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Effects of dietary docosahexaenoic acid (DHA; 22:6*n*-3) on lipid and fatty acid compositions and growth in gilthead sea bream (*Sparus aurata* L.) larvae during first feeding

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ABSTRACT

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The effects of dietary DHA levels on lipid class compositions, total lipid and phosphoglyceride fatty acid compositions and growth were investigated in gilthead sea bream, *Sparus aurata*, larvae during the first 2 weeks after hatching. Different dietary levels of DHA were supplied to the larvae in the rotifer, *Brachionus plicatilis*, previously enriched with (a) *Nannochloropsis gaditana*, (b) a mixture of *N. gaditana*, *Isochrysis galbana* and *Rhodomonas* sp. or, (c) Protein Selco (Artemia Systems). The results showed that growth performance of gilthead sea bream larvae at first feeding was influenced by both total *n*-3HUFA and DHA contents of the diet, the best growth rate being achieved with a high *n*-3HUFA content and a high DHA:EPA ratio. Lipid contents, nutritional status, and total lipid and phosphoglyceride DHA levels of the larvae were directly correlated with dietary DHA levels. The fatty acid compositional data suggested that dietary EPA could not contribute significantly to tissue DHA levels in larval gilthead sea bream, presumably due to low $\Delta 4$ desaturase activity.

ABBREVIATIONS

AA, arachidonic acid (20:4*n*-6); CPL, diradyl (diacyl+alkenylacyl+alkylacyl) glycerophosphocholines; DHA, docosahexaenoic acid (22:6*n*-3); DMA, dimethyl acetal; DPA, docosapentaenoic acid (22:5*n*-3); EPA, eicosapentaenoic acid (20:5*n*-3); EPL, diradyl (diacyl+alkenylacyl+alkylacyl) glycerophosphoethanolamines; HUFA, highly unsaturated fatty acid; PA/CL, phosphatidic acid/cardiolipin; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SM, sphingomyelin.

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INTRODUCTION

The aims in larval nutrition of marine fish during the last decade have focused mainly on determining *n*-3PUFA requirements. Most of the current knowledge has been derived from studies in juveniles (Watanabe et al., 1989a,b), because larval marine fish are generally unwilling to ingest semi-purified or purified diets. However, quantitative requirements of fish larvae may be different from those of the juveniles or adults (Sargent et al., 1989). Studies have been performed with larval stages of species such as red sea bream (Izquierdo et al., 1989a,b), gilthead sea bream (Koven et al., 1990), or striped bass (Tuncer and Harrell, 1992) in order to determine their respective *n*-3PUFA requirements. Special consideration has been given to the comparative efficacy of eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3) as essential fatty acids (Koven et al., 1989; Watanabe et al., 1989c; Takeuchi et al., 1990). Investigations with turbot (Witt et al., 1984), gilthead sea bream (Koven et al., 1989) and dolphin, *Coryphaena hippurus* (Ostrowski and Divakaran, 1990) have revealed that DHA is strongly retained and is essential for larvae of these marine fishes.

Kanazawa et al. (1982) showed that EPA was preferentially incorporated into gall bladder, swim bladder and alimentary tract, and secondly into liver and gill tissue, but few reports indicate a specific role for this fatty acid. In contrast, DHA has been shown to be strongly retained in brain lipids of sea bass *Dicentrarchus labrax* L. (Pagliarani et al., 1986), and brain and retinas of cod (Tocher and Harvie, 1988) and rainbow trout (Tocher and Harvie, 1988; Bell and Tocher, 1989). Moreover, in recent investigations we have demonstrated that the level of DHA increases dramatically in brain lipids of turbot during development of weaned fish (Mourente et al., 1991; Mourente and Tocher, 1992).

In this study we have investigated the effects of different dietary DHA levels, supplied in the rotifer *Brachionus plicatilis*, on lipid class compositions, total lipid and major phosphoglyceride fatty acid compositions and growth of gilthead sea bream larvae during 2 weeks post-hatch.

MATERIALS AND METHODS

The experiments were carried out in the hatchery of the marine fish farm CUPIMAR S.A., Salina de San Juan Bautista, San Fernando (Cádiz), Spain.

Algae and artificial booster

Different species of algae were used to enrich rotifers with PUFA: the Eustigmatophycean *Nannochloropsis gaditana* Lubian, Instituto de Ciencias Marinas de Andalucia, # B3, Puerto Real (Cádiz) Spain; the Haptophycean *Isochrysis galbana* Green, University of Delaware, Leives, # T-ISO, USA;

and the Rhodophycean *Rhodomonas* sp. Algae were all grown in sea water (pH = 7.2 ± 0.2; salinity 32 ± 0.5 mg/g) supplemented with culture medium (Walne, 1966), and maintained in continuous culture in chamber-controlled conditions of temperature (18 ± 0.5 °C) and illumination (15 W m^{-2}). Algae reached densities of 20–30 × 10^6 cells/ml (*N. gaditana*), 10–15 × 10^6 cells/ml (*I. galbana*) and 0.8–1.3 × 10^6 cells/ml (*Rhodomonas* sp.) respectively, and were harvested by centrifugation at 7000 rpm for 15 min. The commercial artificial booster Protein Selco (Artemia Systems) was also used to enhance the nutritional quality of rotifers.

Rotifers

Three different populations of the rotifer *Brachionus plicatilis* O.F. Muller, cultured with baker's yeast *Saccharomyces cerevisiae*, were used. Treatment (a) consisted of rotifers enriched with *N. gaditana*. For treatment (b) rotifers were enriched on a mixture of *N. gaditana*, *I. galbana* and *Rhodomonas* sp. in proportions of 1 : 1 : 1 by volume. In treatment (c), Protein Selco was used as enricher. For treatments (a) and (b), rotifers at a density of 500 individuals/ml were enriched with algae over 12–16 h. The enrichment of rotifers with Protein Selco was carried out according to the manufacturers' instructions.

Gilthead sea bream larvae

Larvae hatched from naturally spawned *Sparus aurata* eggs, originating from a common broodstock, were placed in 1000 l cylindrical tanks at a density of 150 larvae/l. Tanks, four replicates per treatment, had a layer of sand on the bottom and central drainage covered by a 250 μm mesh screen gauze. Underground salt water (sea water well) was used: salinity 32 ± 1 mg/g, temperature 18 ± 0.5 °C and pH 7.3 ± 0.2, circulating at 1.25 volumes/day. Rotifers were added to the tanks twice a day (mornings and evenings) from the 3rd day after hatch onwards, to maintain a concentration of 5–10 individuals/ml. *N. gaditana* was added to treatment (a) tanks, and a mixture of *N. gaditana*, *I. galbana* and *Rhodomonas* sp. (1 : 1 : 1, by volume) was added to treatment (b) and (c) tanks as "green water", to maintain the performance and the nutritional quality of the different rotifer populations. In treatment (a), only *N. gaditana* was used to produce "green water", so that no other algal species could contribute as a DHA source for larvae by drinking, filtration or accidental ingestion (Van der Meeren, 1991). In all cases, algae were at a density of 0.4 × 10^6 cells/ml.

