA requirement for proper membrane functions. The presently proposed interpretation is that DHA can be considered as a marker of the neutral fatty acid absorption. Hence, the above findings are consistent with the idea that PC stimulates the absorption of dietary neutral lipid, with as direct consequences more energy for growth, an increased triglyceride synthesis and an increased incorporation of diet specific FA.

This conclusion is consistent with previous results indicating an impaired lipid export from the intestinal absorptive cells in carp larvae fed PL-free or PI-rich diets instead of PC-rich diets, resulting in smaller liver and hepatocyte size (Fontagné et al. 1998). The link between the latter histological observations and the growth of the PC-fed carp (Geurden et al. 1998) coincides with the link between the increased DHA and triglyceride level and the better growth of PC-fed turbot in present study. An increased absorption of dietary fatty acids is also in accordance with the specific enhancement of the secretion of intestinal lipoproteins by PC, as reported in rat cell lines (Field and Mathur 1995).

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References

Bell, J.G., Castell, J.D., Tocher, D.R., MacDonald, F.M. and Sargent, J.R. 1995. Effects of different arachidonic: docosahexaenoic ratios on phospholipid fatty acid compositions and prostaglandin production in juvenile turbot. Fish Physiol. Biochem. 14: 139–151.

Bell, M.V., Henderson, R.J. and Sargent, J.R. 1985. Changes in the fatty acid composition of phospholipids from turbot (*Scophthalmus maximus*) in relation to dietary polyunsaturated fatty acid deficiencies. Comp. Biochem. Physiol. 81: 193–198.

Bell, J.G., Tocher, D.R., MacDonald, F.M. and Sargent, J.R. 1994. Effects of diets rich in linoleic (18:2n-6) and α-linolenic (18:3n-3) acids on the growth, lipid class and fatty acid compositions and eicosanoid production in juvenile turbot *(Scophthalmus maximus* L.). Fish Physiol. Biochem. 13: 105–118.

Castell, J.D., Bell, J.G., Tocher, D.R. and Sargent, J.R. 1994. Effect of purified diets containing different combinations of arachidonic acid and docosahexaenoic acid on survival, growth and fatty acid composition of juvenile turbot (*Scophthalmus maximus*). Aquaculture 128: 315–333.

Charlon, N. and Bergot, P. 1984. Rearing system for feeding fish larvae on dry diets. Trial with carp (*Cyprinus carpio* L.) larvae. Aquaculture 41: 1–9.

Christie, W.W. 1982. The analysis of fatty acids. *In* Separation, Identification and Structural Analysis of Lipids. pp. 63–90. 2nd Edition, Edited by W.W. Christie. Pergamon Press, Oxford.

Coutteau, P., Van Stappen, G. and Sorgeloos, P. 1995. A standard experimental diet for the study of fatty acid requirements of weaning and first ongrowing stages of European sea bass (*Dicentrarchus labrax* L.): selection of the basal diet. ICES Mar. Sci. Symp. 201: 130–137.

Coutteau, P., Van Stappen, G. and Sorgeloos, P. 1996. A standard experimental diet for the study of fatty acid requirements of weaning and first ongrowing stages of marine fish: a comparison of extruded and extruded/coated diets. Arch. Anim. Nutr. 49: 49–59.

Cowey, C.B., Adron, J.W., Owen, J.M. and Robert, R.J. 1976. The effect of different dietary oils on tissue fatty acid and tissue pathology in the turbot *Scophthalmus maximus*. Comp. Biochem. Physiol. 53B: 399–403.

Dhert, P., Lavens, P., Duray, M. and Sorgeloos, P. 1990. Improved larval survival at metamorphosis of Asian sea bass (*Lates calcarifer*) using w3-HUFA-enriched live food. Aquaculture 90: 63–74.

Field, F.G. and Mathur, S.N. 1995. Intestinal lipoprotein synthesis and secretion. Prog. Lipid Res. 34: 185–198.

Folch, J., Lees, M. and Sloane-Stanley, G.S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509.

Fontagné, S., Geurden, I., Escaffre, A.M. and Bergot, P. 1998. Histological changes induced by dietary phospholipids in intestine and liver of common carp larvae. Aquaculture (in press).

Geurden, I., Charlon, N., Marion, D. and Bergot, P. 1997a. Influence of purified soybean phospholipids on early development of carp (*Cyprinus carpio* L.). Aquacult. Int. 5: 137–149.

Geurden, I. Coutteau, P. and Sorgeloos, P. 1997b. Effect of a dietary phospholipid supplementation on growth and fatty acid composition of European sea bass (*Dicentrarchus labrax* L.) and turbot (*Scophthalmus maximus*) juveniles from weaning onwards. Fish Physiol. Biochem. 16: 259–272.

Geurden, I. Coutteau, P. and Sorgeloos, P. 1997c. Increased docosahexaenoic acid (DHA) levels in total and polar lipid of European sea bass (*Dicentrarchus labrax* L.) postlarvae fed vegetable or animal phospholipids. Mar. Biol. 129: 689–698.

Geurden, I., Marion D., Charlon, N., Coutteau, P. and Bergot, P. 1998. Comparison of different soybean phospholipidic fractions as dietary supplements for common carp larvae. Aquaculture (in press).

Izquierdo, M.S., Watanabe, T., Takeuchi, T., Arakawa, T. and Kitajima, C. 1989. Requirements of larval red seabream *Pagrus major* for essential fatty acids. Nippon Suisan Gakkaishi 55: 859–867.