Sampling

Algae were harvested by centrifugation and washed, frozen in liquid nitrogen, lyophilized and stored at −80 °C until analysis (Mourente et al., 1990). Rotifers were filtered with a 60 μm mesh screen gauze, washed and water

carefully eliminated by blotting the gauze on filter paper for 10 min. Rotifers were then immediately frozen in liquid nitrogen and stored at −80°C until analysis. Ten larvae were randomly sampled from each tank at days 6, 9, 12 and 15, anaesthetized and their lengths determined using a binocular dissecting microscope. Four replicates of gilthead sea bream larvae were also collected at days 6, 9, 12 and 15 after hatching for dry weight determination and elemental composition analysis (C, N, H). Another set of larvae samples was collected on the 15th day for lipid analysis, using a similar procedure to that used to sample rotifers.

Total lipid extraction, lipid class separation and quantification

Total lipid was extracted after homogenization in chloroform/methanol (2:1, *v/v*) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. (1957), as detailed previously (Tocher and Harvie, 1988). Lipid classes were separated by high-performance thin-layer chromatography (HPTLC) using a single-dimension double-development method described previously (Tocher and Harvie, 1988; Olsen and Henderson, 1989). The classes were quantified by charring (Fewster et al., 1969) followed by calibrated densitometry using a Shimadzu CS-9000 dual-wavelength flying spot scanner and DR-13 recorder (Olsen and Henderson, 1989).

Total lipid and major phosphoglyceride fatty acid analyses

Individual phosphoglyceride 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, using nonadecanoic acid (19:0) as internal standard (Christie, 1989). Methyl esters were extracted and purified as described previously (Tocher and Harvie, 1988). The fatty acid methyl esters were analyzed in a Hewlett-Packard 5890A gas chromatograph equipped with a chemically bonded (PEG) Supelcowax-10 fused silica wall coated capillary column (30 m×0.53 mm i.d., Supelco Inc., Bellefonte, USA) using hydrogen as carrier gas with a thermal gradient from 185°C to 235°C. Individual fatty acid methyl esters were identified as described previously (Tocher and Harvie, 1988) and quantified using a Hewlett-Packard 3390A recording integrator.

Dry weight and elemental composition determinations

Four replicates of preweighed samples (approximately 500 mg of rotifer wet weight or 15–25 larvae per sample) were maintained at 110°C for 24 h. The dry weights were determined after cooling in vacuo for at least 1 h. The elemental composition (C, N, H) analyses were performed in an elemental analyzer Carlo Erba 1106, using cyclohexanone 2,4-dinitrophenylhydrazone

as standard. Samples were washed with distilled water, then desiccated to constant weight, and accurately weighed replicates of approximately 1 mg of dry weight were analyzed. Carbon, nitrogen and hydrogen contents (μg/larvae) were obtained by multiplying their respective percentage values by the dry weight per larva. Protein content was obtained by multiplying the nitrogen content by 6.25.

Statistical analysis

Results are presented as means $\pm$ s.d. ($n=3$ or 4). Differences between treatments were analyzed by one-way analysis of variance (ANOVA), followed when pertinent by a multiple comparison test (Tukey). Differences were reported as statistically significant when $P<0.05$ (Zar, 1984).

Materials

Potassium bicarbonate, potassium chloride, BHT and nonadecanoic acid (>99% pure) were from Sigma Chemical Co. (Poole, Dorset, UK). Cyclohexanone 2,4-dinitrophenylhydrazone was from Aldrich Chemical Co. (Gillingham, Dorset, UK). TLC (20×20 cm×0.25 mm) and HPTLC (10×10 cm×0.15 mm) plates precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). Glacial acetic acid, sulphuric acid and all solvents (HPLC grade) were purchased from Fluka Chemicals Co. (Glossop, Derbyshire, UK).

RESULTS

The aim of the present study was to investigate effects of varying levels of dietary DHA on lipid and fatty acid compositions and growth parameters in gilthead sea bream larvae during and following first feeding. It is well established that algal species belonging to the class Eustigmatophyceae have high levels of EPA but essentially lack DHA (Mourente et al., 1990). Therefore, the marine microalga *N. gaditana* was used as the PUFA enrichment regime for rotifers in treatment (a). In treatment (b), rotifers were enriched with a mixture of three different species of marine unicellular algae, two of which (*I. galbana* and *Rhodomonas* sp.) contain significant amounts of DHA (Pohl and Zurheide, 1979; Kurmaly et al., 1989; Mourente et al., 1990). Proportions of (1:1:1, *v/v/v*) were chosen in order to give an intermediate level of DHA in the rotifers. For the highest dietary DHA, rotifers were enriched using Protein Selco (treatment c), a good source of DHA, protein and vitamins (Leger et al., 1989).

Total lipid and fatty acid content of the different marine microalgae and booster (Protein Selco) used for rotifer enrichment regimes are shown in Table 1. *Nannochloropsis gaditana* contained high levels of arachidonic acid (AA; 20:4*n*-6) and EPA, but only trace amounts of DHA. The mixture of *N.*

TABLE 1

Total lipid (percentage of dry weight) and fatty acid content (μg of fatty acid per mg of dry weight) of marine microalgae, *Nannochloropsis gaditana, Isochrysis galbana* and *Rhodomonas* sp., a mixture of the three previous microalgae (1:1:1; *v/v/v*) and Protein Selco (Artemia Systems)

Treatment: Fatty acid	*N. gaditana* (a)	*I. galbana*	*Rhodomonas* sp.	Mixture (b)	Protein Selco (c)
14:0	1.9±0.1	11.5±0.9	3.1±0.3	6.0±0.4	5.9±0.3
15:0	2.8±0.5	2.9±0.3	2.0±0.1	2.1±0.1	2.0±0.1
16:0	13.0±1.4	7.5±0.2	5.9±0.4	7.2±0.3	17.8±0.4
16:1*n*-7	14.3±1.2	2.5±0.1	0.8±0.1	4.0±0.3	11.3±0.4
16:2	0.4±0.1	0.1±0.0	0.2±0.0	0.2±0.0	0.9±0.4
16:3	0.5±0.2	0.6±0.1	0.5±0.0	0.6±0.0	1.2±0.2
16:4	tr	1.2±0.1	0.9±0.1	0.8±0.2	1.7±0.2
18:0	0.1±0.0	0.2±0.0	0.3±0.0	0.2±0.0	6.4±0.2
18:1*n*-9	1.5±0.1	5.2±0.1	1.0±0.1	2.3±0.1	22.1±0.4
18:1*n*-7	tr	1.9±0.6	tr	0.7±0.0	6.5±0.1
18:2*n*-6	1.1±0.1	4.7±0.1	2.1±0.1	2.5±0.1	8.0±0.5
18:3*n*-3	0.1±0.0	6.3±0.1	9.6±0.4	5.1±0.2	1.8±0.1
18:4*n*-3	0.1±0.0	8.9±0.2	8.0±0.4	5.8±0.2	4.3±0.0
20:2*n*-6	0.6±0.3	0.1±0.0	tr	0.2±0.1	1.5±0.1
20:3*n*-6	0.9±0.1	tr	tr	0.1±0.0	0.7±0.3
20:4*n*-6	3.2±0.4	0.2±0.0	0.1±0.0	0.6±0.1	2.1±0.1
20:3*n*-3	0.1±0.0	tr	0.2±0.0	0.1±0.0	0.1±0.1
20:4*n*-3	0.2±0.0	0.3±0.0	0.1±0.0	0.2±0.0	1.7±0.0
20:5*n*-3	23.5±2.7	0.6±0.0	4.2±0.1	5.3±0.2	31.3±0.7
22:5*n*-6	tr	0.9±0.0	0.2±0.1	0.4±0.0	1.0±0.0
22:5*n*-3	tr	tr	0.1±0.0	0.3±0.1	4.3±0.2
22:6*n*-3	0.1±0.1	5.2±0.3	2.9±0.1	2.7±0.1	30.0±0.1
Totals					
Saturated	17.9±1.9	22.8±1.4	11.4±0.8	15.5±0.6	33.4±0.8
Monounsaturated	16.9±1.6	11.2±0.6	2.4±0.2	7.0±0.4	48.4±0.7
Unsaturated	30.9±3.5	29.5±0.7	29.4±1.2	24.9±0.8	94.8±1.5
(*n*-6) HUFA	4.1±0.5	1.4±0.0	0.4±0.1	1.1±0.1	6.4±0.2
(*n*-3) HUFA	24.0±2.7	6.2±0.3	7.5±0.2	8.6±0.2	68.9±0.6
Total lipids (dry wt%)	11.5±0.4	11.1±0.7	7.4±1.1	8.3±0.9	22.8±1.7

Data are means±s.d. ($n=3$). s.d.=0.0 implies an s.d. of <0.05. Totals include some other minor components not shown. tr, trace<0.1%. HUFA, highly unsaturated fatty acids (>C_{20} and with at least 3 double bonds).

gaditana, I. galbana and *Rhodomonas* sp. had the lowest levels of AA, EPA and total *n*-3HUFA, but contained 2.7 μg of DHA per mg of dry weight. In contrast, Protein Selco had the highest levels of total *n*-3HUFA, EPA and DHA (30 μg per mg dry weight), and an intermediate AA content.

Total lipid and fatty acid contents of rotifers *B. plicatilis* enriched by the different regimes are shown in Table 2. Treatment (a) produced rotifers with the highest content of EPA, *n*-3 and *n*-6HUFA and the lowest content of DHA (0.4 μg/mg dry weight) (low DHA:EPA ratio). Rotifers enriched with the mixture of algae showed a content of DHA (1.4 μg per mg dry weight) three-fold greater than rotifers enriched with treatment (a), but with the lowest total *n*-3HUFA content. In contrast, rotifers on treatment (c) had the highest content of DHA (4.6 μg per mg dry weight) i.e. 11.5-fold and 3.5-fold more than rotifers from treatments (a) and (b) respectively, an intermediate level of EPA i.e. half of that in rotifers from treatment (a) but 3-fold greater than rotifers from treatment (b), and showed a total *n*-3HUFA content similar to that of treatment (a). Therefore, rotifers on treatment (c) had a high DHA:EPA ratio.

Gross composition and lipid class composition of sea bream larvae reared on the three treatments are presented in Table 3. There were no significant differences between protein content of larvae from different treatments. In contrast, the lipid content increased in larvae from treatment (a) to (b) to (c). Although the errors on these determinations made the differences not significant statistically, the significantly increased amounts of individual lipid classes from larvae on treatment (c) showed that the trend in total lipid was real. In absolute terms (dry weight percentage), larvae from treatment (c) presented significantly higher content of total polar and total neutral lipids, followed by larvae from treatment (b) and then from treatment (a), although no significant differences were found in total neutral lipid content between larvae from groups (a) and (b). The major polar lipid classes were total diradyl glycerophosphocholines (CPL) and total diradyl glycerophosphoethanolamines (EPL), followed by phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid/cardiolipin (PA/CL) and sphingomyelin (SM). Polar lipids, as a percentage of larvae dry weight, were significantly greater in larvae from treatment (b) compared to treatment (a) and significantly greater in larvae from treatment (c). A similar trend of increasing content from treatment (a) to (b) to (c) was observed in the individual phosphoglyceride classes, with CPL, EPL, PS and PI all significantly greater in larvae from treatment (c).

The major neutral lipid class was cholesterol, followed by triacylglycerol, cholesteryl esters and free fatty acids. As with polar lipids, there was a trend of increasing neutral lipid classes through treatments (a) to (b) to (c), with all classes being significantly greater in larvae from treatment (c).

Table 4 shows the fatty acid compositions of total lipid from larvae reared on the different regimes. Total saturated fatty acids were significantly higher in larvae from treatment (a), whereas total monounsaturates were lower. Total PUFA and total *n*-3HUFA were lowest in larvae from treatment (b). No significant differences were found between dimethyl acetal (DMA) levels.

TABLE 2

Total lipid (percentage of dry weight) and fatty acid content (μg fatty acid/mg of dry weight) of the rotifer *Brachionus plicatilis* cultured with baker's yeast *Saccharomyces cerevisiae* and enriched in polyunsaturated fatty acids with (a) *Nannochloropsis gaditana,* (b) a mixture of *Nannochloropsis gaditana, Isochrysis galbana* and *Rhodomonas* sp. and (c) Protein Selco (Artemia Systems)

Fatty acid	(a)	(b)	(c)
14:0	1.8±0.2	1.5±0.4	1.7±0.7
15:0	2.6±0.4	3.3±0.3	2.1±0.3
16:0	10.2±0.4	5.3±0.2	7.2±0.3
16:1*n*-7	18.7±0.8	12.7±0.8	16.8±0.8
16:2	0.2±0.0	0.1±0.0	0.2±0.0
17:0	0.3±0.0	0.2±0.1	0.2±0.0
16:3	0.4±0.1	0.4±0.1	0.7±0.1
16:4	1.5±0.1	2.0±0.3	1.3±0.2
18:0	2.7±0.1	2.8±0.1	3.2±0.1
18:1*n*-9	12.1±0.8	16.9±1.8	20.9±1.0
18:1*n*-7	5.9±0.1	5.5±1.3	7.2±0.5
18:2*n*-6	2.4±0.0	2.9±0.3	4.2±0.1
18:3*n*-3	0.2±0.0	1.1±0.0	0.6±0.0
18:4*n*-3	0.2±0.0	1.0±0.0	0.7±0.1
20:0	0.1±0.0	0.1±0.0	0.1±0.0
20:1*n*-9	2.2±0.1	2.9±0.1	2.9±0.1
20:1*n*-7	0.8±0.1	0.9±0.1	1.0±0.1
20:2*n*-6	0.9±0.1	1.0±0.1	0.9±0.2
20:3*n*-6	0.9±0.2	0.5±0.0	0.5±0.1
20:4*n*-6	3.0±0.1	0.7±0.1	1.2±0.1
20:3*n*-3	0.2±0.0	0.2±0.1	tr
20:4*n*-3	0.2±0.0	0.7±0.1	0.7±0.1
20:5*n*-3	16.4±0.7	2.8±0.1	9.8±0.5
22:1*n*-11	0.6±0.1	0.5±0.3	0.7±0.1
22:1*n*-9	0.3±0.0	0.4±0.1	0.4±0.1
22:3*n*-3	0.2±0.1	0.2±0.1	0.2±0.1
22:4*n*-3	0.2±0.0	0.3±0.0	0.2±0.1
22:5*n*-6	0.3±0.1	0.4±0.1	0.3±0.1
22:5*n*-3	1.7±0.1	0.4±0.1	1.4±0.1
22:6*n*-3	0.4±0.0	1.4±0.1	4.6±0.2
Totals			
Saturated	17.9±0.7	13.3±0.2	14.6±1.3
Monunsaturated	40.7±1.5	39.9±3.9	50.1±2.1
Polyunsaturated	31.3±1.4	18.8±0.4	29.8±1.5
(*n*-6) HUFA	4.4±0.3	1.9±0.1	2.6±0.2
(*n*-3) HUFA	19.4±0.9	6.2±0.1	16.9±0.8
Dry weight (%)	12.2±0.9	10.6±0.7	11.5±0.5
Total lipids (as dry wt%)	14.7±0.5	14.1±1.5	14.1±0.8

Data are means±s.d. ($n=3$). s.d.=0 implies as s.d. of <0.05. Totals include some minor components (<0.1%) not shown. HUFA, highly unsaturated fatty acids (>C_{20} and with at least 3 double bonds).