Kanazawa, A., 1993. Essential phospholipids of fish and crustaceans. *In* Fish Nutrition in Practice, Les Colloques nr 61, pp. 519–530. Edited by S.J. Kaushik and P. Luquet. INRA, Paris.

Kanazawa, A., Teshima, S., Inamori, S., Iwashita, T. and Nagao, A. 1981. Effects of phospholipids on growth, survival rate and incidence of malformation in the larval ayu. Mem. Fac. Fish. Kagoshima Univ. 30: 301–309.

Kanazawa, A., Teshima, S., Inamori, S.and Matsubara, H. 1983a. Effects of dietary phospholipids on growth of the larval red sea bream and knife jaw. Mem. Fac. Fish. Kagoshima Univ. 32: 109–114.

Kanazawa, A., Teshima, S., Kobayashi, T., Takae, M., Iwashita, T and Uehara, R. 1983b. Necessity of dietary phospholipids for growth of the larval ayu. Mem. Fac. Fish. Kagoshima Univ. 32: 115–120.

Kanazawa, A., Teshima, S. and Sakomoto, M. 1985. Effects of dietary bonito-egg phospholipids and some phospholipids on growth and survival of the larval Ayu, *Plecoglossus altivelis*. Z. Angew. Ichtyol. 4: 165–170.

Léger, Ph., Naessens-Foucqaert, E. and Sorgeloos, P. 1987. Techniques to manipulate the fatty acid profile in *Artemia* nauplii and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M.). *In* Artemia Research and its Applications. Vol. 3, pp. 411-424. Edited by P. Sorgeloos, D.A. Bengtson, W. Decleir and E. Jaspers. Universa Press, Wetteren.

Léger, C., Frémont, L. and Boudon, M. 1981. Fatty acid composition of lipids in the trout. – I. Influence of dietary fatty acids on the triglyceride fatty acid desaturation in serum, adipose tissue, liver, white and red muscle. Comp. Biochem. Physiol. 69B: 99–105.

Léger, C., Gatesoupe, F.J., Métailler, R., Luquet, P. and Frémont, L. 1979. Effect of dietary fatty acids differing by chain lengths and omega-series on the growth and lipid composition of turbot (*Scophthalmus maximus* L.). Comp. Biochem. Physiol. 64B: 345–350.

Le Millinaire, C., Gatesoupe, F.J. and Stéphan, G. 1983. Approche du besoin quantitatif en acides gras longs polyinsaturés de la série n-3 chez la larve de turbot (*Scophthalmus maximus*). C.R. Hebd. Séances Acad. Sci., Paris 296: 917–920.

Lepage, G. and Roy, C.C. 1984. Improved recovery of fatty acid trough direct transesterification without prior extraction or purification. J. Lipid Res. 25: 1391–1396.

Linares, F. and Henderson, R.J. 1991. Incorporation of ^{14}C-labeled polyunsaturated fatty acids by juvenile turbot, *Scophthalmus maximus* (L.) *in vivo*. J. Fish Biol. 38: 335–347.

McEvoy, L.A., Navarro, J.C., Hontoria, F., Amat, F. and Sargent J.R. 1996. Two novel *Artemia* enrichment diets containing polar lipid. Aquaculture 144: 339–352.

Mourente, G., Tocher, D.R. and Sargent, J.R. 1991. Specific accumulation of docosahexaenoic acid (22:6n-3) in brain lipids during development of juvenile turbot *Scophthalmus maximus* L. Lipids 26: 871–877.

Mourente, G. and Tocher, D.R. 1994.*In vivo* metabolism of [1-C^{14}] linolenic acid (18:3(n-3)) and [1-C^{14}] eicosapentaenoic acid (20:5(n-3)) in a marine fish: time-course of the desaturation / elongation pathway. Biochim. Biophys. Acta 1212: 109–118.

Olsen, R.E. and Henderson, B.J. 1989. The rapid analysis of neutral and polar marine lipids using double development HPTLC and scanning densitometry. J. Exp. Mar. Biol. Ecol. 129: 189–197.

Olsen, R.E., Henderson, R.J. and McAndrew, B.J. 1990. The conversion of linoleic and linolenic acid to longer chain polyunsaturated fatty acids by Tilapia (*Oreochromis nilotica*) *in vivo*. Fish Physiol. Biochem. 8: 261–270.

Sargent, J.R., Bell, J.G., Bell, M.V. Henderson, R.J. and Tocher, D.R. 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish. *In* Coastal and Estuarine Studies. Aquaculture: Fundamental and Applied Research. pp. 103–124. Edited by B. Lahlou and P. Vitiello. American Geophysical Union, Washington D.C.

Takeuchi, T., Arakawa, T., Satoh, S. and Watanabe, T. 1992. Supplemental effect of phospholipids and requirement of eicosapentaenoic acid and docosahexaenoic acid of juvenile striped jack. Nippon Suisan Gakkaishi 58: 707–713.

Tocher, D.R. 1993. Elongation predominates over desaturation in the metabolism of 18:3n-3 and 20:5n-3 in turbot (*Scophthalmus maximus*) brain astrological cells in primary culture. Lipids 28: 267–272.

Tocher, D.R. and Mackinlay, E.E. 1990. Incorporation and metabolism of (n-3) and (n-6) polyunsaturated fatty acids in phospholipid classes in cultured turbot (*Scophthalmus maximus*) cells. Fish Physiol. Biochem. 8: 251–260.

Watanabe, T. 1993. Importance of docosahexaenoic acid in marine larval fish. J. World Aquac. Soc. 24: 152–166.

Watanabe, T., Kitajima, C. and Fujita, S. 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34: 115–143.