TABLE 3

Gross composition and lipid class composition (percentage of dry weight) of 15-days-old gilthead sea bream larvae fed with rotifers *Brachionus plicatilis* that had been enriched in polyunsaturated fatty acids with (a) *Nannochloropsis gaditana,* (b) a mixture of *Nannochloropsis garditana, Isochrysis galbana* and *Rhodomonas* sp. and (c) Protein Selco (Artemia Systems)

Composition	(a)	(b)	(c)
Protein (dry wt%)	59.7±1.5	61.5±1.2	61.1±2.6
Lipid (dry wt%)	19.9±2.3	21.3±1.9	26.3±3.5
Lipid class (dry weight %)			
Total polar	10.0±0.2^a	11.2±0.1^b	12.9±0.3^c
Diradyl glycerophosphocholine	4.0±0.2^a	4.2±0.1^a	5.3±0.1^b
Diradyl glycerophosphoethanolamine	3.6±0.1^a	4.1±0.1^b	4.5±0.1^b
Phosphatidylserine	0.9±0.1^a	1.1±0.1^a	1.4±0.1^b
Phosphatidylinositol	0.7±0.0^a	0.7±0.0^a	0.9±0.0^b
Phosphatidic acid/cardiolipin	0.5±0.0^a	0.7±0.1^b	0.5±0.0^a
Sphingomyelin	0.3±0.0	0.3±0.0	0.3±0.0
Total neutral	9.9±0.2^a	10.1±0.1^a	13.4±0.3^b
Cholesterol	4.8±0.2^a	5.0±0.2^a	5.7±0.1^b
Free fatty acids	0.3±0.2^a	0.3±0.1^a	1.0±0.2^b
Triacylglycerol	2.4±0.3^a	2.4±0.1^a	3.8±0.2^b
Cholesteryl esters	2.4±0.2^{ab}	2.3±0.2^a	2.9±0.1^b

Tanks with larvae fed on treatments (a) contained *N. gaditana* at a density of 400 000 cells/ml and those whose larvae were fed on treatments (b) and (c) contained a mixture of *N. gaditana, I. galbana* and *Rhodomonas* sp. at the same density, to maintain the quality of the rotifer. Data are means ± s.d. ($n=4$). s.d. = 0.0 implies an s.d. < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $P<0.05$. If no superscript appears, values are not different.

There was a significant increase in larval total lipid DHA content from treament (a) to treatment (b) and also from treatment (b) to treatment (c) (Table 4). In contrast, EPA and docosapentaenoic acid (DPA; 22:5*n*-3) levels were highest in larvae from treatment (a) and lowest in larvae from treatment (b) with intermediate levels in larvae from treatment (c) (all differences significant). AA was also highest in total lipid from treatment (a) larvae.

The fatty acid compositions of CPL from larvae fed the three different regimes are shown in Table 5. Total monoenes, total PUFA, total *n*-3 and *n*-6HUFA were significantly higher in larvae from treatments (a) and (c), whereas no significant differences were found among total saturated fatty acids. The percentage of DHA was significantly greater in larvae from treatment (c). The percentages of EPA and DPA were greatest in treatment (a) and lowest in treatment (b) with intermediate percentages in treatment (c). AA also showed this pattern in CPL.

In EPL, the percentages of PUFA and HUFA were the greatest of all the

TABLE 4

Total lipid fatty acid composition (percentage of weight) of 15-day-old gilthead sea bream larvae fed with rotifers *Brachionus plicatilis* enriched in polyunsaturated fatty acids with (a) *Nannochloropsis gaditana*, (b) a mixture of *N. gaditana*, *I. galbana* and *Rhodomonas* sp., and (c) Protein Selco (Artemia Systems)

Fatty acid	(a)	(b)	(c)
14:0	1.0±0.1	0.8±0.1	0.9±0.0
15:0	3.4±0.3[a]	4.3±0.2[b]	3.6±0.2[a]
16:0DMA	0.4±0.0	0.4±0.0	0.4±0.0
16:0	14.7±0.2[a]	12.5±0.4[b]	12.5±0.4[b]
16:1*n*-7	7.0±0.3[a]	7.8±0.2[b]	5.9±0.2[c]
16:2	0.6±0.0[a]	0.3±0.1[b]	0.4±0.1[b]
16:3	0.6±0.1	0.5±0.0	0.6±0.1
16:4	0.5±0.1	0.5±0.1	0.5±0.1
18:0DMA	1.9±0.3	1.6±0.2	2.0±0.6
18:1*n*-9DMA	0.1±0.0	0.2±0.0	0.2±0.0
18:1*n*-7DMA	0.1±0.0	tr	tr
18:0	9.6±0.3	9.3±0.2	9.5±0.1
18:1*n*-9	9.4±0.5[a]	13.3±0.8[b]	12.3±0.2[b]
18:1*n*-7	5.6±0.4	5.7±0.2	6.8±1.5
18:2*n*-6	1.9±0.0[a]	2.8±0.2[b]	2.7±0.1[b]
18:3*n*-3	0.1±0.0[a]	0.4±0.0[b]	0.2±0.0[a]
18:4*n*-3	0.7±0.1	0.6±0.1	0.6±0.0
20:0	0.2±0.0	0.2±0.0	0.2±0.0
20:1n-9	1.2±0.0[a]	1.6±0.2[b]	1.4±0.0[b]
20:1*n*-7	0.9±0.1	0.9±0.1	0.9±0.1
20:2*n*-6	0.6±0.2	0.9±0.2	0.6±0.1
20:3*n*-6	0.8±0.1	0.8±0.1	0.6±0.1
20:4*n*-6	4.7±0.1[a]	3.2±0.1[b]	3.1±0.2[b]
20:3*n*-3	0.2±0.0	0.2±0.1	0.3±0.1
20:4*n*-3	0.3±0.0[a]	0.8±0.0[b]	0.9±0.1[b]
20:5*n*-3	10.5±0.5[a]	6.6±0.1[b]	7.4±0.3[c]
22:1*n*-11	0.1±0.0	0.1±0.0	0.2±0.1
22:1*n*-9	0.2±0.1	0.2±0.1	0.3±0.0
22:3*n*-3	0.7±0.1[a]	0.3±0.1[b]	0.4±0.1[b]
22:4*n*-3	0.1±0.1	tr	tr
22:5*n*-6	0.3±0.1	0.5±0.1	0.5±0.0
22:5*n*-3	4.8±0.2[a]	1.8±0.1[b]	2.9±0.1[c]
22:6*n*-3	8.5±0.8[a]	10.0±0.7[b]	12.4±1.2[c]
Totals			
Saturated	29.4±0.1[a]	27.6±0.6[b]	27.3±0.2[b]
Monounsaturated	24.4±0.4[a]	30.3±1.4[b]	27.8±1.6[c]
Polyunsaturated	37.4±0.2[a]	32.4±0.6[b]	35.7±1.5[a]
Dimethyl acetal	2.6±0.3	2.1±0.3	2.5±0.6
(*n*-6) HUFA	6.1±0.1[a]	4.9±0.1[b]	4.4±0.1[c]
(*n*-3) HUFA	25.1±0.2[a]	19.9±0.7[b]	24.3±1.5[a]

Experimental details as in legend to Table 3. Data are means±s.d. ($n=4$), s.d.=0.0 implies an s.d. of <0.05. Totals include some minor components (<0.1%) not shown. tr, trace<0.1%. HUFA, highly unsaturated fatty acid ($\geq C_{20}$ and with at least 3 double bonds). Values within a given row not bearing the same superscript letter are significantly different at $P<0.05$.

TABLE 5

Fatty acid composition (percentage of weight) of total diradyl glycerophosphocholine from 15-day-old gilthead sea bream larvae fed with rotifers *Brachionus plicatilis* enriched in polyunsaturated fatty acids with (a) *Nannochloropsis gaditaña*, (b) a mixture of *N. gaditana*, *I. galbana* and *Rhodomonas* sp., and (c) Protein Selco (Artemia Systems)

Fatty acid	(a)	(b)	(c)
14:0	1.4±0.1	1.3±0.1	1.2±0.1
15:0	0.8±0.1[a]	1.6±0.1	1.1±0.4
16:0	24.9±0.1[a]	21.9±1.4[b]	21.9±0.8[b]
16:1*n*-7	8.2±0.2	8.8±1.1	6.4±0.8
16:2	0.8±0.0	0.5±0.2	0.6±0.1
16:3	0.8±0.1	0.7±0.2	0.7±0.0
16:4	0.3±0.0	0.3±0.2	0.3±0.1
18:0	5.7±0.1[a]	6.6±0.4[b]	6.5±0.2[b]
18:1*n*-9	12.6±0.5[a]	18.9±1.1[b]	16.1±0.6[c]
18:1*n*-7	5.5±0.6[a]	9.2±0.2[b]	6.4±0.6[a]
18:2*n*-6	2.4±0.0[a]	4.0±0.2[b]	3.4±0.1[c]
18:3*n*-3	0.1±0.0[a]	0.4±0.1[b]	0.2±0.1[b]
18:4n-3	0.1±0.0	0.1±0.0	0.1±0.0
20:0	0.1±0.0	0.1±0.0	0.4±0.3
20:1*n*-9	0.8±0.1	1.0±0.2	0.8±0.1
20:1*n*-7	0.7±0.2	0.7±0.1	0.6±0.1
20:2*n*-6	0.7±0.2	0.8±0.2	0.6±0.1
20:3*n*-6	1.0±0.0[a]	0.9±0.1[a]	0.7±0.0[b]
20:4*n*-6	3.9±0.1[a]	1.9±0.1[b]	2.3±0.0[c]
20:3*n*-3	0.6±0.1	0.5±0.2	0.7±0.1
20:4*n*-3	0.3±0.0[a]	0.7±0.1[b]	0.9±0.1[b]
20:5*n*-3	11.9±0.8[a]	5.7±0.6[b]	8.1±0.1[c]
22:1*n*-11	0.1±0.0	0.1±0.0	0.1±0.1
22:1*n*-9	0.1±0.0	0.2±0.2	0.1±0.0
22:3*n*-3	0.5±0.1[a]	0.2±0.1[b]	0.7±0.1[a]
22:4*n*-3	tr	0.1±0.1	0.2±0.1
22:5*n*-6	0.9±0.1[a]	1.6±0.9[a]	2.5±0.1[b]
22:5*n*-3	3.1±0.3[a]	0.9±0.1[b]	1.9±0.1[c]
22:6*n*-3	3.8±0.6[a]	3.5±0.1[a]	6.2±0.8[b]
Totals			
Saturated	33.1±0.2	31.8±2.3	31.4±0.5
Monounsaturated	28.0±1.5[a]	39.1±2.5[b]	30.6±0.7[a]
Polyunsaturated	33.1±1.1[a]	25.8±0.9[b]	31.6±0.3[a]
(*n*-6) HUFA	6.3±0.2[a]	4.8±0.8[b]	6.0±0.1[a]
(*n*-3) HUFA	20.3±1.1[a]	11.7±0.8[b]	18.4±0.7[a]

Experimental details as in legend to Table 3. Data are means±s.d. ($n=4$), s.d.=0.0 implies an s.d. of <0.05. Totals include some minor components (<1%) not shown. tr, trace<0.1%. HUFA, highly unsaturated fatty acid ($\geq C_{20}$ and with at least 3 double bonds). Values within a given row not bearing the same superscript letter are significantly different at $P<0.05$.

TABLE 6

Fatty acid composition (percentage of weight) of total diradyl glycerophosphoethanolamine from 15-day-old gilthead sea bream larvae fed with rotifers *Brachionus plicatilis* enriched in polyunsaturated fatty acids with (a) *Nannochloropsis gaditana*, (b) a mixture of *N. gaditana*, *Isochrysis galbana* and *Rhodomonas* sp., and (c) Protein Selco (Artemia Systems)

Fatty acid	(a)	(b)	(c)
14:0	0.5±0.1	0.4±0.1	0.3±0.0
15:0	2.2±0.3[a]	3.0±0.5[ab]	3.4±0.2[b]
16:0DMA	1.5±0.1	1.3±0.1	1.3±0.0
16:0	9.2±0.1[a]	9.1±0.1[a]	8.3±0.2[b]
16:1*n*-7	1.6±0.6[a]	2.6±0.6[b]	0.9±0.1[a]
16:2	0.3±0.0[a]	0.8±0.1[b]	0.7±0.0[b]
16:3	0.8±0.2	0.5±0.2	0.5±0.0
16:4	0.6±0.2	0.4±0.1	0.4±0.1
18:0DMA	3.9±0.1[a]	2.4±0.3[b]	3.3±0.1[c]
18:1*n*-9DMA	0.8±0.0	0.6±0.1	0.8±0.0
18:1*n*-7DMA	0.5±0.0[a]	0.3±0.0[b]	0.3±0.0[b]
18:0	12.3±0.4[a]	14.3±0.3[b]	12.9±0.2[a]
18:1*n*-9	3.8±0.2[a]	8.4±1.2[b]	5.5±0.5[c]
18:1*n*-7	4.9±0.2	7.0±1.7	5.1±0.2
18:2*n*-6	0.9±0.0[a]	2.0±0.3[b]	1.3±0.0[c]
18:3*n*-3	0.2±0.0	0.2±0.0	0.2±0.0
18:4*n*-3	0.6±0.1[a]	0.2±0.0[b]	0.5±0.0[a]
20:0	0.6±0.3[a]	0.2±0.0[b]	0.2±0.0[b]
20:1*n*-9	0.9±0.1[a]	1.5±0.2[b]	1.2±0.1[b]
20:1*n*-7	0.6±0.2	1.1±0.2	0.9±0.1
20:2*n*-6	0.5±0.4	0.4±0.3	0.4±0.1
20:3*n*-6	0.7±0.0[a]	0.7±0.1[a]	0.5±0.0[b]
20:4*n*-6	6.6±0.1[a]	4.7±0.5[b]	4.5±0.1[b]
20:3*n*-3	tr	0.3±0.1	tr
20:4*n*-3	0.4±0.0[a]	1.0±0.1[b]	1.1±0.1[b]
20:5*n*-3	12.1±0.6[a]	8.7±0.8[b]	8.2±0.2[b]
22:1*n*-11	tr	tr	tr
22:1*n*-9	0.3±0.1	tr	0.1±0.0
22:3*n*-3	0.7±0.3	0.7±0.3	0.7±0.2
22:4*n*-3	0.2±0.0	0.2±0.1	tr
22:5*n*-6	0.6±0.2	1.0±0.6	1.5±0.5
22:5*n*-3	8.3±0.6[a]	2.8±0.3[b]	4.8±0.2[c]
22:6*n*-3	17.3±1.3[a]	17.2±2.1[a]	24.5±1.8[b]
Totals			
Saturated	25.3±0.5	28.0±0.7	26.2±0.2
Monounsaturated	12.3±1.1[a]	20.7±2.9[b]	13.7±0.4[a]
Polyunsaturated	52.2±1.1[a]	44.1±3.7[b]	51.1±0.8[a]
Dimethyl acetal	6.6±0.2[a]	4.6±0.5[b]	5.8±0.1[c]
(*n*-6) HUFA	8.3±0.2[a]	7.5±1.3[ab]	7.0±0.6[b]
(*n*-3) HUFA	39.1±1.2[a]	30.9±2.9[b]	39.4±1.1[a]

Experimental details as in legend to Table 3. Data are means±s.d. ($n=4$), s.d.=0.0 implies an s.d. of <0.05. Totals include some minor components (<0.1%) not shown. tr, trace<0.1%. HUFA, highly unsaturated fatty acid ($\geq C_{20}$ and with at least 3 double bonds). Values within a given row not bearing the same superscript letter are significantly different at $P<0.05$.

TABLE 7

Fatty acid composition (percentage of weight) of phosphatidylserine from 15-day-old gilthead sea bream larvae fed with rotifers *Brachionus plicatilis* enriched in polyunsaturated fatty acids with (a) *Nannochloropsis gaditana*, (b) a mixture of *N. gaditana*, *Isochrysis galbana* and *Rhodomonas* sp., and (c) Protein Selco (Artemia Systems)

Fatty acid	(a)	(b)	(c)
14:0	1.2 ± 0.3	0.6 ± 0.2	0.6 ± 0.2
15:0	5.0 ± 0.1^{a}	9.9 ± 0.5^{b}	8.7 ± 1.3^{b}
16:0	7.6 ± 0.2^{a}	9.5 ± 0.9^{b}	8.1 ± 0.9^{ab}
16:1*n*-7	1.4 ± 0.0^{a}	2.2 ± 0.4^{b}	1.2 ± 0.2^{a}
16:2	1.0 ± 0.1^{a}	1.5 ± 0.1^{b}	0.8 ± 0.1^{a}
16:3	1.0 ± 0.1	1.2 ± 0.3	1.1 ± 0.4
16:4	1.1 ± 0.3	0.6 ± 0.4	1.4 ± 0.4
18:0	25.1 ± 0.3^{a}	27.6 ± 1.3^{b}	26.0 ± 0.4^{ab}
18:1*n*-9	2.8 ± 0.1^{a}	4.3 ± 0.4^{b}	3.5 ± 0.0^{c}
18:1*n*-7	2.6 ± 0.1^{a}	2.4 ± 0.1^{a}	1.9 ± 0.2^{b}
18:2*n*-6	0.4 ± 0.1^{a}	0.8 ± 0.1^{b}	0.6 ± 0.1^{ab}
18:3*n*-3	tr	0.2 ± 0.1	tr
18:4*n*-3	0.5 ± 0.1	tr	0.5 ± 0.1
20:0	0.8 ± 0.0	0.8 ± 0.0	0.5 ± 0.3
20:1*n*-9	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.1
20:1*n*-7	0.5 ± 0.2	0.8 ± 0.1	0.7 ± 0.0
20:2*n*-6	0.5 ± 0.1^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}
20:3*n*-6	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.2
20:4*n*-6	2.5 ± 0.1^{a}	1.4 ± 0.1^{b}	1.5 ± 0.2^{b}
20:3*n*-3	0.7 ± 0.1^{a}	0.3 ± 0.1^{b}	0.2 ± 0.0^{b}
20:4*n*-3	0.3 ± 0.0^{a}	0.6 ± 0.0^{b}	0.6 ± 0.1^{b}
20:5*n*-3	3.7 ± 0.1^{a}	2.3 ± 0.1^{b}	2.1 ± 0.3^{b}
22:1*n*-11	tr	tr	tr
22:1*n*-9	tr	tr	0.3 ± 0.1
22:3*n*-3	1.5 ± 0.1^{a}	0.9 ± 0.1^{b}	0.7 ± 0.1^{b}
22:4*n*-3	tr	tr	tr
22:5*n*-6	0.9 ± 0.4^{a}	1.7 ± 0.7^{b}	2.5 ± 0.2^{b}
22:5*n*-3	10.7 ± 1.1^{a}	3.6 ± 0.2^{b}	5.6 ± 0.1^{c}
22:6*n*-3	19.0 ± 0.9	18.4 ± 0.7	21.1 ± 2.8
Totals			
Saturated	40.5 ± 0.4^{a}	49.6 ± 1.9^{b}	44.9 ± 1.5^{c}
Monounsaturated	8.2 ± 0.2^{a}	10.8 ± 1.1^{b}	8.4 ± 0.3^{a}
Polyunsaturated	46.0 ± 1.6^{a}	35.1 ± 0.8^{b}	40.9 ± 1.6^{c}
(*n*-6) HUFA	5.1 ± 1.0^{ab}	4.1 ± 0.4^{a}	5.5 ± 0.7^{b}
(*n*-3) HUFA	35.9 ± 1.9^{a}	26.1 ± 1.2^{b}	30.4 ± 3.0^{ab}

Experimental details as in legend to Table 3. Data are means $\pm$ s.d. ($n=4$), s.d. $=0.0$ implies an s.d. of <0.05. Totals include some minor components ($<0.1\%$) not shown. tr, trace $<0.1\%$. HUFA, highly unsaturated fatty acid ($\geq C_{20}$ and with at least 3 double bonds). Values within a given row not bearing the same superscript letter are significantly different at $P<0.05$.

TABLE 8

Fatty acid composition (percentage of weight) of phosphatidylinositol from 15-day-old gilthead sea bream larvae fed with rotifers *Brachionus plicatilis* enriched in polyunsaturated fatty acids with (a) *Nannochloropsis gladitana*, (b) a mixture of *N. gaditana, Isochrysis galbana* and *Rhodomonas* sp., and (c) Protein Selco (Artemia Systems)

Fatty acid	(a)	(b)	(c)
14:0	1.4 ± 0.7^{a}	0.5 ± 0.0^{b}	1.1 ± 0.2^{a}
15:0	7.0 ± 0.3	10.9 ± 0.9	8.2 ± 0.7
16:0	8.2 ± 0.6	8.5 ± 0.6	8.1 ± 0.4
16:1*n*-7	2.3 ± 0.2^{a}	1.9 ± 0.1^{a}	1.4 ± 0.2^{b}
16:2	1.2 ± 0.2^{a}	1.5 ± 0.1^{a}	0.7 ± 0.1^{b}
16:3	0.8 ± 0.0^{a}	1.2 ± 0.1^{b}	1.3 ± 0.3^{b}
16:4	1.5 ± 0.0^{a}	0.8 ± 0.2^{b}	1.1 ± 0.1^{b}
18:0	26.9 ± 0.4	26.1 ± 1.4	26.1 ± 3.3
18:1*n*-9	4.6 ± 0.6^{a}	6.3 ± 0.2^{b}	6.9 ± 1.1^{b}
18:1*n*-7	5.2 ± 0.1	4.6 ± 0.4	4.8 ± 0.6
18:2*n*-6	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
18:3*n*-3	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.3
18:4*n*-3	tr	tr	0.4 ± 0.3
20:0	0.8 ± 0.1	tr	0.1 ± 0.1
20:1*n*-9	0.6 ± 0.2	0.8 ± 0.2	0.7 ± 0.1
20:1*n*-7	0.2 ± 0.1	tr	0.3 ± 0.1
20:2*n*-6	0.4 ± 0.1	0.7 ± 0.4	0.5 ± 0.2
20:3*n*-6	0.5 ± 0.1	0.7 ± 0.3	0.5 ± 0.1
20:4*n*-6	14.1 ± 0.5^{a}	10.1 ± 0.4^{b}	11.9 ± 1.5^{b}
20:3*n*-3	tr	tr	tr
20:4*n*-3	0.7 ± 0.2^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}
20:5*n*-3	8.5 ± 0.6^{a}	6.1 ± 0.1^{b}	6.3 ± 0.6^{b}
22:1*n*-11	tr	tr	tr
22:1*n*-9	tr	tr	0.3 ± 0.1
22:3*n*-3	tr	0.2 ± 0.1	tr
22:4*n*-3	tr	tr	tr
22:5*n*-6	0.8 ± 0.4	2.7 ± 0.2	tr
22:5*n*-3	4.7 ± 0.7^{a}	1.6 ± 0.2^{b}	2.9 ± 0.2^{c}
22:6*n*-3	4.2 ± 0.7^{a}	4.2 ± 0.6^{a}	8.2 ± 0.3^{b}
Totals			
Saturated	45.3 ± 0.8	46.8 ± 1.2	44.8 ± 3.4
Monounsaturated	13.3 ± 0.8	13.6 ± 0.2	14.2 ± 1.7
Polyunsaturated	38.4 ± 2.4^{a}	31.4 ± 0.6^{b}	36.2 ± 1.6^{a}
(*n*-6) HUFA	15.6 ± 1.2^{a}	13.6 ± 0.6^{b}	12.4 ± 1.7^{b}
(*n*-3) HUFA	17.9 ± 1.3^{a}	12.3 ± 0.1^{b}	17.7 ± 0.8^{a}

Experimental details as in legend to Table 3. Data are means $\pm$ s.d. ($n=4$), s.d. $=0.0$ implies an s.d. of <0.05. Totals include some minor components ($<0.1\%$) not shown. tr, trace $<0.1\%$. HUFA, highly unsaturated fatty acid ($\geq C_{20}$ and with at least 3 double bonds). Values within a given row not bearing the same superscript letter are significantly different at $P<0.05$.

TABLE 9

Evolution of total length (mm), dry weight, carbon, nitrogen, hydrogen and protein contents (all expressed as μg/larva), and C:N ratios in gilthead sea bream larvae fed with rotifers *Brachionus plicatilis* enriched in polyunsaturated fatty acids with (a) *Nannochloropsis gaditana*, (b) a mixture of *N. gaditana*, *Isochrysis galbana* and *Rhodomonas* sp. and (c) Protein Selco (Artemia Systems)

Time (days after hatching)	6	9	12	15
(a)				
Total length	3.9 ± 0.0^{ab}	4.0 ± 0.0^{a}	4.5 ± 0.0	5.0 ± 0.0^{a}
Dry weight	29.7 ± 0.9^{a}	34.7 ± 0.9^{a}	55.5 ± 4.3	79.2 ± 3.5^{a}
Carbon	10.2 ± 0.5^{a}	13.6 ± 0.2^{a}	20.3 ± 0.9^{a}	30.2 ± 0.8^{a}
Nitrogen	2.3 ± 0.1^{a}	3.3 ± 0.7^{a}	5.0 ± 0.3^{a}	7.5 ± 0.2^{a}
Hydrogen	1.5 ± 0.1^{a}	1.9 ± 0.0^{a}	3.0 ± 0.1^{a}	4.5 ± 0.0^{a}
Protein	14.7 ± 0.7^{a}	20.8 ± 0.4^{a}	31.7 ± 0.7^{a}	47.2 ± 0.9^{a}
C:N ratio	4.4 ± 0.0	4.1 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
(b)				
Total length	3.8 ± 0.0^{a}	4.1 ± 0.0^{a}	4.7 ± 0.1	4.8 ± 0.0^{b}
Dry weight	26.2 ± 1.6^{b}	36.2 ± 0.9^{ab}	62.1 ± 4.9	69.9 ± 1.8^{b}
Carbon	10.4 ± 0.1^{a}	14.4 ± 0.1^{b}	22.8 ± 0.0^{b}	27.5 ± 0.6^{b}
Nitrogen	2.3 ± 0.0^{a}	3.5 ± 0.0^{b}	5.6 ± 0.1^{ab}	6.8 ± 0.1^{b}
Hydrogen	1.5 ± 0.0^{a}	2.0 ± 0.0^{b}	3.4 ± 0.1^{b}	4.1 ± 0.1^{b}
Protein	14.9 ± 0.2^{a}	22.2 ± 0.2^{b}	35.5 ± 0.7^{b}	42.9 ± 0.9^{b}
C:N ratio	4.4 ± 0.1	4.1 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
(c)				
Total length	4.0 ± 0.0^{b}	4.2 ± 0.0^{b}	4.8 ± 0.1	5.1 ± 0.0^{a}
Dry weight	32.3 ± 2.8^{c}	41.1 ± 2.9^{b}	67.9 ± 7.6	86.5 ± 4.3^{a}
Carbon	11.7 ± 0.5^{b}	15.6 ± 0.6^{c}	24.9 ± 0.4^{b}	33.9 ± 0.4^{c}
Nitrogen	2.6 ± 0.1^{b}	3.9 ± 0.0^{c}	6.2 ± 0.4^{b}	8.4 ± 0.3^{c}
Hydrogen	1.7 ± 0.0^{b}	2.3 ± 0.0^{c}	3.7 ± 0.2^{b}	5.2 ± 0.2^{c}
Protein	16.6 ± 0.5^{b}	24.6 ± 0.3^{c}	39.0 ± 0.2^{b}	52.9 ± 0.4^{c}
C:N ratio	4.4 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0

Data are means ± s.d ($n=4$; except for total length $n=10$). s.d. = 0.0 implies an s.d. < 0.05. Values belonging to the same subject (.e. dry weight, carbon, nitrogen, hydrogen or protein) of the same age (within a given column) not bearing the same superscript letter are significantly different at $P<0.05$.

phosphoglycerides irrespective of treatment (Table 6). Total monoenes were significantly higher and total PUFA, *n*-3 and *n*-6HUFA and DMA were generally significantly lower in larvae from treatment (b) but there were no significant differences in total saturates. The percentage of DHA was significantly highest in larvae from treatment (c). The percentages of EPA and DPA were significantly higher in treatment (a) larvae. Although EPA was not significantly different between larvae from treatments (b) and (c), DPA pre-

sented higher percentages in larvae from treatment (c). The percentage of AA was also significantly highest in larvae from treatment (a).

Fatty acid compositions from PS are presented in Table 7. Total saturated and monounsaturated fatty acids were highest in larvae from treatment (b). The percentages of DPA were highest in PS, compared to other phosphoglycerides, irrespective of treatment. However, the percentage of DPA was highest in larvae from treatment (a), lowest in larvae from treatment (b) and intermediate in treatment (c). Both EPA and AA were significantly higher in larvae from treatment (a). The percentage of DHA was greatest in larvae from treatment (c) although, on this occasion, this was not statistically significant.

Fatty acid compositions of PI are shown in Table 8. No significant differences were found in total saturates or total monoenes between the dietary groups, whereas total PUFAs were significantly lower in treatment (b). Compared to other phosphoglycerides, PI showed the highest percentages of *n*-6HUFA and AA. However, the percentage of AA was highest in larvae from treatment (a) and lowest in larvae from treatment (b). This pattern was also observed for EPA and DPA. The percentage of DHA was significantly higher in larvae from treatment (c).

Table 9 shows data for larval growth (total length and dry weight), elemental composition (C, N, H) and protein content. The larvae from treatment (c) had the highest values for total length, dry weight and carbon, nitrogen, hydrogen and protein content, indicating the greatest growth overall. The group (c) larvae were significantly larger by day 6 and they maintained that advantage throughout the experimental period. Similar trends were observed for carbon, nitrogen and hydrogen contents. C/N ratios showed higher values at day 6, then decreased and remained constant until day 15.

DISCUSSION

Larvae from treatment (c) showed the highest content of total lipid. Total polar lipid and total neutral lipid content correlated with dietary DHA levels, but not with the total *n*-3HUFA content of the diet. The main increase observed in neutral lipid was due to significantly higher levels of all classes, including TAG and cholesterol, in larvae from treatment (c). The magnitudes of TAG (Fraser et al., 1987), total polar lipid and cholesterol (Håkanson, 1989a,b) are useful indicators for determining the nutritional condition of laboratory-reared or natural populations of marine fish larvae. Using these criteria, gilthead sea bream larvae from treatment (c) having the highest total polar lipid, TAG and cholesterol content, appeared to have the best nutritional status, followed by larvae from treatment (b) and then the treatment (a) larvae. Therefore, nutritional condition correlated with the level of DHA in the diets of the larvae. These experiments were performed under the nor-

mal operating conditions at the fish farm using sand on the bottom of the tanks and 150 000 larvae per tank. This made the collection of reliable mortality data impossible but no obviously different mortalities were observed between the treatments.

Similarly, with regard to the growth parameters shown in Table 9, it is noteworthy that the largest larvae were obtained by feeding with rotifers from treatment (c), which contained the highest values of DHA, but not the highest total *n*-3HUFA content. It is noteworthy that the improved growth rate for group (c) larvae was primarily in the first 6 days. The poorest growth parameters were found in larvae fed with rotifers enriched by treatment (b), which contained the lowest total *n*-3HUFA content but intermediate DHA content. Rotifers from treatment (b) contained 6.2 μg *n*-3HUFA/mg of dry weight. This is greater than the minimum established for the same age gilthead sea bream larvae by Koven et al. (1990) (5.1 μg/mg of dry weight), or the minimum established by Tuncer and Harrel (1992) for striped bass larvae (5.7 μg/mg of dry weight). Better growth response was obtained in larvae fed with rotifers enriched by treatment (a), which was low in DHA, but had the highest total *n*-3HUFA content of 19.4 μg/mg of dry weight. This is consistent with the conclusion that a high *n*-3HUFA content in the diet (well over minimum requirements) significantly improves growth of gilthead sea bream larvae during first feeding. The growth of larvae reared on rotifers from treatment (a) showed that the presence of DHA in the diet may not be strictly necessary, presumably because larvae could strongly retain DHA from the yolk (Koven et al., 1989), at least in the minimum amounts needed to cover the requirement for proper development of the neural system during the first 2 weeks of life. However, the results with larvae reared on rotifers from treatment (c) show that increasing the DHA:EPA ratio of the total *n*-3HUFA correlates significantly with improved overall growth parameters, particularly in the first week of weaning. It is possible, however, that growth could also have been improved in group (c) larvae by the protein supplement contained in Protein Selco, which may provide a better level of essential amino acids than algae-enriched rotifers (Mourente, 1989).

The C:N ratio is considered as an indicator of the lipid:protein ratio (Anger, 1988) and the patterns found in larvae fed the three different regimes suggest a more rapid lipid accumulation (C:N=4.4) at day 6 after hatching, followed by a period in which lipid and protein increase at similar rates. No significant differences were detected between the C:N ratios from the different treatments. This implies that in gilthead sea bream larvae, after yolk sac absorption and when beginning to feed, there is proportionally a greater accumulation of lipid than of protein. Lipids, especially DHA, may be particularly necessary at this time for rapid development of the neural system (Neuringer et al., 1988; Bazan, 1990). High dietary *n*-3HUFA, with a high DHA content, may result in improved development of the nervous systems includ-

ing greater visual acuity (Neuringer et al., 1988). This may enhance the detection and capture of prey and the net energy gain by the larvae (Noakes and Godin, 1988) and so indirectly affect growth parameters.

Gilthead sea bream larval lipids show a characteristic distribution of fatty acids between the different phosphoglyceride classes. CPL was characterized by high 16:0 and relatively low levels of HUFA, whereas EPL showed the highest level of HUFA, with similar levels of both C_{20} and C_{22} HUFA, and intermediate levels of saturates and monoenes. PS was characterized by high HUFA that was predominantly C_{22} HUFA, and high 18:0. High 18:0 was also characteristic of PI, along with the highest total of C_{20} HUFA and 20:4*n*-6 specifically. Similar distributions of fatty acids between the phosphoglyceride classes in fish systems have been reported previously (Tocher and Sargent, 1984; Tocher and Harvie, 1988; Tocher, 1990; Tocher and Mackinley, 1990; Mourente et al., 1991) but the present data are noteworthy as they demonstrate that this distribution is largely unaffected by dietary influences.

The results of the present study suggest that bioconversion of EPA to DHA during this critical period of first feeding is low. It is not possible from compositional data alone to evaluate exactly how much DHA has been conserved in larvae from yolk sac reserves (Koven et al., 1989) or has been bioconverted from its precursor, EPA. However, the rate of DHA biosynthesis from DPA appears to be low, since DPA (the elongation product of EPA), accumulated in larval total lipids and phosphoglycerides in amounts related to dietary EPA levels. In contrast, DHA levels in phosphoglycerides were related to dietary DHA and not dietary EPA. This may indicate that $\Delta 4$ desaturase activity is low in larval gilthead sea bream at the time of first feeding.

In conclusion, the results from the present study have shown that growth performance of gilthead sea bream larvae at first feeding was influenced by both total *n*-3HUFA and DHA content of the diet, the best growth rate being achieved with a high *n*-3HUFA content and a high DHA:EPA ratio. Lipid contents of the larvae, nutritional status, and total lipid and phosphoglyceride DHA levels were directly correlated with dietary DHA levels. The fatty acid compositional data supported the finding that dietary DHA was important in that it suggested that dietary EPA could not contribute significantly to tissue DHA levels in larval gilthead sea bream, presumably due to low $\Delta 4$ desaturase activity.

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